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Acupuncture Treatment in Patients with Low Back Pain

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Abstract

Low back pain is the most common medical problem and very commonly treated condition with acupuncture today. The pain can arise from muscles, tendons, ligaments, bones or intervertebral discs. The pain is usually in the lower back region, gluteal region with or without radiation in the legs, with muscle tension and stiffness, limited movement or sometimes tingling and burning sensation. In the term of Traditional Chinese Medicine (TCM), pain appears if there is no good Blood and Qi flow in the body. Pain caused by Qi stagnation usually appears due to strong emotional and mental changes and stress. In this research, we included 60 patients, 28 males and 32 females, on age from 18 to 88, all treated with acupuncture for low back pain. All patients were cured with certain number of treatments. Most of the patients needed less than 5 treatments. Treatments were made with normal and fire needle and combined. The best results were achieved with fire needle acupuncture treatment. In the treatment, we used local Ashi *i.e.* trigger points and meridian points: BL24 (QiHaiSu), BL25 (DaChangShu), BL26 (GuanYanShu), BL27 (XioChangShu), BL28 (PangGuangShu), GB30 (HuanTiao), BL40 (WeiZhong), BL57 (ChengShen), GB37 (GuanMing) and BL60 (KunKun). Acupuncture as a treatment for low back pain is a very effective treatment giving very satisfying and positive results in a short time of period. The percentage of cured patients (relieved of the symptoms) is 100% which is a clear indicator of the success of acupuncture in the treatment of low back pain.

Keywords

Traditional Chinese Medicine, Acupuncture, Treatment, Lumbar, Pain

1. Introduction

Low back pain is the most common medical problem and very commonly

treated condition with acupuncture today. The pain can arise from muscles, tendons, ligaments, bones or intervertebral discs. The pain is usually in the lower back region, gluteal region with or without radiation in the legs, with muscle tension and stiffness, limited movement or sometimes tingling and burning sensation [1]. Low back pain affects both women and men and increases in the aging population. The pain can be also influenced by the psychological factors: anxiety, stress and depression [2]. Low back pain can be defined as:

1) Acute pain—present pain for less than 6 weeks;

2) Subacute pain—present pain between 6 weeks and 3 months;

3) Chronic pain—present pain for longer than 3 months [1].

In the term of Traditional Chinese Medicine (TCM), pain appears if there is no good Blood and Qi flow in the body. Pain caused by Qi stagnation usually appears due to strong emotional and mental changes and stress. If there is Blood stasis, there will be present sharp pain at a specific location and painful swelling. If there is insufficiency of Qi and Blood, the pain is not severe but it lasts longer and becomes worse after rest. The pain is worse after rest, because there is insufficient Blood and Qi to keep the flow moving. Movement helps to improve the Qi and Blood flow. Before starting the treatment, the practitioner must know if the pain is due to insufficiency of blockage, Blood stasis or Qi stagnation, what is the cause and the meridians that are affected. Therefore, every patient in TCM is given an individual treatment based on the pattern of disharmony.

The Qi and the Blood flow can be affected by the external pathogenic factors: wind, cold, dampness, heat and etc. and when they invade the low back, they cause low back pain. If they are present in the upper body, cold or flu appears. Low back pain caused by wind is very achy, and caused by cold, the pain is located in one point, very sharp and severe. Cold is also causing Blood stasis. Pain caused by dampness is fixed, but very heavy and usually chronic. If there is swelling, it results from too much fluid accumulation. Heat causes swelling, redness and inflammation, especially in the joints. A person also must have strong Liver and Kidney, so the invasion of the external pathogens will be short and acute.

Internal factors that can cause low back pain are the emotions. Liver depression caused by frustration and stress can cause sciatica pain. Kidney can be damaged by constant fear and make the low back area weak. Worry and overthinking can damage the spleen and cause Blood insufficiency [3].

The principle treatment in TCM is to get the Blood and Qi flow free as soon as possible, remove the blockages from the Blood, expel the exogenous pathogenic factors, warm and invigorate the Blood, eliminate the inflammation and improve the function of whole body [4].

2. Material and Methods

In this research are included 60 patients, 28 males and 32 females, on age from 18 to 88, all treated with acupuncture for low back pain.

The patients were randomly selected from the archive of the clinic treated for low back pain in a period of three years 2015, 2016 and 2017. The number of pa-

tients, age and sex were randomly selected. Patients are mostly from a city urban area with a sedentary lifestyle.

Inclusion criteria. In the treatment are included patients who have finished the treatments, patients with low back pain with or without radiation of the pain in the leg, patients with hip pain, patients with acute and chronic pain, patients with osteoporosis, trauma injury, discus hernia, sciatica pain, muscle sprain, degenerative diseases, spondylolysis, spondylitis and vertebral spondylolisthesis.

Exclusion criteria. In the treatment were not included patients who haven't finished the treatments, cancer patients, pregnant patients, children and patients with localized pain on other parts of the body.

Ethical clearance of the study was claimed from all the patients before starting the treatment.

The patients were treated with acupuncture treatment in a clinic for TCM and acupuncture in Skopje, Macedonia by a doctor specialist in acupuncture. Treatments were made with normal and fire needle acupuncture and combined. Treatments with normal needle were with duration of 35 - 40 minutes on Ashi and meridian points. Treatments with fire needle were with duration of 5 - 10 minutes on local Ashi points. Treatments were done indoor, on a room temperature, once a week, ten treatments in one session. In the treatment were used fine sterile disposable needles 0.25 × 25 mm manufactured by Wuijuiang City Medical & Health Material Co., LTD.

In the treatment were used local Ashi *i.e.* trigger points and meridian points: BL24 (QiHaiSu), BL25 (DaChangShu), BL26 (GuanYanShu), BL27 (XioChangShu), BL28 (PangGuangShu), GB30 (HuanTiao), BL40 (WeiZhong), BL57 (ChengShen), GB37 (GuanMing) and BL60 (KunKun).

3. Results and Discussion

After treatments, patients were monitored and were coming for doctoral examinations. During controls, patients explained how they feel after treatments, whether there is more pain and other accompanying symptoms. All patients were cured (relieved of the symptoms) from the low back pain with certain number of treatments and recurrence of pain was not reported by any patient, although it is possible to happen in some cases. If there is pain recurrence, patients are encouraged to report it always and come for more treatments.

47% or 28 patients needed less than 5 treatments to achieve the results. In **Table 1** it is shown the number of patients and treatments made. 33% or 20 patients needed 5 to 10 treatments, 9 patients (15%) needed 10 to 15 treatments and only 3 patients (5%) needed more than 15 treatments.

The best results were achieved with fire needle acupuncture treatment. 48 patients were treated with fire needle, 5 patients with normal needle and 7 patients combined with fire and normal needle. The same results are shown in **Table 2**. In our practice the result are showing that the treatment with fire needle gives better results that the treatment with normal needle in conditions like low back pain, knee pain, elbow and other.

Of the treated patients, 32 were female and 28 male, but these numbers are irrelevant. The patients were on age from 18 to 88. Patients were divided in 7 age groups. Most of the patients (27%, 16 patients) were older than 70. The results for age groups are shown in Table 3.

30 of the treated patient were also taking pain medications occasionally. 10 of the patients had insomnia, and 5 patients had constipation. Of the other symptoms were present those typical for low back pain syndrome: radiating pain in the knees, legs and hips, restricted movement, pain when lifting heavy objects, numbness, tingling, burning sensation, edema, stiff muscles and etc. Most patients had pain in the level of L4/L5 and L5/S1.

The duration of the pain was different in all patients. Some have the pain for 15 - 20 years, for 1 - 2 years, 3 months, 2 weeks or few days. 37 patients had chronic pain and 23 had acute pain. Patients with acute pain were treated with normal needle acupuncture and combined treatment. Patients treated for chronic pain needed in average 7 treatments. Patients treated for acute pain needed in average 5 treatments.

The treated acupoints were chosen with aim to help the patients to eliminate the pain, remove the accompanying symptoms, to restore the function of the

Table 1. Number of treatments made.

Number of treatments	Number of patients
<5	28 (47%)
5 - 10	20 (33%)
10 - 15	9 (15%)
>15	3 (5%)

Table 2. Number of patients who used the different type of acupuncture treatment.

Type of acupuncture treatment	Number of patients
Fire needle	48 (80%)
Normal needle	5 (8%)
Combined	7 (12%)

Table 3. Age groups.

Age group	Number of patients
<20	1 (2%)
20 - 30	2 (3%)
30 - 40	8 (13%)
40 - 50	11 (18%)
50 - 60	9 (15%)
60 - 70	13 (22%)
>70	16 (27%)

muscles, tendons, ligaments, joints and bones, to ensure free and painless movement of the body and remove all the blockages from the Blood and Qi flow. The points are located on the upper back, middle and lower back, hips and legs [5].

Acupuncture benefits all parts of the body. Acupuncture can significantly reduce the pain and inflammation in the affected area, reduce the disability, improve the function, reduce the functional limitations and correct the Blood and Qi flow in the affected area [4] [6] [7].

4. Conclusion

Acupuncture as a treatment for low back pain is a very effective treatment giving very satisfying and positive results in a short time of period. The percentage of cured patients (relieved of the symptoms) is 100% which is a clear indicator of the success of acupuncture in the treatment of low back pain.

Conflicts of Interest

The authors declare that there are not conflicts of interest.

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Clinical Observation of 242 Cases of Polycystic Ovary Syndrome

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Abstract

Objective: To compare the clinical effect and safety between letrozole (LE) and clomiphene citrate (CC) stimulated cycles in women with polycystic ovary syndrome (PCOS). To evaluate the effectivenesses and benefits of letrozole for ovulation induction in infertile women with PCOS. **Methods:** We retrospectively analyze the clinical data of 242 cases of the first ovulation induction cycle patients with PCOS, who referred to the Department of Reproductive Medicine, The First Affiliated Hospital of Yangtze University from June 2016 to June 2018, and were randomly divided into letrozole group and control group. The experimental group received Letrozole 2.5 mg/d for 5 days during days 3 - 7 of menstrual cycle. The control group was given clomiphene citrate 100 mg/d for 5 days during days 3 - 7 of menstrual cycle. Progynova will be used when the follicular diameter is 14 mm. **Results:** Letrozole group had less mature follicles, lower estrogen levels, thicker endometrium and higher ovulation rate in HCG day. But there is no difference between two groups in clinical pregnancy rate, single pregnancy rate, abortion rate, prenatal pregnancy delivery and newborns. **Conclusion:** Letrozole and clomiphene citrate have similar effect on ovulation induction, but we still need a lot of clinical data of letrozole about the safety of follicle, embryo, fetus and newborns.

Keywords

Polycystic Ovary Syndrome, Clinical Pregnancy Rate, Letrozole, Clomiphene Citrate

1. Introduction

Polycystic ovary syndrome (PCOS) is caused by a variety of factors. Non-ovulation

or rare ovulation is one of the main reasons for infertility. Although the etiology of PCOS has not been completely clarified, there have been many years of historical experience in the induction of ovulation therapy for PCOS. Clomiphene citrate (CC) is the first-line ovulation-promoting drug recommended by the World Health Organization (WHO). However, during the clinical observation process, CC resistance was found in many patients, affecting cervical mucus, and affecting the side effects of submucosal intima, thereby affecting pregnancy outcomes. Letrozole (LE) is a third-generation selective non-steroidal aromatase inhibitor that induces ovulation by inhibiting estrogen production. The ovulation effect and safety of LE are in the end.

According to a recent review, letrozole appears to improve live-birth and pregnancy rates in anovulatory women with PCOS, compared to CC. Letrozole is not associated with any anti-estrogenic effects on endometrium. This is supported by studies reporting adequate endometrial thickness during letrozole treatment [1].

In this study, 242 cases of ovulation-promoting patients treated with ovulation therapy were prospectively controlled to explore the clinical application of LE.

2. Materials and Methods

2.1. Research Object

242 cases of PCOS patients from July 2016 to June 2018 in the work were collected. We performed the first cycle of ovulation in this center, randomly divided into LE group and CC group.

Inclusion criteria: PCOS diagnostic criteria according to Rotterdam criteria (ovulation thinning or anovulation, ovarian polycystic changes and hyperandrogenic laboratory tests or clinical manifestations, ruled out other cases), age 20 - 33 years, luteinizing hormone 3 - 12 mmol/L, normal prolactin, at least one side of tubal patency (hysterosalpingography/peritoneal laparoscopy), normal uterine cavity imaging (uterine hysterosalpingography/color ultrasound/hysteroscopy), normal semen (fifth version of semen standard) And normal sexual life.

Exclusion criteria: Bilateral hydrosalpinx, tuberculosis, ovarian endometriosis, ovarian cyst, adenomyosis, endometrial polyps, intrauterine adhesions, history of uterine and ovarian surgery, and other factors that have been found to affect pregnancy.

2.2. Treatment Program

LE group menstrual 3 - 7 days to give 2.5 mg orally, began to monitor follicular growth after discontinuation, when the follicle diameter reached 14 mm plus progynova 1 mg/day until the follicle mature (18 - 20 mm), injection HCG10000u. Guide the same room and give support to the corpus luteum after ovulation. The CC group received oral administration of 100 mg on the 3rd to 7th days of menstrual period. The other processes of monitoring were the same as above.

2.3. Monitoring Indicators

Follicle monitoring was performed after discontinuation of the patient. The diameter of the dominant follicles was less than 14 mm. Monitoring was performed every 2 to 3 days. When the diameter of the dominant follicles was greater than 14 mm, daily monitoring was performed and urine LH strips were combined until the dominant follicle diameter was greater than 18 mm, injection of HCG. Monitoring of the patient's endometrium thickness, number of mature follicles, HCG injection on the day of the female two peak level, ovulation rate, clinical pregnancy rate, early pregnancy abortion rate. When more than three dominant follicles are mature, it is recommended that the patient cancel the cycle. Gonadotropin should be added when follicles grow less than 1 mm for more than 3 days. LH peaks are given to HCG injection for follicles larger than 14 mm, and LH peaks (early-onset LH peaks) are eliminated when the follicles are larger than 14 mm cycle. The above conditions are not included in the experimental statistics.

2.4. Statistical Analysis

SPSSI3.0 software was used for statistical analysis. Count data were used for paired sample t-test; measurement data were tested with X^2, $P < 0.05$ was considered statistically significant.

3. Results

3.1. The Basic Situation

PCOS is characterized by hyperandrogenism, insulin resistance, and chronic anovulation and affects 5% - 10% of women in reproductive age. It is the commonest cause of anovulatory infertility accounting for >80% of all cases [2]. There was no significant difference in age, BMI, basic follicle number, and baseline LH. There was a statistically significant difference in the length of infertility and the level of basal FSH in the LE group (Table 1).

3.2. Treatment Outcomes

There were more follicles in the CC group than in the LE group. The HCG day in the LE group had lower levels of female sensation, thicker endocardium, and higher ovulation rate, the differences were statistically significant (Table 2). The clinical pregnancy rate, single pregnancy rate, and early pregnancy were all significant (Table 3).

Table 1. Basic conditions.

	BMI (kg/m*)	Average age (years)	Infertility period (years)	Basic follicle number (one)	Basic FSH level (TU/ml)	Foundation LH level (TU/ml)
CC	24.04 ± 2.1	26.46 ± 4.6	3.7 ± 2.8	12.4 ± 3.2	6.53 ± 2.27	9.31 ± 3.76
LE	24.39 ± 2.98	27.2 ± 3.5	4.9 ± 2.6	11.9 ± 4.5	7.08 ± 1.6	8.88 ± 1.23
P value	P > 0.05	P > 0.05	P < 0.01	P > 0.05	P < 0.05	P > 0.05

Table 2. Number of ovulation monitoring.

	Cases	(Units) > 14 mm follicular	Intimal thickness	HCG daily estradiol	Ovulation rate (%)
CC	121	187	8.1 ± 1.3	326.12 ± 57.3	91 (75.2)
LH	121	161	9.5 ± 2.1	256. 63 ± 62.71	107 (88.43)
P value			P < 0.01	P < 0.01	P < 0.01

Table 3. Pregnancy outcomes.

	Total clinical pregnancy rate (%)	Ovulation cycle pregnancy rate (%)	Twin pregnancy rate (%)	Number of childbirths (one)
CC	16 (13.22)	16 (17.58)	1 (6.25)	16
LH	25 (20.66)	25 (23.36)	1 (4.00)	24
P value	P < 0.05	P < 0.05		

3.3. Pregnancy

The pregnant women of the two groups had no obvious abnormalities except prenatal examinations of 3 cases of early pregnancy, 3 cases of early pregnancy, mid-late pregnancy and late pregnancy. In the CC group, there were 16 cases of pregnancy, 1 case of miscarriage in early pregnancy, 1 case of twin preterm birth, and the remaining 14 cases were single pregnancy and full-term delivery without complications. In the LE group, there were 25 cases of pregnancy, 2 cases of miscarriage in the first trimester and 1 case of twin preterm birth. One case of neonatal hypoxic-ischemic encephalopathy complicated with neonatal hypoxic-ischemic encephalopathy improved after active treatment. The remaining 22 cases were single pregnancy and full term delivery was absent (**Table 3**). Pregnancy and Neonatal Conditions were similar as shown in **Table 4**, there was no statistical difference in abortion rate between the two groups.

4. Discussion

Miwally M.F., Casper, R.F. [3] reported that 12 cases of anovulatory infertility and 10 cases of ovary infertility in PCOS patients after induction of ovulation were very thin (≤5 mm), given to promote ovulation after LE. The inner membrane can reach an average of 8 mm. Our study also found that the endometrial thickening in the LE group may be related to factors such as LE that do not affect the endometrial estrogen receptors, the short half-life of LE, and the increase of estrogen levels at follicular maturation. Wallace K.L., *et al.* [4] reported from the molecular biology level that compared with CC ovulation-elevating LE, it can increase the endometrium LIF, DKKI. LIFR and FGF-22 gene expression and improve endometrial receptivity. Baruah J., *et al.* [5] found that LE does not affect estrogen receptors. Binding of estrogen receptors can promote the proliferation of endometrial epithelial cells and stroma, improving the blood flow of the endometrium. Although most of the literature reported thicker endometrium in

Table 4. Pregnancy and neonatal conditions.

	Birth weight (Kg)	Score (minutes)	Complication rate (%)	Premature delivery (%)	Abortion rate (%)
CC	3.17 ± 1.54	9.57 ± 0.92	0	1 (6.67)	1 (6.25)
LH	3.14 ± 1.39	9.38 ± 0.87	1 (4.17)	1 (4.35%)	2 (8.00)
P value	P > 0.05	P > 0.05			

the LE group, the pregnancy rates reported in various literatures were not consistent. Xiao Jinsong [6] analyzed the literature on the effectiveness of letrozole in ovulation in the past decades systematically. They believe that there may be differences in the ovulation therapy of letrozole and clomiphene in many ways, as endometrium thickness, number of dominant follicles, ovulation rate, pregnancy rate, but the difference was not statistically significant. Theoretically, Letrozole can make up for the shortcomings of clomiphene ovulation therapy and provide an alternative for clinicians. However, there is still a lack of large-scale, multi-center systematic research. In 2014, Richard S., Legro, M.D. [7] found that the LE group had higher cumulative ovulation rate and birth rate than the CC group. There was no significant difference between the two groups of women in ovulation-pregnant women during pregnancy and newborns. We found that the rate of ovulation in the LE group was 88.43% higher than that in the CC group, which was 75.2%. The clinical pregnancy rate in the two groups was 20.66% compared with 13.22%, and the ovulation cycle pregnancy rate was 23.36% compared to 17.58%. There were no significant differences. The current mechanism of high LE ovulation rate is not very clear, may be related to the microenvironment of the ovary, autocrine and paracrine mechanisms, and the thickness of the endometrium is not the only factor to evaluate the endometrial receptivity, not to affect the outcome of pregnancy the only factor, therefore, the endometrial thickness is not positively correlated with the clinical pregnancy rate. In this study, multiple follicular development in LE group was less than that in CC group, and the level of estrogen in HCG day was low. Similar to the results of Polyzos *et al.* [8], it is considered that LE is beneficial to the development of single follicle in ovulation induction, reducing the multiple pregnancy rate, and reducing ovarian hypertrophy. Especially for some patients with particularly sensitive ovary, LE micro-stimulation can be used to promote ovulation, to obtain a suitable pregnancy outcome and reduce the occurrence of complications.

One possible limitation of this trial may be argued to be using pregnancy rather than live birth rate as the primary outcome. However, we believe that pregnancy rate is clinically important. A further slight concern is that more than one sonologist had done the serial ultrasonography for evaluation of endometrial thickness and follicular growth. We need to pay more attention to adverse events as well.

5. Conclusion

In summary, this study suggests that LE and CC have similar ovulation-promoting

effects, and LE may be better than CC for less multifollicular development during ovulation induction. However, whether LE can replace CC for ovulation treatment of PCOS still requires a larger sample of multicenter studies. At the same time, LE still requires a large amount of clinical data for the safety of follicles, embryos and offspring.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Oncolytic Herpes Simplex Virus ICP47 Deletion Reverses Tumor Immune Evasion

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Abstract

Herpes simplex virus (HSV) is an enveloped, double-stranded DNA virus that has been used with modification as oncolytic viruses (OVs) against a number of tumor types. OVs represent a new class of therapeutic agents that promote anti-tumour responses through a dual mechanism of action that is dependent on selective tumor cell killing and the induction of systemic anti-tumour immunity. Among OVs, HSVs preferentially replicate in and lyse cancer cells, leading to *in situ* autovaccination, adaptive anti-virus and anti-tumor immunity. Suppression of antitumor immunity after OV therapy has been observed and the molecular and cellular mechanisms of action are recently reported. ICP47, a small protein produced by the herpes simplex virus, is considered as an important factor in the evasion of cellular immune responses in HSV-infected cells. Therefore, reviewing the research status of ICP47 is certainly helpful to improve the anti-tumor effect of oncolytic HSVs (oHSVs). Here, this review will focus on the following contents: 1) Anti-tumor mechanism of OVs; 2) Functions of early HSV genes; 3) The mechanism of immune escape of ICP47; 4) Recombinant HSV against cancer; 5) The functional verification of ICP47 deletion. This review highlights the current understanding of recombinant HSVs against cancers.

Keywords

Oncolytic Virus, HSV, ICP47, Anti-Tumor Immunity

1. Introduction

Tumors are originated from transformed cells in tissues or organs, which contain heterogeneous cancer cells, such as tumor stem cells etc. [1] [2] [3] [4] [5], and tumor stromal cells, for instance immune cells etc., in tumor microenvironment [6]. And cancer is still a serious danger to human health [7]. Conventional chemotherapy and radiotherapy achieve limited efficacy leading to the search for novel ways to treat cancer [8]. The concept of using viruses as a cancer treatment drug dates back to the beginning of the last century, when it was observed that patients with different kinds of malignant tumors who underwent rabies vaccination or experienced viral diseases exhibited spontaneous tumor regression [9]. However, the use of wild-type viruses for cancer therapy was associated with serious adverse events. So with genetic-engineering of virus vectors to reduce pathogenicity, oncolytic viruse (OV) therapy became a promising therapeutic strategy [10]. Replication-competent oncolytic HSV (oHSV) vectors target actively dividing neoplastic cells while sparing normal cells and can be exploited as a therapeutic strategy for the selective destruction of tumors without damaging adjacent normal tissue [11]. The infected neoplastic cells, which are killed by the replicating viruses, release progeny virions [12]. This model of viral amplification and lateral cell-to-cell transmission lead to the further destruction of surrounding cancer cells. As these viruses destroy tumor cells by oncolysis, cross-resistance with other therapy approaches, such as radiotherapy, chemotherapy and hormonal therapy, typically does not arise [13]. Thus oHSV selectively propagates in cancer cells while displaying minimal adverse effects in healthy cells, making it one of the most promising treatments [14]. Thus, the research of the ICP47 status, involving in both viral replication and immune escape, is beneficial to the function enhancement of oHSV.

2. Anti-Tumor Mechanism of Oncolytic Virus

Oncolytic viruses are therapeutically useful viruses that selectively infect and damage cancerous tissues without causing harm to normal tissues [15]. It can kill infected cancer cells in many different ways, ranging from direct virus-mediated cytotoxicity through a variety of cytotoxic immune effector mechanisms [16]. Oncolytic viruses typically takes over and controls the molecular cell death machinery of the infected cancer cells, allowing death to occur only after available cellular resources have been maximally exploited for the replication and assembly of new viruses [17]. In addition to killing infected cells, oncolytic viruses can mediate the killing of uninfected cancer cells through indirect mechanisms such as destruction of tumor blood vessels, amplification of specific anticancer immune responses, or the specific activity of proteins expressed from engineered viruses encoded by transgenes [18] (**Figure 1**).

3. Functions of Early HSV Genes

HSV transcription is complexly regulated by both viral and cellular factors [19].

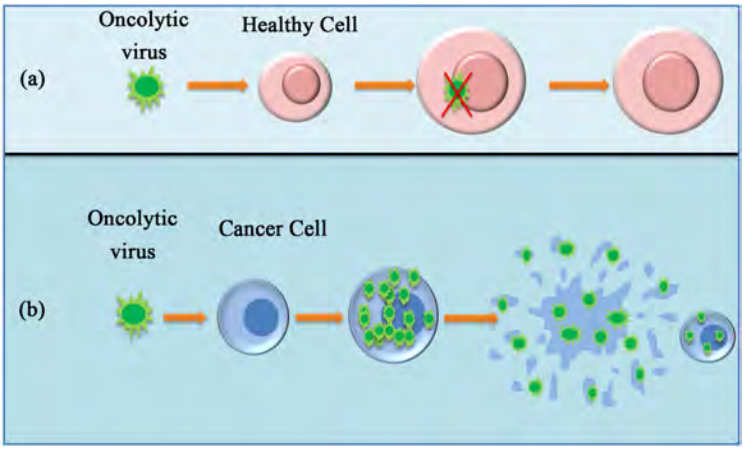

Figure 1. Infection and killing of tumor cells by an oncolytic virus. (a) An oncolytic virus cannot replicate in normal, healthy cells; (b) An oncolytic virus targets a cancer cell, multiplying within the cells before destroying them, and virus replication leads to cell lysis (direct effect) and the release of progeny virions, resulting in virus spread throughout the tumor, causing the body's immune response.

There are five immediate-early proteins in HSV: infected cell polypeptide (ICP)0, ICP4, ICP22, ICP27 and ICP47. The first four are involved in the regulation of viral transcription [19]. ICP4 is a transcription factor that recruits cellular complexes, including TFIID, to viral DNA to enhance transcription initiation and can also function to repress transcription of some viral genes [20]. ICP0 is an immediate early viral protein crucial in both lytic and latent HSV-1 infection [21]. The main function of ICP0 is to offset the cellular frontline antiviral defenses and consequently to enhance downstream viral gene expression. The most important functional domain of ICP0 is a RING-type E3 ubiquitin ligase located in its second exon, which targets several host factors for proteasome-dependent degradation. Some of the ICP0 E3 substrates are part of the host intrinsic defenses. Their degradation contributes to the augmentation of viral DNA expression and DNA replication [22]. ICP27 is involved in the nuclear export of viral mRNAs and has a role in recruiting RNA Pol II to viral genes. ICP27 is the first viral protein shown to activate cryptic polyadenylation signals (PASs) in introns [23]. ICP22 plays a role in recruiting elongation factors like the complex to the HSV-1 genome to allow for efficient viral transcription elongation late in viral infection and ultimately infectious virion production. In the absence of ICP22, viral production is reduced globally in the late stages [24]. ICP47 effectively blocks the major histocompatibility complex class I (MHC I) antigen presentation pathway. ICP47 binds with high affinity to the human transporter associated with antigen presentation (TAP) and blocks its binding of antigenic peptides [25]. Association of ICP47 precludes substrate binding and prevents nucleotide-binding domain closure necessary for ATP hydrolysis. By blocking viral antigens from entering the endoplasmic reticulum, HSV is hidden from cytotoxic T lymphocytes (CTL), which may contribute to establishing a lifelong infection in the host [26].

4. The Mechanism of Immune Escape of ICP47

Cellular immunity against viral infection and tumour cells depends on antigen presentation by MHC I molecules [27]. Intracellular antigenic peptides are transported into the endoplasmic reticulum by the TAP and then loaded onto the nascent MHC I molecules, which are exported to the cell surface and presented to the immune system. CTL recognize non-self peptides and kill the infected or malignant cells. Defects in TAP account for immunodeficiency and tumor development. To escape immune surveillance, some viruses have evolved strategies either to down regulate TAP expression or directly inhibit TAP activity [28]. HSV evades $CD8^+$ T-cells by producing ICP47, which limits immune recognition of infected cells by inhibiting TAP [29]. ICP47 is targeted at TAP, one of the important proteins that determine the efficiency of antigen presentation by MHC-I. ICP47 through competition with immunological peptides in combination with the TAP peptide binding sites, reduces the peptide transport function of the TAP, resulting in instability of no-load MHC I molecules. The expression of MHC I on tumor cell surface was significantly decreased, which directly interferes with the MHCI mediated CTL activation. Then HSV avoid the host immune clearance, even become a virus of reserve in the body [30] (**Figure 2**).

5. Recombinant HSVs against Cancers

HSV offers particular advantages for use as an oncolytic virus. The engineered

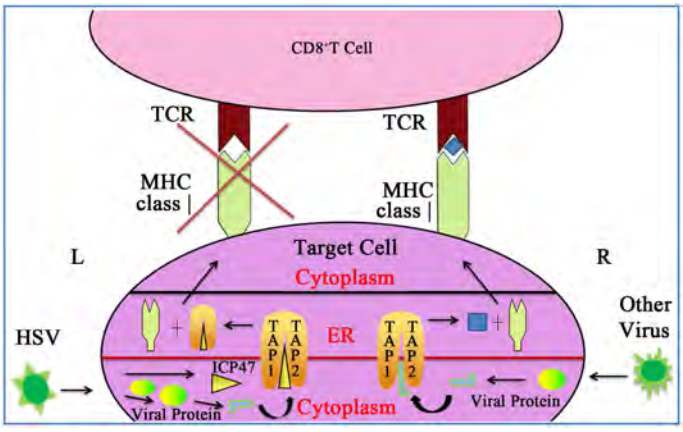

Figure 2. Mechanism of immune escape of HSV via ICP47. R—Under normal circumstances, after invading target cells, viruses transfer their own genetic material into the host cell, and use the transcription and translation elements of the host to synthesize foreign antigen, namely exogenous antigen. Exogenous antigens were degraded into antigenic peptides, which are transferred to the endoplasmic reticulum through TAP, then bind to MHC I molecules after modification. The MHC-peptide complexes are transported to the cell surface for $CD8^+$ T cell recognition. Recognition can lead to an immune response to the virus. L—Due to the strong affinity between HSV ICP47 and TAP, TAP will be preferentially bound by ICP47, resulting in the emergence of empty carrier MHC I molecules. Therefore, $CD8^+$ T cells could not recognize them, and HSVs could avoid the immune responses.

oHSVs have demonstrated remarkable safety in clinical trials, with some evidence of efficacy [31]. The first recombinant HSV strain directed against cancer, dlsptk, was generated through the deletion of the UL23 gene encoding thymidine kinase (TK) [32]. TK processes nucleotides to facilitate replication of viral DNA. In the absence of HSV TK, HSV-1 infecting normal cells would fail to replicate at a rate sufficient to sustain infection. While the efficacy and selectivity of dlsptk established a proof of principle for the use of HSV-1 genome deletions to achieve tumor selectivity through selective attenuation, the TK deletion was ultimately problematic from the standpoint of clinical application, as it rendered the strain impervious to first-line anti-herpes medications. This resistance represented to many the loss of a crucial safety control for clinical experimentation with viral therapies [33]. Thus, dlsptk and its TK deletion were abandoned. HSV-G207 features a single deletion of UL39 (ICP6) and double deletion of RL-1 (ICP34.5). ICP34.5 is the major gene determinant of HSV neurovirulence [34]. ICP34.5 precludes the shut-off of host protein synthesis in infected cells [35]. ICP6 is the large subunit of viral ribonucleotide reductase, a key viral enzyme for DNA synthesis that is necessary for virus replication in normal non-dividing cells. These two generations of oHSVs are designed to reduce viral replication in non-cancer cells. A third-generation oHSVs vector, G47Δ, was created by adding to the UL39 and RL1 deletions of G207 a deletion of the HSV-1 gene *α*47 (ICP47) (**Figure 3**).

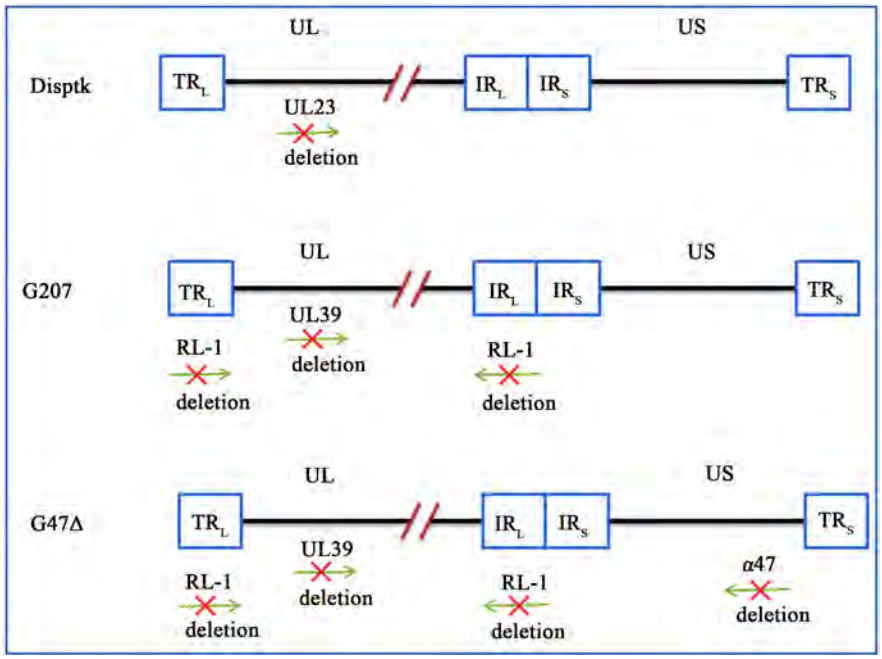

Figure 3. Recombinant HSVs against cancers. The first recombinant HSV strain directed against cancer, dlsptk, was generated through the deletion of the UL23 gene encoding thymidine kinase (TK). HSV-G207 features a single deletion of UL39 (ICP6) and double deletion of RL-1 (ICP34.5). A third-generation oHSV vector, G47Δ, was created by adding to the UL39 and RL1 deletions of G207 a deletion of the HSV-1 gene *α*47 (ICP47).

6. The Functional Verification of ICP47 Deletion

Because the ICP47 inhibits TAP, which translocates peptides across the endoplasmic reticulum, the down-regulation of MHC class I that normally occurs in human cells after infection with HSV-1 does not occur when the *α*47 gene is deleted. G47Δ-infected human cells in fact presented higher levels of MHC class I expression than cells infected with other HSV-1 vectors [36]. Further, human melanoma cells infected with G47Δ were better at stimulating their matched tumor-infiltrating lymphocytes *in vitro* than those infected with G207. The deletion also places the late US11 gene under control of the immediate-early *α*47 promoter, which results in suppression of the reduced growth phenotype of γ34.5-deficient HSV-1 mutants including G207. In the majority of cell lines tested, G47Δ replicated better than G207, resulting in the generation of higher virus titers, and exhibiting greater cytopathic effect [37]. Therefore, the verification of ICP47 deletion is particularly complex and important. There are two main methods: start with the HSV DNA/RNA sequence to verify the integrity of HSV and verify the functional changes of oHSV to indirectly prove whether ICP47 is deleted [36] (**Figure 4**).

Southern Blot: southern blot is a method used in molecular biology for detection of a specific DNA sequence in DNA samples. Southern blotting combines transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization. Thus, it validates the presence of the ICP47deletion in HSV.

Virus Yields of Replication: because of the overlapping transcripts encoding ICP47 and US11, the deletion in *α*47 also places the late US11 gene under control of the immediate-early *α*47 promoter. This alteration of US11 expression enhances the growth of g34.5 mutants by precluding the shutoff of protein synthesis. Therefore, deletion of ICP47 facilitates virus replication.

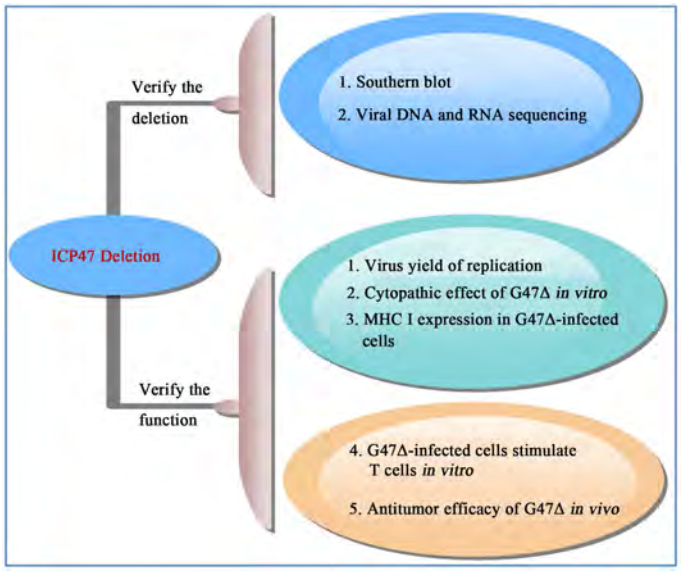

Figure 4. The functional verification of ICP47 deletion.

Cytopathic Effect of G47Δ *in Vitro*: G47Δ, a third-generation oHSV vector, was created by adding to the UL39 and RL1 deletions of G207 a deletion of the HSV-1 gene *α*47. Therefore, G47Δ should be significantly more efficient at destroying tumor cells than G207.

MHC I Expression in G47Δ-Infected Cells: ICP47 inhibits the function of TAP in translocating peptides across the endoplasmic reticulum in human cells. Thus, G47Δ-infected cells should have no down-regulation of MHC I expression.

G47Δ-Infected Cells Stimulate T Cells *in Vitro*: due to the strong affinity between ICP47 and TAP, and will be preferentially bound, resulting in the emergence of empty carrier MHC I. Therefore, $CD8^+$ T cells could not recognize infected cells, and HSV could avoid the immune killing effect [31]. However, G47Δ, deletion of ICP47, infected tumor cells would stimulate T cells to a greater extent than G207-infected tumor cells.

Antitumor Efficacy of G47Δ *in Vivo*: ICP47 deletion restores MHC I and allows tumor cells to present antigens to T cells in response to infection. Therefore, the antitumor effect of G47Δ would be enhanced.

G47Δ has been shown efficacious in animal tumor models of a variety of cancers including brain tumors, prostate cancer, breast cancer and schwannoma [36] [38] [39] [40]. Moreover, the above seven methods have been used in detail by researchers, and compared with G207, G47Δ have enhanced the anti-tumor effect [36].

7. Conclusion

To enhance oncolytic efficacy, yet maintain safety, a third-generation vector, G47Δ, was constructed from G207 by a deletion within the nonessential ICP47 gene. Normally, HSV-1 infection causes down regulation of MHC I expression on the surface of infected cells, with the binding of ICP47 to TAP blocking antigenic peptide transport in the endoplasmic reticulum and loading of MHC I molecules [41]. ICP47 binds to TAP in a species-specific manner, with the affinity for murine TAP being about 100-fold less than for human TAP. Disruption of ICP47 results in increased MHC I expression in G47Δ-infected cells compared with G207-infected cells, with enhanced stimulation of antiviral and antitumor T cell activity; and enhanced virus growth and cytotoxicity in a variety of tumor cell lines in culture and in glioma xenograft models *in vivo* because of deletion-associated immediate-early expression of the late US11 gene [42]. Currently, accumulating evidence indicated that ICP47 was associated with viral replication and immune escape. Therefore, deleting ICP47 of oncolytic virus will exert promising anti-tumor effects in cancer therapy. Indeed, an ICP47 deletion mutant has been approved for clinical usage [43] [44].

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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The Beneficial Effect of Enriched Environment on Pathogenesis of Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a common neurodegenerative disease, its main clinical symptoms are the progressive decline of cognitive and memory functions. Enriched Environment (EE) achieves the goal of improving brain cognitive reserve by enhancing the multi-directional stimulation on movement, sensory and cognitive systems of animals. And EE can regulate the levels of various trophic factors in the brain, promote synaptic regeneration and enhance neural plasticity to reduce the loss of neurons induced by inflammation. At present, there is still no effective treatment for AD and the clinical intervention drug is expensive. So it is essential to actively explore non-drug treatment. This review will explain the effects of EE on learning ability, memory ability and mental behavior in AD, and provide a new direction for the treatment and rehabilitation of AD.

Keywords

Alzheimer's Disease, Enriched Environment, Microtubule Associated Protein Tau, β-Amyloid Precursor Protein, Neurogenesis

1. Introduction

1.1. Enriched Environment (EE)

1.1.1. The Origin and Concept of EE

Enriched environment (EE) was first studied by Donald Hebb, who raised rats in his home and later showed that their performance of Morris water maze was superior compared with animals raised in laboratory [1]. Later, Rosenzweig *et al.* confirmed that EE can promote adult hippocampus neurogenesis and cognitive ability in rats [2] [3] [4]. EE was originally defined in 1978 as a complex of in-

*These authors have same contribution.

animate objects and gregarious stimuli. Compared with Standard environment (SE), EE involves voluntary exercise, environmental enrichment, social interaction and spatial complexity, which are beneficial for improving cognitive reserve. The construction of EE is abundant and novel, which triggers the changes in growth factor synthesis, dendritic modifications, synaptic plasticity and neurogenesis [5] [6]. Horticultural therapy is the exercise of encouraging people to participate in planting or gardening activities to improve their bodies, minds, and spirits [7] [8]. In some ways, horticultural therapy is similar to EE, both of which provide patients with a more complex living environment, create a comfortable life to relieve the patient's mental stress and encourage patients to exercise more to enhance the various body systems.

1.1.2. The Construction and Effect of EE

Various forms of stimulation are the basic characteristics of EE, whose constructive principle is providing more chances for experiment animals to exercise and communicate. And EE is not only the copy of natural environment, but needs to be based on the living habits of the experiment animals, such as sleep habits and dietary habits. To promote the development of the behavior and the nervous system, it is important to provide experiment animals with a comfortable and relaxing living environment [9] [10] [11].

The construction of EE varies according to the aims of researchers and experimental animals. In general, EE cages are larger than that of SE. There is nothing except food and water in cages of SE. But there is a variety of sports equipment in EE cages, such as ladders, running wheels, platforms, tunnels, boxes, balls, blocks, swings, etc. (**Figure 1**). All of these are to ensure appropriate physical stimulation and spatial complexity, and encourage animals exercise voluntarily.

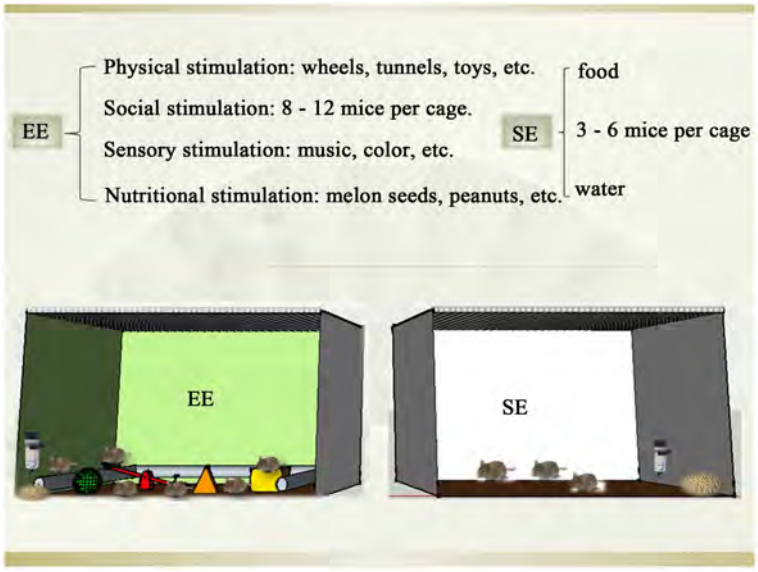

Figure 1. The construction of EE and SE. Compared with SE, EE is more complex, which contains running wheels, colorful tunnels, different toys and more animals except food and water. Abbreviations: EE, enriched environment; SE, standard environment.

In order to maintain environmental novel, the layouts should be replaced 2 - 3 times a week. Some other forms of stimulations are added into experiment according to the research's needs, such as playing different types of music to promote animals' sensory perception and feeding more nutritive foods to promote animals' growth [12] [13] [14] [15] [16].

1.1.3. Effects of EE on Neurogenesis and Cognitive Function in Normal Mice

The EE has been widely used to explore the connection between cognition and neurogenesis since the concept of EE was proposed. Many studies have revealed EE can alter the morphology and molecular biology of animal brains [17] [18] [19] [20]. And EE can increase the number and volume of neuron in the visual cortex and hippocampus. Dendritic branches and density of dendritic spines are both increased to enhance neuronal plasticity and delay the decline of memory in aged rats [21] [22] [23].

As early as 1964, Altman *et al.* demonstrated that the glial cells in cortex of enriched rats increasing of 75 percent, which mainly differentiate into a large number of oligodendrocytes and a small amount of astrocytes [24]. In 1997, Epp *et al.* proposed that EE can increase the number of hippocampal neurons in adult mice and promote the survival of neuronal precursor cells in the hippocampal dentate gyrus (DG) [25] [26]. Later, Kempermann *et al.* confirmed that growth of old rats' hippocampal neurons was increased by 5 times and most of the new cells differentiated into astrocytes, when rats raised 10 to 20 months old under EE. That's to say, EE can induce neurogenesis, enhance the neural plasticity and decrease the aging rate of the nervous system even in middle-aged and old rats [27] [28] [29].

Emerging evidence have indicated that the expression of a variety of cellular growth factors are significantly increased in brain, which promote adult neurogenesis, such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), glial cell source, glial cell derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF) and neurotrophin-3 (NT-3), etc. [30] [31] [32] [33]. And the EE animals could be less anxious in the presence of complex environment, because EE can lead neuronal endocrine changes and reduce the reactivity of the animal's hypothalamic-pituitary-adrenal axis (HPA axis) through regulating the neurotransmitter transmission of dopaminergic and glutamatergic neurons [34] [35]. Williams *et al.* demonstrated EE can promote the expression of calmodulin in hippocampus, which leads to increased phosphorylation of endogenous cAMP response element binding protein (CREB). And CREB is the key protein mediated spatial learning ability of memory and conducive to the consolidation of new memories [36] [37] [38]. Sharafi Z. *et al.* confirmed that noradrenergic neurons (NA) and serotonin (5-HT) neurons can be activated in the EE, which promote the release of monoamine neurotransmitters to enhance learning and synaptic plasticity in adult brains [39]. All of above factors are beneficial for brain's neurogenesis and improvement of neural plasticity. It also

enhances the animal's learning and spatial memory ability to delay the progress of AD.

2. Alzheimer's Disease (AD)

2.1. The Concept and Clinical Symptoms of AD

AD was discovered by German doctor Alois Alzheimer in 1906, which is one of the most common chronic degenerative diseases of the central nervous system. The main clinical symptoms are the progressive decline in cognitive and memory functions of patients, including various psychiatric symptoms and behavioral disorders [40]. With the aging of the global population, the incidence of AD in the world is increasing year by year. According to the research of World Alzheimer Reports 2018, there are 50 million people worldwide living with dementia and the number will increase to 152 million by 2050. In 2018, the cost of treating AD is estimated to exceed $1trillionand may reach $2 trillion by 2030 [41]. AD seriously endangers the physical and mental health of the elderly and imposes a heavy burden on the society and the patients' family. It has become a serious social and medical problem.

2.2. The Pathogenesis of AD

The abnormal production and deposition of β-amyloid (Aβ) in senile plaques and hyper-phosphorylated Tau protein (p-Tau) in neurofibrillary tangles (NFTs) are hallmark features in the brains of AD patients. But pathological changes of AD are complex and diverse. There are also some other hypotheses about AD, such as inflammatory, autophagy, oxidative stress, insulin signaling pathway dysfunction and mitochondrial dysfunction [42] [43] [44] (**Figure 2**).

2.2.1. β-Amyloid

Aβ is produced by the sequential cleavage of the amyloid precursor protein (APP) via a series of APP cleaving enzyme, such as α-secretase, β-secretase and γ secretase (**Figure 2**). The pathways of degradation of APP have both non-amyloidogic pathway (NAP) and amyloidogic pathway (AP) [45]. In NAP, APP is cleaved by α-secretase to form soluble APP (α-sAPP), which is nutritive and protective for neurons. But in AP, APP is cleaved by β and γ secretase to form insoluble Aβ peptides, which are neurotoxic for neurons. Furthermore, α-secretase appears to compete with β-secretase for the initial disintegration of APP, thus the expression and activity of both enzymes may affect the Aβ level. In general, most APP is cleaved by α-secretase, but the activity of β and γ secretase in AD patients is much higher than α-secretase, leading to excessive insoluble Aβ deposition. And the common types of Aβ are Aβ40 and Aβ42. In normal conditions, APP is cleaved to produce a large amount Aβ40 and a small amount Aβ42. Although there is a little Aβ42, it's likely to deposit easily, because Aβ42 is more insoluble than Aβ40. Once metabolism of Aβ42 is abnormal, it is easy to deposit in the patients' brain to form neurotoxic SPs, induce dendrite and axon retraction and neuronal apoptosis [46] [47]. On the other hand, Aβ can activate

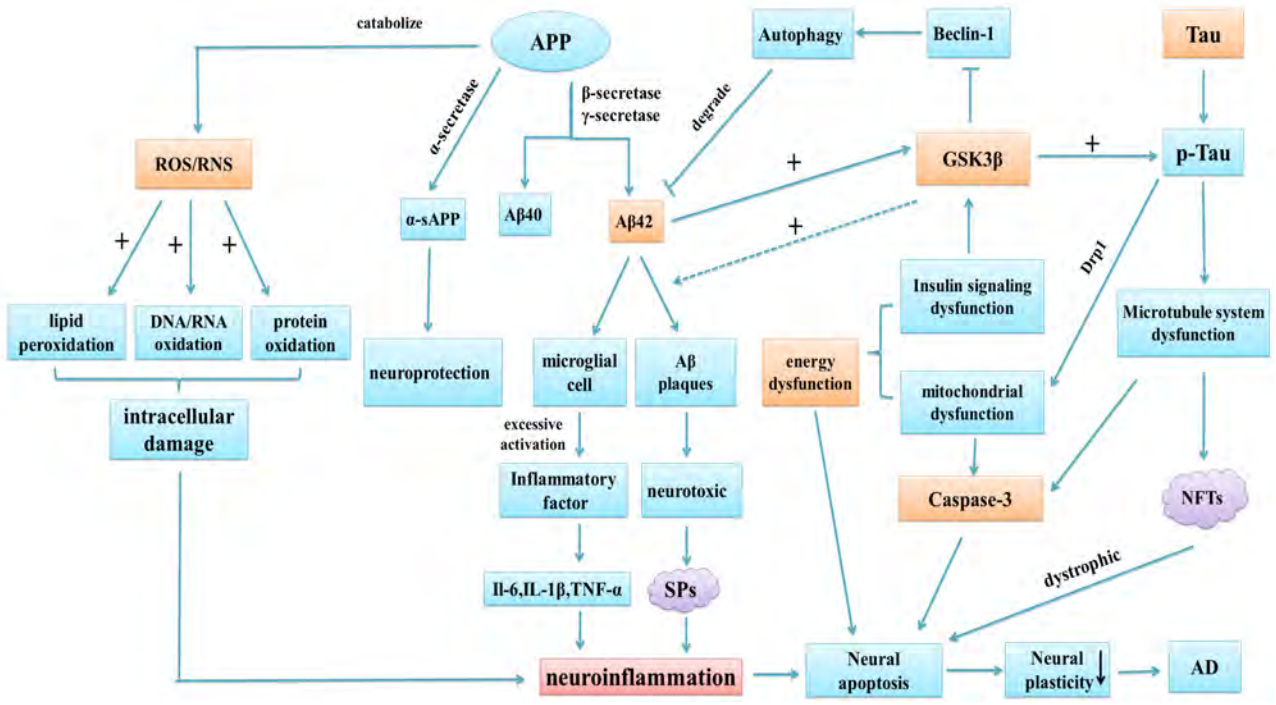

Figure 2. The major pathological mechanisms of AD. 1) SPs formed of insoluble Aβ42 and NFTs formed of hyper-phosphorylated Tau can lead a large number of neuronal apoptosis induced by inflammation, which reduces the synaptic plasticity of neurons and exacerbates the occurrence of AD. 2) GSK-3β is the key enzyme that mediates Aβ42 and p-Tau proteins, which regulates cell proliferation, survival and apoptosis by regulating multiple signaling pathways. 3) ROS/RNS can lead to the neuroinflammation through leading to the lipid peroxidation damage of cell membrane, destroying the oxidative modification of intracellular proteins, nuclear and mitochondrial DNA/RNA. In summary, multiple inflammatory pathways are involved in the development and progression of cognitive deficits in AD. Abbreviations: Drp1, dynamin related protein 1; Caspases, cysteine proteolytic enzymes; APP, amyloid precursor protein; p-Tau, hyper-phosphorylated Tau protein; SPs, senile plaques; NFTs, neurofibrillary tangles; α-sAPP, soluble APP; ROS/RNS, reactive nitrogen species/reactive oxygen species.

monocytes and peripheral microglia via tyrosine kinase pathway to produce IL-1β, IL-6, IL-8, TNF-α and other pro-inflammatory factors to trigger nervous system inflammatory immune response and accelerate neuronal apoptosis which directly lead to impairment of memory and cognitive [48] [49] [50] [51] [52].

2.2.2. Tau Protein

Tau protein is an important microtubule-associated protein expressed in neuron axons of the central nerve system, regulating the balance of phosphorylation and non-phosphorylation to keep the stability of the neuronal cytoskeleton. Researches have shown that main component of NFTs is abnormal p-Tau proteins. The clinical data also show that p-Tau proteins in cerebrospinal fluid (CSF) are significantly elevated in AD patients [53] [54]. Thus, tangle formation and Tau release might contribute to the increased levels of Tau in CSF, suggesting that Tau level in CSF could be a good predictor of the early neurofibrillary changes occurring in brain of AD patients. And NFTs can reduce the affinity of microtubules, block the nutrient transport pathway of the microtubules and lead to atrophic apoptosis of neuronal dendrites and axons [55]. Moreover, Aβ can improve contents of p-Tau proteins and lead to an increase of NFTs, while Tau

protein can also mediate neurotoxic lesions of Aβ. Both of them accelerate the development of AD [56] [57] [58].

2.2.3. Oxidative Stress

The dynamic balance between production and elimination of reactive nitrogen species (RNS) and reactive oxygen species (ROS) is broken in body, leading to excessive accumulation of ROS. And ROS can not only directly lead to lipid peroxidation damage of cell membrane, but destroy the oxidative modification of intracellular proteins, nuclear and mitochondrial DNA/RNA (**Figure 2**). In the early stage, ROS can induce hyper-phosphorylation of Tau protein and production of Aβ, indicating that ROS occurs earlier than the mild cognitive impairment, SPs and NFTs, thus to lead to cell and tissue damages and promote the disease progression. In addition, inflammatory responses induced by ROS also directly lead to neuronal necrosis or apoptosis [59] [60] [61].

2.2.4. Autophagy

Autophagy is a highly primitive protective mechanism mediated by autophagy associated genes (ATG) to maintain intracellular homeostasis [62] [63]. Varo *et al.* have shown that many autophagic vesicles are accumulated in the Aβ plaques, while few autophagic vesicles exist in normal brain tissue, indicating that the clearance of Aβ is associated with the level of autophagy [64]. Beclin-1 is an important regulator in the formation of autophagic vesicles, decreased expression of which not only affects the process of phagocytosis, but also reduces the cleared ability of microglia (**Figure 2**). In addition, the stable microtubule system is essential to transport autophagic vacuoles to lysosomes efficiently, which ensures the normal function of autophagy. However, hyper-phosphorylated Tau protein loses the ability to correctly bind to microtubules of neurons, making autophagosomes unable to bind to tubulin and transport to lysosome, thus, leading to autophagy dysfunction [65] [66] [67]. This may indicate that autophagy can protect the brain's neurogenesis and cognitive function by removing abnormally accumulated proteins in the cell.

2.3. Current Treatments of AD

At present, the common drugs of treatment for AD are cholinergic drugs, neuroprotective drugs, antioxidants, non-steroidal anti-inflammatory drugs (NSAIDs) and drugs for improving cerebral circulation or brain metabolism [68] [69] [70] [71]. Up to now, acetylcholinesterase inhibitors are the most effective drugs, which can inhibit the activity of acetylcholinesterase to reduce the breakdown of acetylcholine in the brain [72]. Monoclonal antibody vaccines against Aβ and Tau proteins have been studied popularly in the past decades, and have achieved remarkable results in animal experiments. Unfortunately, major pharmaceutical companies have failed in phase III clinical trials, despite the inoculation of Aβ antibodies can reduce the content of Aβ in the brain, but it does not improve patients' cognitive impairment, and even bring huge side effects to patients [73]

[74] [75] [76] [77]. Therefore, the vaccine research on Aβ and Tau protein has fallen into a bottleneck. How to improve the specificity of Aβ antibody and evade the adverse effects of vaccine on the autoimmune system will become the future research direction. Another popular research topic is autophagy, as a widely existed and conserved protective mechanism in cells, playing an important role in regulating clearance of Aβ plaques and p-Tau protein [78] [79]. Therefore, future's research on autophagy regulators has provided a new direction for the treatment of AD [80]. Furthermore, the research of earlier non-drug treatment methods (rehabilitative garden therapy and horticultural therapy) has become a hot topic in current research because of the clinical drugs are expensive and the effect is not good. The research of EE on AD has also been developed gradually in China in recent decades [81].

3. The Applications of EE

3.1. The Applications of EE in AD Mice

3.1.1. The Effect of EE on Aβ

The SPs formed of excessive Aβ plaques is the typical pathological feature of AD. Therefore, how to efficiently degrade and alleviate the accumulation of Aβ becomes an important method of treating AD. The NAP is the main pathway for the normal hydrolysis of APP in the brain, while α-secretase is an important hydrolase which mediated the pathway of NAP hydrolysis. Hence, the APP in the brain will not hydrolyze normally and abnormal hydrolysis pathway will be activated by α-secretase abnormal. Furthermore, ADAM9, ADAM10 and ADAM17 are three key genes to regulate α-secretase, which play important roles in the normal hydrolysis of APP, and ADAM10 directly exerts the α-secretase site degradation of APP. The expression of ADAM10 was decreased in hippocampus of APP transgenic mice, suggesting that it may be one of the important reasons for the excessive accumulation of APP in the brain [82] [83]. And researches have indicated that expression of ADAM10 increased significantly in the hippocampal of APP transgenic mice via chronic running exercise in EE for five months ($P < 0.05$). This indicate that ADAM10 may promotes the normal hydrolysis of APP and significantly reduces the content of insoluble Aβ42, thus to reduce its excessive deposition [84]-[90] (**Figure 3**). Mainardi *et al.* [91] and Maesako *et al.* [92] demonstrated that EE can reduce the Aβ plaques in the brain of aging rats by increasing the expression of Aβ-degrading enzymes. Li and his college also confirmed that EE can significantly reduce the contents of Aβ in hippocampal CA1 region of SAMP8 mice and effectively reduce neuronal apoptosis [93].

3.1.2. The Effect of EE on Protein Tau

Tau protein plays an important role in the stabilization of the microtubule system of neurons. The NFTs which formed by hyper-phosphorylated Tau protein accumulated in cerebral cells is the significant pathological feature of AD. Lazarov *et al.* [94] demonstrated that EE can reduce Aβ levels and amyloid deposition

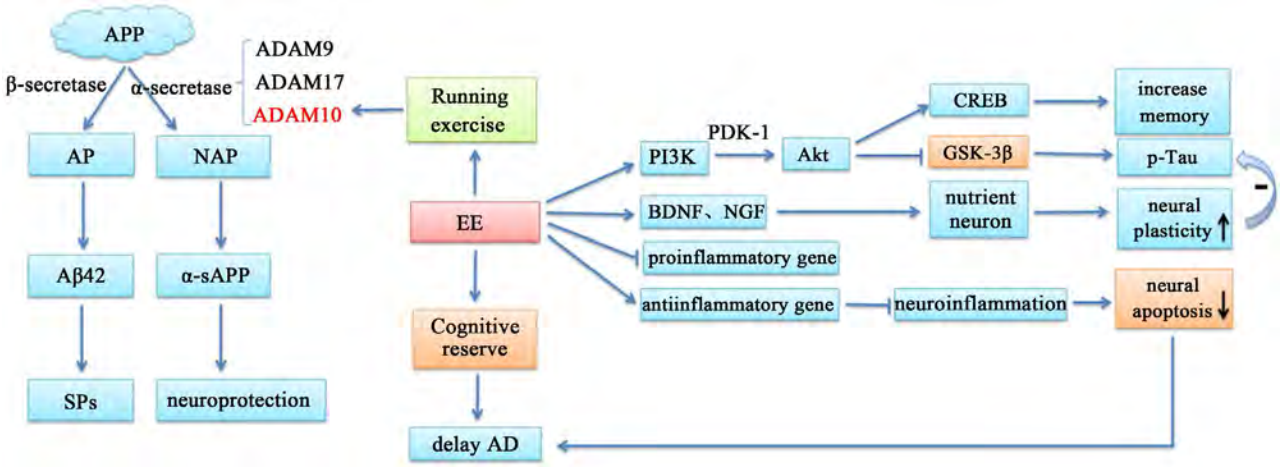

Figure 3. The mechanisms of EE on AD mice. 1) EE is beneficial for improving animals' cognition reserve to attenuate pathologic symptoms in Alzheimer's disease. 2) Long-term exposure in EE, proliferation of hippocampal cells is enhanced significantly. 3) Voluntary chronic exercise in EE activates the genes that mediated the degradation of APP. 4) EE rescues impaired neurogenesis and enhances synaptic plasticity by promoting the expression of various neuronal nutrient factors and regulating PI3K signal pathway. Abbreviations: CREB: cAMP response element binding protein; Akt/PKB: protein kinase B; PI3K: phosphatidylinositol kinase-3; NAP: non-amyloidogic pathway; AP: amyloidogic pathway; SPs: senile plaques.

in Transgenic Mice. Hu *et al.* [95] have shown that Tau transgenic mice have lower phosphorylated Tau protein in the EE compared with SE. In addition, the expression of BDNF in enriched group is significantly higher than that in standard group, which means that BDNF is likely to participate in decreased expression of Tau protein [96]. And chronic endurance exercise can enhance the antioxidant enzyme system of Tau transgenic mice, increase the expression of a series of antioxidant enzymes such as catalase, and activate phosphatidylinositol kinase-3 (PI3K). At the same time, PI3K can activate PDK-1, an activator of the upstream pathway of serine/threonine-protein kinase (Akt/PKB), to activate the enzymatic activity of Akt, while Akt can inhibit the hyper-phosphorylation of Tau via inhibiting the activity of GSK-3β in the downstream pathway [97] [98] (**Figure 3**).

3.1.3. The Effect of EE on Neural Plasticity

Complex environment is beneficial for activating voluntary exercise, increasing cognitive reserve and promoting proliferation and differentiation of hippocampal neonatal neural precursor cells in AD mice (**Figure 3**). Many researchers have indicated that APP transgenic mice in EE have lower levels of phosphorylated Tau protein in enriched group than standard group, which is associated with increased expression of a series of trophic factors such as BDNF, VEGF and NGF in APP transgenic mice. And the trophic factors can enhance protection and regeneration of neurons, promote the proliferation and phagocytic level of glial cells to improve the axonal transport, which is useful for degradation of Aβ plaques and p-Tau [99] [100]. Furthermore, EE reduces the expression of pro-inflammatory genes while increasing the expression of anti-inflammatory genes, greatly reducing the level of neuronal apoptosis induced by inflammation

[101] [102]. In short, long-term exposure in EE, proliferation of hippocampal cells is enhanced significantly and matured to become new neurons and glia, while enhanced neurogenesis was accompanied by a significant reduction in levels of hyper-phosphorylated Tau and Aβ plaques.

3.2. The Application of EE in AD Patients

In recent decades, a large number of animal experiments are shown that EE has a good effect on the emotional, mental behavior and cognitive memory in AD animals. Therefore, some hospitals began to combine with the constructive principles of EE to establish a comprehensive rehabilitation garden, which provide AD patients with a variety of simple games and exercise methods to improve patients' living ability, such as Chongqing Ge-Le-Shan Geriatric Rehabilitation Hospital. Music therapy, exercise therapy, planting flowers, jigsaw puzzles and other simple rehabilitation methods are used to encourage patients to do exercises. It is found that the cognitive function, mood and living ability of patients can be improved significantly, via various comprehensive exercises in the EE. In short, the main role of EE is to increase the brain's cognitive reserve by constantly trying new things, to promote brain's neurogenesis and enhance neuronal plasticity, thus to delay the decline of cognitive function [103] [104] [105].

3.3. The Application of EE in Other Neurodegenerative Diseases

The improvement of EE on neurodegenerative diseases was first discovered in Huntington's disease (HD) [106]. Researches indicated that EE can delay the atrophy of the brain and the onset of HD symptoms, and restore the neuronal regeneration function of SVZ region [107]. The study of EE on transient whole-cerebral ischemia rats and AD mouse model also found that the sensory movement, learning ability and memory function were enhanced after enriched stimulations. And the secretion of NGF, BDNF, GDNF and other nutrient factors increased significantly than the standard group, which means that neuronal regeneration may be associated with neurotrophic factors [108] [109]. Furthermore, compared with mice grown in the SE, the visual acuity of mice which long-term lived in EE increased by 18%, suggesting that EE is conducive to the development of the visual system [110]. EE can also improve non-spatial memory defects in N-methyl-D-aspartate receptor knockout mice and promote cognitive and spatial memory in aged rats via enhancing glutamate decarboxylase activity and hippocampal synaptic density [111] [112] [113]. In addition, EE has a positive effect on the recovery of depression, Parkinson's disease (PD) and other neurological diseases [114] [115] [116] [117].

4. Conclusions

Although morbidity of AD is very high in the old people, it may have produced unknown pathological changes at the early stage, which means that AD may be slowly formed over a long period of time. When obvious AD symptoms are

found, it is generally beyond the scope of drug intervention to reach an irreversible level. Due to the large individual differences and the difference between the exogenous input of Aβ and the Aβ formed pathologically, it has brought great difficulties to the modeling of AD, which directly leads to the difference between actual pathological situation and the model. Hence, the results of the study are not applicable to clinical research, which may be an important reason why the research on AD has not made substantial progress over the years. As one of the emerging rehabilitation methods, EE has been proven to promote the secretion of various neurotrophic factors and improve neurogenesis in the study of neurological diseases, such as cerebral ischemia and hypoxic brain damage, craniocerebral trauma, PD and AD. Although some researchers' experimental results did not achieve the expected results, this may be related to the differences in established models and environmental settings. There are still many needs to be improved based on the current research on EE. For example, environmental setting, interventional optimal time, intensity, age range and duration for different groups of people need to be further explored.

In addition, SPs formed of Aβ deposition and NFTs formed of p-Tau in brain tissue induce different degrees of neuronal inflammation in AD patients. The immune response induced by inflammation accelerates aging and apoptosis of neurons, but long-term living in EE can increase cognitive reserve, promote the secretion of a variety of neurotrophic factors and enhance synaptic reconstruction after enriched stimulations. Therefore, EE can rescue impaired neurogenesis and enhance synaptic plasticity efficiently to delay the rate of degeneration of the nervous system and improve cognitive impairment. In future studies, we should explore the inflammatory mechanism and neuro-immune response mechanism of EE on AD, and EE will play a major role in the treatment and recovery of AD.

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

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Abbreviations

Aβ: Amyloid-β
AD: Alzheimer's Disease
APP: Amyloid Precursor Protein
IL-6: Interleukin-6
IL-1β: Interleukin-1β
TNF-α: Tumor Necrosis Factor-α
NSAIDs: Non-Steroidal Anti-Inflammatory Drugs
CREBcAMP: Response Element Binding Protein
Akt/PKB: Protein Kinase B
PI3: Kphosphatidylinositol kinase-3
NAP: Non-Amyloidogic Pathway
AP: Amyloidogic Pathway
SPs: Senile Plaques
EE: Enriched Environment
SE: Standard Environment
Drp1: Dynamin related protein 1
Caspases: cysteine proteolytic enzymes
p-Tauhyper: phosphorylated Tau protein
NFTs: Neurofibrillary Tangles
α-sAPP: soluble APP
RNS: Reactive Nitrogen Species
ROS: Reactive Oxygen Species

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Carbonic Anhydrase III S-Glutathionylation Is Necessary for Anti-Oxidant Activity

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Abstract

Carbonic anhydrase isozyme CA3 protects cells against oxidative stress. Ectopic expression of murine Ca3, but not Ca2, protects proto-oncogene Evi1 expressing Rat1 fibroblast cells (ca3low) against hydrogen peroxide (H_2O_2) induced stress. Ca3 is S-glutathionylated via glutathione adducts with cysteines 181 and 186. Substitution of both Ca3 cysteines with serine fails to protect cells from oxidative stress. Insertion of cysteine at 181 and 186 in Ca2 is insufficient for conferring efficient anti-oxidant activity. This shows for the first time that S-glutathionylation of cys181 and cys186 residues is required for Ca3 anti-oxidant activity but that additional factors are also required.

Keywords

CA3, CAIII, Carbonic Anhydrase III, S-Glutathionylation, Apoptosis, Anti-Oxidant

1. Introduction

Intracellular pH is partly regulated by a family of carbonic anhydrase (CA, EC4.2.1.1) enzymes that reversibly catalyse the hydration of carbon dioxide to bicarbonate and hydrogen ions [1]. The *α*-CAs comprise 15 distinct zinc metalloenzymes that can be sub-divided into cytoplasmic (CAI, CAII, CAIII, CAVII, CAXIII), membrane bound (CAIV, CAIX, CAXII, CAXIV, CAXV), mitochondrial (CAV), non-catalytic (CAVIII, CAX, CAXI) and secreted (CAVI) [2]. These ubiquitous enzymes are of significant importance for many physiological processes and are implicated in various pathological conditions including atherosclerosis [3], retinitis pigmentosa [4], myasthenia gravis [5] and cancer [6].

Carbonic anhydrase III (CA3) is unique in this family as it has low hydratase activity [7] but it is very abundant in liver, skeletal muscle and adipose tissue.

However, surprisingly for such an abundant protein (e.g. 2% of wet weight in slow oxidative muscle (type 1) [8]) its function remains an enigma. Until recently Ca3KO mice were believed to lack any functional deficit [9] but have now been shown to display impaired mitochondrial ATP synthesis [10]. In addition, these mice show changes in expression of genes involved in oxidative stress [11]. This implies CA3 might participate in the cellular response to oxidative stress.

There is mounting evidence that strongly suggests CA3 is an anti-oxidant. Tissue in which it is abundant is those whose metabolic activity results in considerable oxidative stress, including aerobic respiration in skeletal muscle and lipid metabolism in adipose tissue. CA3 expression is co-induced with established anti-oxidant genes such as superoxide dismutase following endurance training in elite athletes when skeletal muscle is exposed to increased oxidative stress [12] [13].

Functional molecular evidence, in addition to the associated expression studies above, shows that CA3 has anti-oxidant activity. Enforced CA3 expression in NIH3T3 cells protects them from hydrogen peroxide (H_2O_2)-induced apoptosis [14]. EVI1 proto-oncoprotein transformed Rat1 fibroblasts have repressed ca3 expression and either transgene mediated restoration of Ca3 in these cells or direct RNAi mediated Ca3 KD in parental Rat1 cells (high endogenous ca3) protects and sensitizes cells to H_2O_2 induced apoptosis respectively [15]. Insight into a possible mechanism of CA3 anti-oxidant activity has been obtained from post-translational modification observed in cells during oxidative stress. CA3 is modified by S-glutathionylation in response to t-butylhydroperoxide or menadione in cultured hepatocytes [16] as well as in stressed skeletal muscle [11]. Crystal structure studies and site directed mutagenesis reveal two cysteine residues, cys181 and cys186, are available for the addition of glutathione adducts via transient formation of oxidised cysteine sulfenic acid intermediates [17] [18].

S-glutathionylation of CA3 is believed to help protect and aid recovery of cells from the damaging effects of oxidative agents. Previous studies showing that CA3 has anti-oxidant activity and that the protein is S-glutathionylated has led to speculation that the two processes are connected [18]. Direct functional evidence for this is lacking. In this study advantage is taken of our previous analysis in Rat1 fibroblast cells [15]. Derivative Evi1 proto-oncoprotein expressing Rat1 fibroblast cells, designated 5.61, have low ca3 and increased sensitivity to oxidative stress but become resistant upon restoration of ca3 levels by ectopic expression of Ca3. 5.61 cells are used here to explore the relationship between CA3 S-glutathionylation and CA3 anti-oxidant activity.

2. Materials & Methods

Cell Culture

Rat1 fibroblast cells and Evi1 expressing Rat1 cell population 5.61 cells have been described previously [15]. Rat1 and 5.61 cells were cultured in complete

medium (CM) comprising Dulbecco's Modified Eagle's Medium (Lonza Group Ltd, Basel, Switzerland, BE12-604F) supplemented with 5% newborn calf serum (Sigma-Aldrich, Poole, UK, N4637) and 2.5 mM glutamine, 50 μg/ml penicillin, 50 units/ml streptomycin (Lonza Group Ltd., BE17-605E & BE17-603E), 500 μg/ml G418 (5.61 cells only, Invitrogen, Paisley, UK), 37˚C, 5% CO_2. For hydrogen peroxide (H_2O_2) treatment, cells were incubated in CM supplemented with 750 mM H_2O_2 (Sigma-Aldrich, 21676) for 16 hrs.

Preparation of Plasmid DNA

Plasmids pCMVsport6Ca3 (I.M.A.G.E. Id 4195712), pCMVsport6Ca2 (I.M.A.G.E. Id 6479187) and pRLCMV have all been described previously (Source Bioscience, geneservice, Cambridge, UK; Stratagene, La Jolla, CA, USA). Plasmid DNA's were prepared by affinity chromatography using Nucleobond® PC500EF gravity flow columns according to manufacturer's instruction (Macherey-Nagal GmbH & Co. Kg, Düren, Germany).

Site Directed Mutagenesis

Point mutations of Ca2 and Ca3 gene sequences were created by Quick Change™ XL site directed mutagenesis using either pCMVsport6Ca2 or pCMVsport6Ca3 DNA, according to the manufacturer's instructions (Agilent technologies, USA). Briefly, 50 ng of plasmid DNA was mixed with 125 ng forward and reverse primers, 1 μl dNTP's, Pfu Turbo DNA polymerase (2.5 U) in 50 μl and incubated 95˚C, 30 sec then 14 cycles of 95˚C 30 sec, 55˚C 1 min followed by 68˚C 6 min in a programmable thermocycler (MJ Scientific, Hampton, New Hampshire, USA, PTC-100). N186C (Ca2) and C181S (Ca3) mutant plasmid DNA's were used as template for site directed mutagenesis to create double mutants S181CN186C (Ca2) and C181SC186S (Ca3) respectively. Oligonucleotide primers for site directed mutagenesis were designed using the site directed mutagenesis primer design tool (Agilent technologies) and synthesized by Integrated DNA Technologies (BVBA, Leuven, Belgium). Ca2 S181C FP: GCTAACTTTGATCCTTGCTGCCTTCTTCCTGGAAAC; Ca2 S181C RP: GTTTCCAGGAAGAAGGCAGCAAGGATCAAAGTTAGC; Ca2 N186C FP: TTGCTCCCTTCTTCCTGGATGCTTGGACTACTGGACATAT; Ca2 N186C RP: ATATGTCCAGTAGTCCAAGCATCCAGGAAGAAGGGAGCAA;

Ca2 S181CN186C FP: GATCCTTGCTGCCTTCTTCCTGGATGCTTGGACTAC; Ca2 S181CN186C RP: GTAGTCCAAGCATCCAGGAAGAAGGCAGCAAGGA

TC; Ca3 C181S FP: TTTTACACACTTTGACCCATCAAGCC

TGTTCCCTGCTTGCCG; Ca3 C181S RP: CGGCAAGCAGGGAACAGGCTTGA

TGGGTCAAAGTGTGTAAAA; Ca3 C186S FP: CATGCCTGTTCCCTGCTAGCC

GGGACT; Ca3 C186S RP: AGTCCCGGCTAGCAGGGAACAGGCATG;

Ca3 C181SC186S FP: CAAGCCTGTTCCCTGCTAGCCGGGACTATTGGACCT

ACC; Ca3 C181SC186S RP:
GGTAGGTCCAATAGTCCCGGCTAGCAGGGAACAGGCTTG.

Sequencing

Point mutation of Ca2 and Ca3 gene sequences were verified by partial sequencing of plasmid DNA (DNA Analysis Facility, Human Genetics Unit, Ninewells Hospital, Dundee, UK). DNA sequence analysis was performed using FinchTV version 1.4.0.

DNA Mediated Transfection

5.61 cells were transfected with 1 μg plasmid DNA using Fugene6® (Roche Diagnostics GmbH, Mannheim, Germany, 11815091001). 1×10^5 cells were incubated with a 1:6 ratio DNA: FuGENE6®, prepared as described by the manufacturer, for 48 hrs.

Caspase 3 Assay

Cells were incubated in 96 well dishes (Costar 3917) and apoptosis determined by the Caspase 3/7-Glo® assay according to the manufacturer's instructions (Promega, G8090), measuring luminescence with a Fluostar OPTIMA luminometer (BMG LABTECH).

Western Blot Analysis

Protein extracts, SDS polyacrylamide gel electrophoresis and western blotting were performed as described previously [15] with either *α*-ca3 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA, E-19), *α*-ca2 (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA, C-10) or *α*-gapdh (Fitzgerald Industries, North Acton, MA, USA, 6C5) and diluted 1/1000 (E-19), 1/200 (C-10) or 1/5000 (6C5). Appropriate HRP conjugated *α*-goat (Sigma-Aldrich, A5420) or *α*-mouse (Sigma-Aldrich, A9044) IgG secondary antibodies were used at 1/5000 dilutions and detection was performed by enhanced chemiluminescence (Pierce, Rockford, IL, USA, 32209).

Statistical Analysis

Statistical significance was determined by two-way ANOVA using GraphPad PRISM® 7.0c software. $P \leq 0.05$ was considered significant.

3. Results

Ca3 but not Ca2 protects cells from oxidative stress

Murine Ca2 and Ca3 proteins show 60% amino acid identity (**Figure 1**). Initially we examined and compared the anti-oxidant activity of these two proteins in cells. Evi1 transformed Rat1 cells (5.61) were chosen for this purpose as they have previously been shown to express low levels of ca3 [15] and they are −ve for ca2. Both genes were transiently expressed for 48 hrs in 5.61 cells using pCMVsport6Ca2 and pCMVsport6Ca3 expression vectors as described in materials and methods. Western blot analysis with *α*-ca3 (Santa Cruz E-19) antibodies shows elevated levels of Ca3 in pCMVsport6Ca3 transfected 5.61 cells relative to parental (UT) or empty vector (pRCCMV) transfected cells (**Figure 2(a)**). Similarly, western blot analysis with *α*-ca2 (Santa Cruz D-8) shows abundant

```
Ca3     MAKEWGYASHNGPEHWHELYPIAKGDNQSPIELHTKDIRHDPSLQPWSVS 50
Ca2     -SHH---SKS----NW-KEF---N--R---VDID-GTAQ-------LLIC 50

        YDPGSAKTILNNGKTCRVVFDDTFDRSMLRGGPLSGPYRLRQFHLHWGSS 100
        --KVAS-S-V---HSFN-E---SQ-FAV-KE-----S---I---P----- 100

        DDHGSEHTVDGVKYAAELHLVHWNPKYNTFGEALKQPDGIAVVGIFLKIG 150
        -GQ------NKK-----------T--GD--K-VQH------L------- 150

        REKGEFQILLDALDKIKTKGKEAPFNHFDPSCLFPACRDYWTYHGSFTTP 200
        PASQGL-KITE--HS------R-A-AN---CS-L-GNL-----P--L--- 200

        PCEECIVWLLLKEPMTVSSDQMAKLRSLFASAENEPPVPLVGNWRPPQPI 250
        -LL--VT--V----I----E--SHF-K-NFNS-G-AEELM-D----A--- 250

        KGRVVRASFK 260
        -N-KIK---- 260
```

Figure 1. Comparison of the primary amino acid sequence of murine Ca2 (Accession number NM_009801) and Ca3 (Accession number NM_007606) proteins. Identity of amino acid sequences are indicated using the single letter code. Regions of Ca2 identical with Ca3 are indicated by –. Cysteine residues 181 and 186, present in Ca3 but absent from Ca2 are highlighted by a boxed **C** in bold.

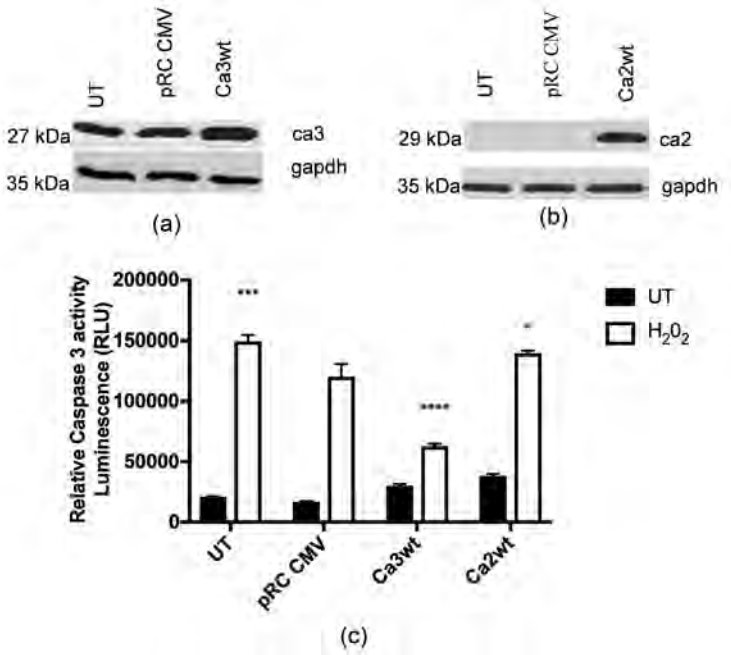

Figure 2. Caspase 3 activity in H_2O_2 treated 5.61 cells with enforced expression of Ca2 and Ca3. (a) and (b) show representative examples of western blot analysis of whole cell extracts derived from untransfected (UT) 5.61 cells or 5.61 cells transfected with empty vector (pRC CMV), Ca2 (Ca2wt) or Ca3 (Ca3wt) expression vectors for 48 hrs with *α*-ca2 (C-10), *α*-ca3 (E-19) and *α*-gapdh (6C5) antibodies. The expected 29 kDa Ca2, 27 kDa Ca3 and 35 kDa gapdh proteins are indicated; (c) shows a histogram of relative caspase 3 catalytic activity in untransfected (UT), empty vector (pRC CMV) transfected and Ca2 (Ca2wt) or Ca3 (Ca3wt) overexpressing 5.61 cells + (white columns) or – (black columns) 16 hrs H_2O_2. Histogram is the mean value of three measurements and the error bars the SEM. Statistical analysis shows two-way ANOVA and Dunnetts multiple comparisons test of H_2O_2 treated pRC CMV vs UT or Ca3wt or Ca2wt, *P < 0.05, ***P < 0.001, ****P < 0.0001.

Ca2 protein in pCMVsport6Ca2 transfected 5.61 cells which is absent from parental (UT) and empty vector (pRCCMV) transfected cells (**Figure 2(b)**). In each case, western blot analysis with *α*-gapdh (Santa Cruz 6C5) confirmed even loading of proteins.

Next, we investigated the anti-oxidant activity of Ca2 and Ca3 in 5.61 cells. Cells were transiently transfected with Ca2 or Ca3 expression vectors as described above and then treated with 750 μM H_2O_2 for 16 hrs. H_2O_2 induced apoptosis was examined by measuring caspase 3 catalytic activity as described in materials and methods. Ca2, Ca3 or empty vector control cells all show low caspase 3 catalytic activity in the absence of H_2O_2 treatment (**Figure 2(c)**). Caspase 3 catalytic activity is induced in each case following H_2O_2 treatment but is significantly less in Ca3 expressing cells (**Figure 2(c)**, pRC CMV vs Ca3wt, ****P < 0.0001). In contrast, Ca2 expression does not protect 5.61 cells from H_2O_2 induced apoptosis (**Figure 2(c)**, Ca2wt). These data confirm that Ca3 protects cells from oxidative stress induced apoptosis and that this property is not shared by the Ca2 isozyme.

Cysteines 181 and 186 are necessary for Ca3 anti-oxidant activity in Rat1 cells

Reversible oxidative stress induced ca3 S-glutathionylation occurs on cysteine residues 181 and 186 [18]. A full length wild type (wt) Ca3genecDNA in pCMVsport 6 was mutated by site directed mutagenesis (materials & methods) to encode Ca3serine at position 181 (Ca3C181S), Ca3serine at position 186 (Ca3C186S) single mutant or Ca3 serine at position 181 and 186 (Ca3C181SC186S) double mutant proteins, that have previously been shown to inhibit S-glutathionylation [18]. To confirm their expression, the wt and mutant Ca3 proteins were each transiently over-expressed by transfection of the various constructs in 5.61 cells. Western blot analysis of whole cell extracts derived from the transfected cells with *α*-ca3 shows over-expression of wt and mutant 27 KDa Ca3 proteins in transfected cells (**Figure 3(a)**) relative to the endogenous protein in empty vector transfected 5.61 cells (**Figure 3(a)**, pRCCMV). In each case, western blot analysis with *α*-gapdh confirmed even loading of proteins.

The mutant Ca3 proteins were next examined for anti-oxidant activity. 5.61 cells transiently transfected with constructs encoding either wt or mutant Ca3 proteins were examined for caspase 3 catalytic activity (materials and methods) either with or without 750 μM H_2O_2 treatment for 16 hrs. The results show low caspase 3 enzyme catalytic activity is significantly induced following exposure to H_2O_2 in each case (**Figure 3(b)**). As before, there is a highly significant reduction in caspase 3 enzyme catalytic activity in cells with enforced expression of wt Ca3 (**Figure 3(b)** pRC CMV vs Ca3wt, ****P < 0.0001) confirming anti-oxidant activity. However, the H_2O_2 induced caspase 3 enzyme catalytic activity is induced to either a greater (**Figure 3(b)** pRC CMV vs UT ****P < 0.0001 or Ca3C181S **P < 0.01 or Ca3C186S **P < 0.01) or similar extent (**Figure 3(b)** pRC CMV vs Ca3C181SC186S) in all other cells that are either untransfected (UT) or

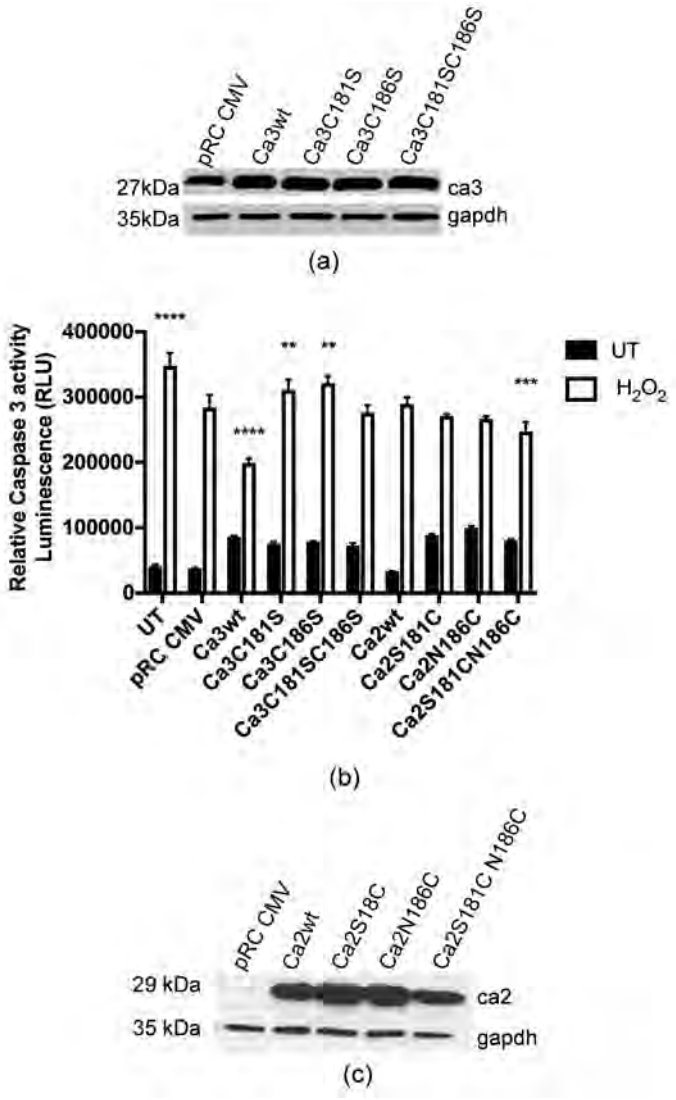

Figure 3. Caspase 3 activity in H_2O_2 treated 5.61 cells with enforced expression of Ca3 and Ca2 encoding wt, cysteine 181 and 186 mutant proteins. (a) shows western blot analysis of whole cell extracts derived from transfected 5.61cells with the empty vector control pRC CMV or over expressing the indicated Ca3wt and mutant proteins. The antibodies used and the Ca3 and gapdh proteins detected are as indicated in figure legend 2; (b) shows a histogram of relative caspase 3 catalytic activity in untransfected (UT), empty vector (pRC CMV), Ca3wt, indicated Ca3 cysteine mutant, Ca2wt and indicated Ca2 serine or asparagine mutant overexpressing 5.61 cells + (white columns) or − (black columns) 16 hrs H_2O_2. Histogram is the mean value of three measurements and the error bars the SEM. Statistical analysis shows two-way ANOVA and Dunnetts multiple comparisons test of H_2O_2 treated pRC CMV vs UT or Ca3wt or C181S or C186S or C181SC186S or Ca2wt or Ca2S181C or Ca2N186C or Ca2S181CN186C, **P < 0.01, ***P < 0.001, ****P < 0.0001.

transfected with one of the three Ca3 mutant proteins. This shows mutant Ca3proteins are no longer protective in Rat1 cells exposed to oxidative stress.

Cysteines 181 and 186 are insufficient to confer anti-oxidant activity on Ca2 in Rat1 cells

The Ca2 amino acids serine 181 (Ca2S181C) and asparagine 186 (Ca2N186C) were converted to cysteine residues, by site directed mutagenesis of pCMVsport6Ca2 expression vector DNA, to see if this would confer anti-oxidant activity on the Ca2 protein. Ca2wt, Ca2S181C, Ca2N186C single mutant and Ca2S181CN186C double mutants were each transiently expressed in 5.61 cells (materials and methods). Western blot analysis of cell extracts with *α*-ca2 shows the presence of similar amounts of the expected wt and mutant 29KDa proteins in transiently transfected cells which is absent from cells transfected with the empty vector control (**Figure 3(c)**). In each case, western blot analysis with *α*-gapdh confirmed even loading of proteins (**Figure 3(c)**). The mutant Ca2 proteins were examined for antioxidant activity in H_2O_2treated cells as before. No significant difference was observed in H_2O_2 induced caspase 3 catalytic activity when compared to empty vector control (pRC CMV) cells (**Figure 3(b)**) but there was a minor but significant reduction with the double mutant protein (**Figure 3(b)** pRC CMV vs Ca2S181CN186C ***P < 0.001).

4. Discussion

It is now widely accepted that CA3 undergoes S-glutathionylation [19] and that this post-translational modification is elevated by either chemical [16] [20], including H_2O_2 [21] or exercise [11] mediated oxidative stress in cultured cells and intact tissues in various mammalian species examined. X-ray crystallography shows that both rat ca3 and S-glutathionylated ca3 are structurally very similar and that two surface exposed cysteine residues (C181 and C186) participate in adduct formation [17]. Substitution of both C181 and C186 for serine completely abolishes S-glutathionylation of Ca3 [18]. We show in this study that Ca3 C181 and C186 are both essential for *in vivo* Ca3 anti-oxidant activity and that S-glutathionylation is therefore an essential feature of the mechanism by which this protein participates in protecting cells from oxidative stress.

Mutation of either C181, C186 or both to serine residues are equally effective at inhibition of Ca3 anti-oxidant activity. Previous studies show that C186 is preferentially S-glutathionylated relative to C181, suggesting that this residue might be more important in anti-oxidant activity [18]. However, the same studies also show that the efficiency of C186 S-glutathionylation is significantly reduced in the C181 mutant Ca3 protein (70% reduction). These observations are consistent with our results that mutation of either one or both of these cysteine residues to encode a serine has a significant impact on Ca3 biological activity as an anti-oxidant.

Our results also confirm previous studies [14] that the closely related Ca3 protein, Ca2, does not share anti-oxidant activity. Furthermore, we show that the introduction of cysteines at positions 181 and 186 of the Ca2 protein is insufficient to confer anti-oxidant activity although there is a minor increase in the presence of both residues. This result suggests other factors, which might impact on the efficiency of S-glutathionylation, are required for anti-oxidant activity.

The S-glutathionylated cysteine residues in Ca3 have a low pKa [18] and S-glutathionylation is affected positively and negatively by lysine 211 and glutamic acid 188/aspartic acid 212 respectively. The pKa of the cysteine residues in mutant Ca2 have not been determined, however the lysine, aspartic acid and glutamic acid amino acids are conserved in Ca2. The S-glutathionylation process can occur spontaneously (reviewed in [22]) but can be catalysed. Glutathione-S-Transferase π (GSTP) catalyses S-glutathionylation of both 1-CYS peroxiredoxin [23] and cardiac aldose reductase [24]. Homozygous KO mice depleted of GSTP show a general reduction in oxidative stress induced protein S-glutathionylation [25] suggesting this protein might be involved in conjugation of glutathione to other proteins, including Ca3. Therefore, there might be other molecular determinants of Ca3 S-glutathionylation besides C181 and C186 that are absent from Ca2.

The CA3 C181 and C186 residues are conserved in all mammalian species examined, including human, rat, murine and bovine, but only C181 is found in xenopus and neither residues are observed in chicken or zebrafish (data not shown). This suggests that only mammalian CA3 has evolved anti-oxidant activity, but this would need to be tested experimentally as other cysteine residues might be S-glutathionylated in non-mammalian species. For example, the CA3 isozyme CAVII is also S-glutathionylated but at cysteine residues C183 and C217 [26].

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Conflicts of Interest

The authors declare no conflict of interest.

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Clinical Study of Aspirin in the Prevention of Thrombosis in Breast Cancer Patients with Postoperative Chemotherapy after PICC Insertion

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Abstract

Objective: To study the clinical effect of aspirin in the prevention of venous thrombosis in breast cancer patients with postoperative chemotherapy after peripherally inserted central catheter (PICC) insertion. **Methods:** 240 cases of female breast cancer patients with postoperative chemotherapy after PICC insertion in The First People's Hospital of Jingzhou from June 2014 to December 2017 were selected and divided into experimental group (n = 120) and control group (n = 120) according to the length of stay. The modified Seldinger technique was used in both groups. The experimental group had oral Aspirin enteric-coated tablet on the day of PICC insertion, 100 mg/day until the PICC catheter was removed, while the control group did not take anticoagulant drugs. The therapeutic effects were evaluated by color Doppler ultrasound, Coagulation analysis, and complete blood count. **Results:** There were three cases with venous thrombosis in the experimental group, and the incidence rate was 2.5%, while ten cases in the control group developed venous thrombosis, and the incidence rate was 10%. There was a statistically significant difference in the incidence of thrombosis between the two groups ($P < 0.05$). **Conclusion:** This study showed that oral Aspirin can effectively reduce the incidence rate of venous thrombosis in breast cancer patients with postoperative chemotherapy after PICC insertion. Therefore, it is worthy of clinical application.

Keywords

Aspirin, Breast Cancer, Peripherally Inserted Central Catheter, Venous Thrombosis

1. Introduction

Currently, the multi-cycle adjuvant chemotherapy has become the most important treatment for breast cancer surgery, and peripherally inserted central catheter (PICC) has been widely used in the chemotherapy of malignant tumors [1]. Because patients after breast cancer surgery usually have edema in their affected limbs, which is caused by Lymphatic reflux obstruction, the clinical treatment of infusion and blood collection of the affected limbs is avoided. Therefore, breast cancer patients with chemotherapy have higher requirements for care after PICC insertion compared with other patients. As 6 - 8 cycles of adjuvant chemotherapy is mostly used in breast cancer patients, long-term indwelling PICC can induce venous thrombosis. It has been reported in the literature that the incidence of venous thrombosis can be as high as 38% after long-term indwelling PICC and even the severe venous thrombosis can be a life-threatening [2]. Therefore, it is essential to prevent the formation of thrombosis after PICC insertion. At present, it is inconclusive whether the preventive anticoagulant therapy can prevent PICC-related upper extremity venous thrombosis. Some foreign scholars have found that no use of any form of anticoagulant or antiplatelet drugs is mildly correlated with PICC-related thrombosis risk (odds ratio 1.93, P = 0.036, 95% confidence interval, 1.025 - 3.602) [3]. Findings in another study suggest that the preventive use of anticoagulant drugs can reduce the incidence of venous thrombosis in patients with PICC [4]. In this study, patients with PICC were given small doses of Aspirin tablets to observe the preventive effects on thrombosis after the insertion of PICC.

2. Material and Methods

2.1. General Data

A total of 240 female breast cancer patients in The First People's Hospital of Jingzhou from June 2014 to December 2017 were selected, aged 32 - 57, with a median age of 45 years old. Inclusion criteria: modified radical surgery for breast cancer; postoperative pathological diagnosis of breast cancer; pathological stage I-II; postoperative 6 - 8 cycles of adjuvant chemotherapy; previous history of no digestive ulcer; coagulation analysis result was normal; PICC insertion informed consent was signed; PICC insertion was successful, and the chest radiograph confirmed the catheter insertion site was normal. Exclusion criteria: infections occurred at insertion site during PICC insertion; accidental or artificially induced catheter shedding; there was bleeding tendency during anticoagulation and allergic reactions could not be tolerated.

2.2. Method

All patients were divided into experimental group (n = 120, June 2014-December 2015) and control group (n = 120, January 2016-December 2017). There was no significant difference in the general data between the two groups (P > 0.05). See **Table 1** for details. Both groups were given catheter insertion by professionally

Table 1. General data comparison between experimental group and control group.

Group	Number of cases	Age		Chemotherapy scheme		
		30 - 45	45 - 60	CEF	TEC	EC-T
Experimental group	120	64	56	38	54	28
Control group	120	62	58	35	60	25
χ^2		0.067	0.067	0.177	0.602	0.218
P		0.796	0.796	0.674	0.438	0.641

Note: CEF is cyclophosphamide + epirubicin + fluorouracil, TEC is docetaxel + epirubicin + cyclophosphamide, EC-T is epirubicin + cyclophosphamide sequential docetaxel Match.

trained nurses who have obtained PICC insertion qualifications, and the modified Seldinger technique was used in both groups to improve success rate and reduce complications [5]. A chest X-ray was taken after catheter insertion to confirm the site of the catheter tip and daily care was performed in accordance with the uniform standard: 1) change the dressing once every 7 days, keep local part clean, and the dressing should be changed immediately if it is loose or contaminated; 2) replace the heparin cap once every 7 days; 3) flush the catheter and keep the catheter smooth, once every 7 days during the treatment interval. The heparin dilution was used to seal the catheter during the insertion. The experimental group had oral Aspirin enteric-coated tablet on the day of the insertion, 100 mg/day until the PICC was removed, while the control group did not take anticoagulant drugs.

2.3. Evaluation Indicator

Both groups of patients underwent color Doppler ultrasound on 3rd day, 7th day, 15th day, 30th day after PICC insertion and before extubation or when the patient developed redness, soreness and pain in the limb of insertion, so as to confirm the presence of venous thrombosis. Coagulation analysis and complete blood count were performed every 7 days to learn about the patient's blood changes and coagulation function.

2.4. Statistical Method

Statistical analysis was performed by using SPSS19.0 software. The count data were analyzed by χ^2 test. $P < 0.05$ indicated that the difference was statistically significant.

3. Results

In both groups, except for some patients whose hemoglobin and thrombocytopenia were observed to be reduced during chemotherapy, considering the myelosuppression caused by chemotherapy drugs, the situation was improved after the subcutaneous injection on the patient with leukocyte drug. The experimental group showed no obvious skin bruising, bleeding tendency such as gum bleeding and Aspirin allergic reaction, and the coagulation analysis was normal. There

were three cases with venous thrombosis in the experimental group, and the incidence rate was 2.5%, including one case on the 5th day after the insertion, one case on the 12th day after the insertion, one case on the 27th day after the insertion, while ten cases in the control group developed venous thrombosis, and the incidence rate was 10%, including four cases occurred within 7 days after the insertion, three cases occurred within 7 - 15 days after the insertion, three cases occurred within 15 - 30 days after the insertion, and two cases occurred on the 48th day after the insertion. Consequently, the incidence of thrombosis on both groups was statistically different ($P < 0.05$). The incidence of venous thrombosis and the time of occurrence of the two groups of patients are shown in Table 2.

4. Discussion

At present, breast cancer has become the most common cancer in women, and the postoperative chemotherapy for breast cancer is mostly a chemotherapy regimen by focusing on using a multi-cycle anthracyclines. Since anthracyclines are foaming agents, once extravasation occurred, it can cause redness, blistering, hardening and severe necrosis of surrounding tissues, resulting in the serious consequences [6]. Therefore, PICC insertion or implantable intravenous infusion is mainly used in clinical practice to provide patients with relatively safe infusion channels. However, due to the hypercoagulable state of blood of cancer patients, studies have shown that patients with cancer have a higher risk of thrombosis embolism, and the incidence rate can reach 5%, which is an increasing trend year by year [7]. But the risk of venous thrombosis in patients with breast cancer is five times to that of normal people especially those patients that have undertaken standardized chemotherapy are higher than the former [8]. Thus, while maintaining effective and safe venous access, PICC insertion has had the biggest complication of venous thrombosis. Especially in the long-term insertion, the risk of thrombosis increases due to the change of blood flow status at the catheter site. Therefore, the prevention of the formation of venous thrombosis and reduction of incidence of venous thrombosis after PICC plays a decisive role in the patient's condition and quality of life.

There are three main reasons for the formation of venous thrombosis in breast cancer patients with PICC: First is the hypercoagulable state of the blood: tumor cells can directly or indirectly activate the coagulation system in the body

Table 2. Venous thrombosis occurrence time and incidence of experimental group and control group.

Group	Number of cases	Occurrence time of thrombosis					Incidence rate of thrombosis
		3 d	3 - 7 d	7 - 15 d	15 - 30 d	>30 d	
Experimental group	120	0	1	1	1	0	2.5%
Control group	120	0	4	3	3	2	10%
χ^2							5.760
P							0.016

to produce thrombin by synthesizing various procoagulant substances. In addition, it can also secrete higher fibrinolytic inhibitors to inhibit the body's fibrinolytic activity, and produce platelet membrane glycoprotein and other substances that lead to platelet aggregation, while the anti-thrombin and anticoagulant system activity of cancer patients is significantly lower than normal people. Second, the damage of the blood vessel wall: the growth of tumor cells can directly infiltrate or compress the blood vessels, resulting in damage of vascular endothelial cells, releasing various cytokines to damage the vascular endothelium. Surgery, venipuncture, chemotherapy, and interventional therapy can also cause direct or indirect toxic effects on the vascular endothelium, thereby leading to the body in a state of thrombosis. Third is the venous blood flow stasis state: the tumor itself or the metastatic lymph nodes of the metastasis on the blood vessels results in slow local blood flow and formation of eddy current, and limb muscle relaxation causes blood flow stagnation due to the patient's long-term bed rest, thereby making the blood activated clotting factor clearance slowed down, endothelial cells impaired by hypoxia, platelets activated and aggregate and blood viscosity increase, which is prone to venous thrombosis [9].

For the prevention of venous thrombosis, the Guide of American Society of Clinical Oncology for the Prevention and Treatment of Venous Thromboembolism in Cancer Patients recommended the conventional prescription of anticoagulant drugs to prevent the formation of venous thrombosis for patients with cancer and without bleeding or other anticoagulation contraindications [10]. At present, clinically oral warfarin is used and the patient's INR level is adjusted between 1.5 and 2.5 to prevent the risk of the thrombosis after long-term insertion. However, there is a significant risk of bleeding in the use of warfarin, and patients need to repeatedly perform coagulation analysis and monitoring to adjust the dosage, the process is cumbersome with poor patient compliance. Aspirin enteric-coated tablets are a kind of the non-steroidal anti-inflammatory drug produced by Bayer AG. Because of its inhibitory effect on platelet aggregation, it is clinically used to prevent the formation of thrombosis. Some foreign scholars believe that warfarin and aspirin are with the similar effect in indwelling venous catheter patency in the long-term [11] [12]. The studies conducted by Tufano *et al.* have shown that after patients with platelet dysfunction have oral Aspirin for 24 months, their risk of venous thrombosis recurrence is about 40% lower than before, and there is no increase in bleeding incidence [13]. However, a foreign study on prevention of PICC-related thrombosis found that each method of preventing thrombosis has a protective trend, but no single method can achieve statistical significance alone [3]. Chinese scholar Wan *et al.* found that it is effective in controlling thrombosis after PICC insertion without significant side effects to give 997 lung cancer patients with PICC a small dose of preventive oral aspirin [14], which is consistent with our findings. Therefore, we believe that Aspirin is safe and effective in being chosen to prevent the formation of venous thrombosis after PICC insertion. However, the research objects in this study are breast cancer patients with PICC, so further research is needed on the relation-

ship between tumor type and chemotherapy regimen, and between coagulation system and anticoagulant drugs.

5. Conclusion

In this study, breast cancer patients with PICC postoperative adjuvant chemotherapy after PICC insertion were enrolled as the research objects, and oral Aspirin enteric-coated tablets were given to prevent the occurrence of venous thrombosis after long-term insertion. The results showed that oral Aspirin can effectively reduce the incidence rate of venous thrombosis in breast cancer patients with chemotherapy after PICC insertion. All patients have no obvious adverse reactions, the cost is low and patients are with good compliance. We believe that it is effective and safe, and it is worthy of clinical application.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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The Treatment of Essential Hypertension Based on Health Education

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Abstract

Objective: To investigate the treatment effect of essential hypertension based on health education. **Methods:** A randomized controlled field intervention trial was used in this study, patients with essential hypertension treated in community health services were subjects investigated, and psychological intervention should be carried out based on pharmaceutical services, to evaluate the effect of psychological intervention model based on pharmaceutical care on blood pressure control and overall health improvement in patients with essential hypertension. **Results:** Before treatment, systolic and diastolic blood pressure had no significant difference between two groups, $P > 0.05$. After treatment, SBP and DBP in two groups were significantly decreased compared to that before treatment in the same group. After treatment, SBP and DBP in the intervention group were significantly decreased compared with normal group, $P < 0.05$. Before treatment, there was no significant difference in SRHMS scores of physical, psychological and social health between two groups ($P > 0.05$). After treatment, the scores of the physiological health subscale and the total scale of the control group, the scores of each subscale and the total amount of the intervention group were significantly higher than those before the same group, the difference was statistically significant ($P < 0.05$), and the scores of the mental, social health subscales and the total scale of the intervention group were significantly higher than those in the control group, and the differences were all statistically significant ($P < 0.05$). **Conclusion:** Under the background of the new round of medical and health system reforms, this model will help health workers and community residents establish new types of doctor-patient relationship and improve the quality of life of chronic diseases such as hypertension.

Keywords

The Essential Hypertension, Health Education, Community, Psychological, Intervention

1. Introduction

In recent years, the prevalence of hypertension in China is on the rise. The latest survey of nutritional and chronic conditions in the population showed that the prevalence of hypertension in adults who aged 18 years and above in China in 2012 was 25.2%, which was significantly higher than that in 2002. The etiology of hypertension is complicated, the course of disease is prolonged, the cure rate is low, and there are many complications. Patients and their families have been subjected to tremendous psychological and economic pressure. Psychological stress has become one of the risk factors to human essential hypertension. In most patients hypertension is often accompanied with anxiety, depression and other psychological disorders, which seriously affect their quality of life. Related studies have shown that the application of psychological intervention to the prevention and treatment of hypertension can avoid the inducement of bad society and psychological factors to the patients, reducing the incidence and mortality of hypertension. Pharmaceutical services provide drug-related services to the public with pharmaceutical expertise and tools, and the goal is to get positive results that improve the quality of patient's life [1]. As a main component of chronic non-communicable diseases in communities, the treatment of hypertension is a long-term process and needs drug combination. Practice in developed countries has proved that pharmaceutical care provided by community pharmacists can alleviate adverse drug reactions caused by long-term medication, reduce medical costs, and ensure safe and effective use of drugs, thereby improving the quality of life of patients [2]. However, the combination of psychological intervention and pharmaceutical care, was carried out on patients with essential hypertension in the community. This kind of practice activity is still rare in our country. Therefore, a randomized controlled field intervention trial was used in this study. Patients with essential hypertension treated in community health services were investigated and psychological intervention was carried out based on pharmaceutical services. The aim was to evaluate the effect of psychological intervention model based on pharmaceutical care on blood pressure control and overall health improvement in patients with essential hypertension.

2. Materials and Methods

2.1. Research Object

The investigation was approved by the Ethics Committee of Yangtze University. 100 patients with primary hypertension treated in this community were selected as the research object. For the research purposes, we set up inclusion and exclusion criteria, and inclusion criteria were: 1) According to the diagnostic criteria of WHO for hypertension; 2) the history of hypertension was more than 6 months, taking antihypertensive drugs or receiving antihypertensive treatment; 3) age between 50 and 80; 4) voluntary participation in informed consent. Exclusion criteria: 1) severe visual and hearing impairment; 2) complicated complications (such as Grade III and above in heart failure, severe renal failure, malig-

nant tumor, etc.); 3) previous or present mental and mental disorders, with severe mental and cognitive impairment, and being involved in other intervention research experiments. The patients were randomly divided into the control group and the intervention group, with 50 cases in each group. There were 50 cases in the control group, including 24 male patients and 26 female patients, aged between 51 and 78, the average age was (65.5 ± 5.2) years, and the range of course was 1 - 3 years. There were 50 cases in the intervention group, including 22 male patients and 28 female patients. Aged between 50 and 77, the average age was (64.8 ± 5.4) years, and the range of course was 1 - 3 years. There were no other malignant tumors in two groups, and their mental state was normal. There was no significant difference in clinical data between the two groups ($P > 0.05$), which was comparable. All patients were suffered antihypertensive medication following the doctor's advice.

2.2. Intervention Methods

According to the related standards and regulations in "Guidelines for the management of hypertension in China" (revised in 2014), the control group was given routine antihypertensive drug therapy (dihydropyridine calcium antagonists) and routine management, while the intervention group was given pharmaceutical services (including hypertensive medication) and psychological intervention on this basis for 6 months (October 2013-March 2014). The team consisted of four community pharmacists and four psychological consultants, and it was divided into four groups, which had one community pharmacist and one psychological consultant. Our team trained community pharmacists and psychologists for two weeks, formulated the uniform pharmaceutical and psychological interventions to ensure each group intervention consistency with the content, step, and frequency. The intervention process was strictly controlled. Pharmaceutical services and psychological interventions were as follows:

1) Rational use of education was conducted by community pharmacists on regular health knowledge and drug therapy lectures. It was commonly introduced as basic information of used drugs, including the expected effect of drug treatment, adverse drug reaction and emergency treatment methods for patients. Files about drug use and health were established for each patient. Patients were supplied with regular door-to-door service, solving the problem for use, drug interactions and adverse drug reaction monitoring, timely correcting the wrong drug habit, increasing medication compliance, eliminating the unreasonable drug use factors. The patients were intervened 1 time every 2 weeks, and 1 hour per time. The total intervene is 12 times.

2) Health education was carried out by community pharmacists in the health education room, including: a) Giving out propaganda materials and the knowledge of hypertension lectures. Let patients understand the relationship between bad habits (smoking, drinking, high salt, high fat, high cholesterol diet, etc.) and cardiovascular disease, and motivate them to quit smoking, eat properly and exercise moderately. b) Strengthen successful experience. Patients were encouraged

to recall and share the experience of correcting unhealthy life habits and their influence on hypertension control, and to strengthen patients' willingness to quit smoking and limit alcohol in a timely. c) Enhance the influence of model and enhance its confidence. d) Set goals and formulate action plans. Intervene 1 time every 2 weeks, 2 hours per time. The total intervene is 12 times.

3) Psychological intervention of cognitive behavior by psychological counselors, namely cognitive behavioral therapy (CBT). Through oneself and self-awareness to identify and understand the patient's mental state, and according to the result of their psychological questionnaires, using evaluation and challenge the idea, evaluate assumptions and rules, promote and transfer stimulus control, behavioral skills training and the habit of twisting therapy method, towards key issues (how to control and adjust negative emotions, how to enhance social adaptability, etc.) to carry out targeted treatment. The intervention was carried out in group mode, with 15 people in each group, and 10 to 15 times in 6 months, 1.5 h per time.

4) Music and relaxation therapy were selected for soothing. Beautiful music was played for 20 minutes before rational use, healthy education and psychological intervention. Guided by community pharmacists and psychologists, patients learned to relax scientifically and maintain peace of mind, to reduce the excitability of sympathetic nerve, alleviate the tension of blood vessel wall. This was carried out by group or team, group intervention not more than 60 people, team intervention not more than 20 people. This intervention can be used during lectures, group training. Patients can also carry out their own, unlimited number of times.

5) Social support is conducted by means of household visits, exchange of experience, telephone follow-up etc. including: a) Family support by encouraging family members to participate in the treatment of hypertensive patients, encouraging their active care, encouraging patients, and monitoring their medication as well as blood pressure control. b) Peer support. Using community health service center as a place to promote communication between patients and patients to share experiences in the prevention and treatment of hypertension and encourage and learn from each other. c) Community support: Post information on the prevention and treatment of hypertension in community health service center and neighborhood committees, 1 theme per issue, 1 for every 2 weeks. Open contact information of community pharmacists to facilitate timely communication between patients. Carry out telephone follow-up to guide patients with medication, and follow up on their blood pressure control. Social support run through the whole process of the study and was jointly completed by community pharmacists and psychological consultants.

2.3. Blood Pressure Measuring Tools

Blood pressure was measured by a community pharmacist with a standard mercury sphygmomanometer for the patient's right arm sitting blood pressure, 3

times a week.

2.3.1. Evaluation of Medication Compliance

The compliance of patients was directly related to the development and therapeutic effect of hypertension [3]. The modified Morisky questionnaire [4] was used to evaluate the medication compliance of two groups. The investigation included: 1) Have you ever forgotten to take medication? 2) Do you sometimes do not pay attention to take medicine? 3) Do you stop the medicine when you feel your condition is improving? 4) Do you stop the medicine when you feel your condition is worsening? The above 4 questions were answered by "yes" or "no". If the 4 questions were all "no", then the medication compliance was "good". If one or more of them were "yes", then the medication compliance was "bad". The medication compliance evaluation was conducted 1 month after the intervention began and ended.

2.3.2. The Evaluation of Knowledge of Drug Use

Evaluating the knowledge of hypertension drug use in patients in two groups by self-made questionnaire. 10 questions were set in the questionnaire, including the commonly used hypertension drugs' name, usage and dosage, medication time, treatment goals, drug preservation methods, main adverse reactions and prevention, drug-food interactions, drug-drug interactions and complications. The patient answered 1 question correctly, with 1 point, and the full mark is 10. 9 - 10 points are judged to be excellent, 6 - 8 are good, and less than 6 are bad. The knowledge of drug use was investigated before and after treatment.

2.3.3. Evaluation of Health Status [5]

The health status of hypertensive patients was evaluated by the self-assessment scale (SRHMS) [6]. The table consists of three evaluation forms, namely physiological, psychological and social health, there are 48 entries, the maximum value of each item is 10, and the lowest value is 0; The theoretical maximum value of this table is 170, 150, 120 and 440, which is applicable to the health measurement of the population of 14 years or older. The score of SRHMS can directly reflect the health of the patient. The higher the score, the better the health is. SRHMS were distributed to both groups before and after treatment. After the patient truthfully fills in, the researcher receives on the spot.

2.4. Statistical Methods

The data were statistically analyzed by SPSS 17.0 software. The metering data was expressed as $x \pm s$, comparison between groups was conducted with independent sample t-test, in the group before and after treatment was conducted with paired t-test; the count data was expressed by the rate, and the comparison between the groups was conducted by X^2 text. And SRHMS scores have a Wilcoxon test for statistical results. $P < 0.05$ was a statistically significant difference.

3. Results

3.1. The Comparison of Control and Intervention Groups on Antihypertensive Effect

Before treatment, as regards the SBP and DBP there was no significant difference between the two groups, $P > 0.05$. After treatment, SBP and DBP in the two groups were significantly decreased compared with before treatment in the same group. After treatment, SBP and DBP in intervention group were significantly decreased compared with control group, $P < 0.05$. The detailed data showed in Table 1.

3.2. The Comparison of SRHMS Scores in Control and Intervention Groups

Before treatment, there was no significant difference in SRHMS scores of physical, psychological and social health between two groups ($P > 0.05$). After treatment, the scores of the physiological health subscale and the total scale of the control group, the scores of each subscale and the total amount of the intervention group were significantly higher than those before in the same group, the difference was statistically significant ($P < 0.05$), and the scores of the mental, social health subscales and the total scale of the intervention group were significantly higher than those in the control group, and the differences were all statistically significant ($P < 0.05$). The SRHMS scores of the two groups before and after treatment were compared in Table 2.

Table 1. Comparison of antihypertensive effect between groups.

Groups	n	SBP/mmHg		DBP/mmHg	
		before	after	before	after
Control	50	155.5 ± 14.6	137.2 ± 12.5*	98.3 ± 8.3	84.2 ± 7.2*
Intervention	50	156.4 ± 13.8	130.2 ± 10.4*#	98.0 ± 7.99	79.4 ± 8.4*#

Note: *P < 0.05 vs. before, #P < 0.05 vs. control group.

Table 2. Comparison of SRHMS scores between two groups before and after treatment.

		Control	Intervention
Physical score	Before	84.2 ± 18.9	100.3 ± 21.5
	After	84.9 ± 20.3*	111.5 ± 18.6*
Psychological score	Before	97.9 ± 27.5	95.6 ± 31.2
	After	98.5 ± 29.7	120.5 ± 26.6*#
Social health score	Before	76.8 ± 22.7	77.3 ± 25.0
	After	78.4 ± 27.3	103.4 ± 22.1*#
Total score	Before	259.5 ± 57.8	262.0 ± 59.3
	After	278.4 ± 57.6*	329.4 ± 43.6*#

Note: *P < 0.05 vs. before; #P < 0.05 vs. control group.

4. Discussion

Hypertension is the most common chronic disease, which seriously jeopardizes national health and the quality of patient's life. Providing pharmacological services for hypertensive patients as well as implementing psychological interventions can help them eliminate negative emotions, stabilize their mentality, build confidence to overcome disease, and prevent adverse emotions from harming their own health, thereby improving the effectiveness of drug treatment and quality of patient's life. However, at present, the prevention and treatment of hypertension in China rarely combines pharmacy services with psychological interventions, and most of the subjects studied are hospitalized patients in large hospitals, fewer in the community [7]. Therefore, this study uses randomized-controlled and on-site intervention trials, and takes pharmacological services performed by community pharmacists as a vehicle to provide psychological intervention for patients who have essential hypertension in the community and promote the rational use of drugs in hypertensive patients, as well as strengthen the antihypertensive effect, then improve the patient's health.

Pharmacy services can improve the patients' drug compliance and the level of drug knowledge. In this study, the pharmacy service team led by community pharmacists went deep into the community to conduct on-site consultations, and introduced drug knowledge to patients by organizing lectures on rational use drug and distributing promotional materials, and through the improved Morisky questionnaire and self-prepared questionnaires to assess the drug compliance and the level of drug knowledge. Before treatment, the quantity of better drug compliance in both groups patients had less than 30%, more than 60% of patients had poor level of medical knowledge and their understanding only stayed on the basic knowledge, such as drug name, usage, medication time, treatment goals and so on. After 6 months of pharmacy services, the proportion of patients in the intervention group who adhered to medications was significantly higher, and more patients could become familiar with common adverse reactions of antihypertension drugs and preventive measures, drug-drug interactions, drug-foods interactions and so on, indicating that good community pharmacy services can effectively improve the patient's drug compliance and the level of drug knowledge.

The psychological intervention based on pharmacy services can enhance the antihypertensive effect of patients. Modern medicine believes that the influence of psychological factors goes through the entire process of the disease. Negative emotions such as depressed mood, mental depression can inhibit the normal function of the autoimmune system and reduce body resistance to disease. Studies have shown that psychological factors such as anxiety, which introduced into the brain as stress, can lead to mental stress, depression, or indecision, and then cause negative emotions such as anxiety. The negative emotions represented by anxiety and depression can increase the risk of developing hypertension, which is not conducive to the control of blood pressure. The results of this study

showed that the antihypertensive effect of the intervention group was significantly better than that of the control group. The reason is possible that the control group only used conventional drugs, while the intervention group also increased the pharmacy services and psychological intervention, as well as reduced the effects of anxiety and depression on blood pressure, and these make patient smoothly depressurized. The results of this study are like those investigations [8], suggesting that the implementation of psychological interventions based on pharmaceutical care can enhance the antihypertensive effect of patients.

Psychological interventions based on pharmacy services can improve the psychological and social health of patients. Studies have shown that various psychological factors are closely related to the occurrence, development, and outcome of hypertension [9]. In this study, patients in the intervention group performed cognitive therapy, social support, and other interventions in addition to pharmaceutical care based on conventional drug therapy to improve the patient's psychological state, relieve their anxiety, and increase their confidence and ability in overcoming the disease. After 6 months of intervention, the scores of the physical health subscales and total tables of the control group were higher than those of the same group before the treatment; while the scores of the physical health subscale and total table increased in the intervention group, The score of the healthy subscale was also significantly higher than that of the same group before treatment, suggesting that the psychological intervention measures achieved the desired effect.

Implementing psychological intervention on the basis of pharmacy services enriches the connotation of community health service practice. In China, community health services usually do not include pharmacists providing face-to-face professional services to patients. This study not only introduced pharmacy services in the prevention and treatment of chronic diseases in the community. At the same time, it also incorporates the practice of psychological intervention. Four community pharmacists and four psychological counsellors work together to complete the intervention. All four pharmacists graduated from pharmaceutical or clinical pharmacy majoring in domestic medical colleges. They have received psychology-related subject education such as doctor-patient communication and pharmacy services during their undergraduate study. They have already worked in pharmacy practice positions at least five years, with certain communication skills and work experience; four psychologists have passed the national psychological counsellor's secondary exam, obtained corresponding certificates, and received basic medical and health knowledge training. During the intervention process, the division of pharmacists and psychologists was clear, and both used their own expertise to provide services for patients with hypertension. Through practice, it has been proved that the comprehensive intervention model of pharmaceutical services and psychological intervention has achieved good results, can effectively promote blood pressure control and health recovery of patients, increase the level of rational drug use, and improve the psychological state of patients, which is worthy of promotion and application in community

health services. Under the background of the new round of medical and health system reforms, this model will help health workers and community residents establish new types of doctor-patient relationship and improve the quality of life of patients with chronic diseases such as hypertension.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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The Effects of Colostrum on Gastrointestinal Function and Related Diseases in Premature Infants: A Comprehensive Meta-Analysis of Randomized Controlled Trials

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Abstract

Aim: To systematically evaluate the effects of colostrum intervention on gastrointestinal function and related diseases in premature infants. **Methods:** A randomized controlled trial was conducted in the Cochrane Library, PubMed, EBSCO, CINAHL, Embase, Medline, CBMDISC, CNKI, Wan Fang and VIP databases on the effects of colostrum and oral intervention on gastrointestinal function and related diseases in premature infants. Literature screening, quality evaluation and data extraction were conducted, and data analysis was conducted using Revman 5.3. **Results:** A total of 7 references were included, including 392 subjects. The combined results showed that: colostrum can effectively reduce the incidence of nosocomial infection [RR = 0.42, 95% CI (0.25, 0.73), P = 0.002]. **Conclusion:** Colostrums oral intervention can effectively reduce the incidence of nosocomial infection in premature infants. However, there was no advantage in feeding intolerance, necrotizing enters colitis and length of hospital stay.

Keywords

Colostrums Oral Intervention, Gastrointestinal Function, Meta-Analysis, System Review

1. Introduction

The World Health Organization (WHO) reported that 15 million premature babies born every year in May 2012 [1]. However, due to the immature digestive system of premature infants, most of them adopt nasal feeding or total parenter-

al nutrition to support life. Although meeting the nutritional needs of premature infants, feeding intolerance, necrotizing enter colitis and other diseases are easy to occur. Therefore, safe and effective oral feeding becomes the ultimate goal of premature infants [2]. Studies have shown that oral interventions are sensory stimulation of the lips, jaw, tongue, soft palate, and throat. It can effectively shorten the time from tube feeding to oral feeding for premature infants. If the colostrums are combined with oral intervention, the abundant cytokines in colostrums and immunocompetent cell such as lactoferrin can be fully absorbed in the cheek of premature infant [3] [4] [5] [6]. It can not only strengthen the resistance of premature infants to pathogenic microorganisms but also effectively improve the feeding process [7] [8]. However, due to different intervention measures, frequency and duration of studies, the effects of colostrum intervention on gastrointestinal function and related diseases in premature infants are different. The related studies at home and abroad are collected and summarized to provide evidence basis for clinical nursing. The report is as follows.

2. Material and Methods

2.1. Search Strategy and Study Selection

According to the keywords ("premature OR immature delivery OR low birth weight (LBW) OR very low birth weight (VLBW)") AND ("colostrum oral feeding OR colostrum oral intervention OR colostrum oral drip OR colostrum oral daub OR Oropharyngeal Administration") AND ("gastrointestinal function OR digestive function"). With the language limited to English and Chinese, we retrieved the related articles from Cochrane Library, PubMed, EBSCO, CINAHL, Embase, Medline, Sino Med, CNKI, Wan Fang and VIP from their database inception to April 2018.

Retrieve the steps: 1) System review or meta-analysis of the oral colostrum intervention in the Cochrane Library was searched. 2) Relevant original documents included in Chinese and English databases such as PubMed and EBSCO were searched, and the title, abstract and key words of the obtained articles were analyzed to further determine the keywords. 3) Retrieve the above database using mesh words OR keywords, and use the "AND", "OR", "NOT" operators if necessary. 4) Manually search for references in the published literature.

Study selection was required to meet the following inclusion criteria: 1) The study must be Randomized Controlled trials (RCT). 2) The object of study were premature infants, A live birth of a baby less than 37 week of gestational age, weight is less than 2500 g. 1500 g - 2500 g are called low weight children, <1500 g are extremely low weight children [3] [4]. 3) The intervention method must have two conditions, one is the use of colostrums, and the other is oral intervention. The frequency, dose and duration of intervention should be described. 4) outcome indicator: a) Gestational age at the time of complete non-retention; b) Incidence of feeding intolerance, Patients with feeding intolerance without a clear definition are characterized by gastric retention, vomiting and abdominal dis-

tension [8]; c) Incidence of nosocomial infections, septicemia [9]; d) The incidence of necrotizing enterocolitis; e) LOS (length of stay); f) Secretary immunoglobulin A (sIgA); g) lactoferrin.

Exclusion criteria: 1) The studied that do not include outcome measures; 2) Repeated publication; 3) Unable to extract data or obtain full text; 4) The babies with congenital diseases or malformations or whose guardians cannot provide colostrums.

2.2. Quality Assessment and Date Extraction

All literature quality evaluations were completed by 2 researchers trained in an evidence-based nursing course. Firstly, the included literature was evaluated independently according to the evaluation criteria of Cochrane evaluation manual 5.1.0 [10]. When disagreements arise, discuss inclusion or exclusion. If the above criteria are fully met, the probability of occurrence of various biases is small, grade A. if partial; the probability of occurrence of bias is moderate, grade B. The probability of bias is higher, which is grade C. A, B can be used, C can be excluded. Recommended level: A > B > C.

Two researchers developed standardized tables based on literature content and independently extracted data. Including the year, author, sample size, intervention measures, frequency of intervention, dose used for intervention, intervention method, and duration and outcome index of the included literature.

2.3. Statistical Analysis

The Revman 5.3 software was used for Meta analysis, Weighted Mean Differences (WMD) was used to analyze the continuous data of the same measurement tools, and otherwise Standardized Mean Differences (SMD) was used. Calculation of relative risk (RR) for binary classification data, all analyses calculated 95% CI, for studies without methodological and clinical heterogeneity; statistical heterogeneity was determined by the calculation of I^2 by Cochrane Q test. If the heterogeneity between studies was small ($P > 0.1$, $I^2 < 50\%$), fixed model was used. If the heterogeneity is large ($P \leq 0.1$, $I^2 \geq 50\%$), the random model is used. If the sources of heterogeneity cannot be determined, or heterogeneity is significant, only descriptive analysis is conducted.

3. Result

3.1. Search Results and Study Characteristics

By April 2018, a total of 2682 literalness had been retrieved, Repeated publication, literature review, and irrelevant literature (n = 2324) were deleted, with 358 remaining articles, Read the title, abstract, and exclude the descriptive study and the study without control group (n = 256), read the full article to exclude data incompleteness, remaining 5 articles, 2 articles were obtained by manually searching references, and 7 articles were finally included [9] [11]-[16]. There are 5 Chinese [12] [13] [14] [15] [16] articles and 2 English articles [9] [11]. The

screening process is shown in Figure 1. In one article [15], the intervention group was divided into high frequency group and low frequency group, In order to make the data more clear and split into two RCT, the basic features of the included articles are shown in Table 1.

3.2. Quality Evaluation of Included Literature

The included RCT was evaluated and graded according to the evaluation criteria of Cochrane evaluation manual 5.1.0. The methodological quality of the included articles is mostly medium, and the specific evaluation indicators and results are shown in Table 2.

Table 1. Basic characteristics of the included literature.

study	year	sample size		intervention measure			outcome indicator
		IG	CG	IG	CG	frequency	
F. T. Ji [12]	2016	28	27	Oral drip of colostrum	Saline drip	Once every 4 h, 0.2 ml each time, for seven days	A, B
X. J. Zhao [13]	2017	20	20	Oral drip of colostrum	Saline drip	Once every 4 h, 0.2 ml each time, for seven days	A
C. Y. Chen [14]	2018	46	46	Oral drip of colostrum	Saline drip	Once every 4 h, 0.2 ml each time, for seven days	A, B, C, D
S. X. Cao [15]	2018	20	20	Colostrum oral smear	Sterile water smear	Once every 3 h Once every 6 h	B, E
L. F. Chen [16]	2017	30	30	Colostrum oral smear	Normal saline smear	Once every 3 h, for two weeks	B, C, D, E
Y. X. Zhang [9]	2017	27	28	Oral drip of colostrum	Saline drip	Once every 4 h, 0.2 ml each time, For seven days	C, E, G, H
Glass K. [11]	2017	17	13	Oral drip of colostrum	Sterile drop injection	Once every 3 h, 0.2 ml each time	C, E, G, H

Note: IG: Intervention group. CG: Control group. A: Gestational age at the time of complete non-retention. B: Incidence of feeding intolerance. C: Incidence of nosocomial infection. D: The incidence of necrotizing enters colitis. E: LOS (length of stay). G: Secretary immunoglobulin A (slgA). H: lactoferrin.

Table 2. Methodological quality evaluation for inclusion of RCT.

Sample	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blind of outcome assessment	Incomplete outcome date	Selective reporting	Other sources of bias	Quality grade
F. T. Ji [12]	U	U	L	L	H	L	U	B
X. J. Zhao [13]	H	U	L	L	L	L	U	B
C. Y. Chen [14]	L	U	L	L	L	L	U	B
S. X. Cao [15]	L	U	L	L	L	L	U	B
L. .F Chen [16]	U	U	L	L	L	L	U	B
Y. X. Zhang [9]	L	L	L	L	L	L	L	A
Glass K. [11]	L	U	L	L	L	L	U	B

Note: L, Low risk of bias. U, Unclear risk of bias. H, High risk of bias.

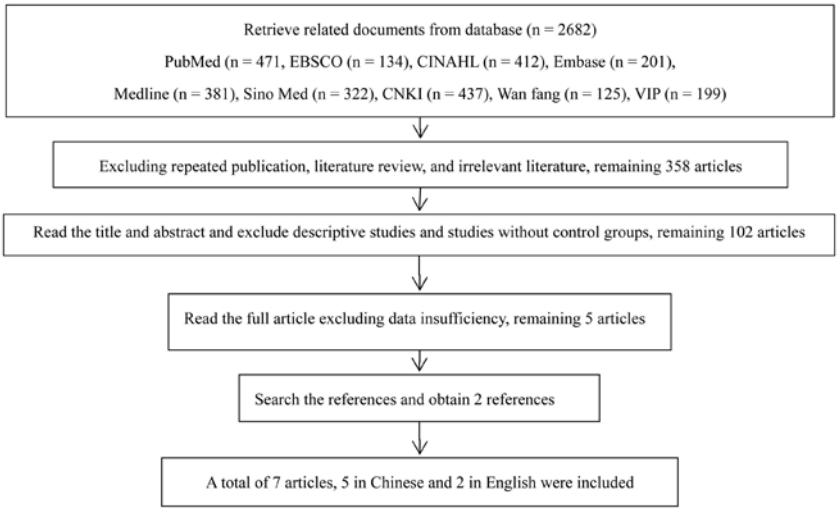

Figure 1. Literature screening flow chart.

3.3. Basic Features of the Included Literature

A total of 392 premature infants were included in 7 literatures. 3 [12] [13] [14] reported gestational age at the time of complete non-retention, 5 RCT [12] [14] [15] [16] reports feeding intolerance, 4 articles [9] [11] [14] [16] reported hospital infection rate, 4 articles [9] [11] [15] [16] reported hospitalization duration, 2 articles [14] [16] reported the incidence of necrotic enter colitis, and 2 articles [9] [11] reported lactoferrin and sIgA. The basic characteristics of the included articles are shown in **Table 1**.

3.4. Effect Evaluation of Colostrums Oral Intervention

3.4.1. Effect on Gestational Age at Complete Non-Retention

3 [12] [13] [14] reported the effect of colostrums intervention on the gestational age of premature infants with complete non-retention. The results of data combination showed that there was heterogeneity between the studies ($P = 0.13$, $I^2 = 50\%$), and the random model was adopted. The results showed that the intervention of colostrums can reduce the time of complete non-retention and improve the gastrointestinal function of premature infants. The difference between the two groups was statistically significant [MD = −1.13, 95% CI (−2.17, −0.10), P = 0.03]. As shown in **Figure 2**.

3.4.2. Effect of Colostrums Oral Intervention on Nosocomial Infection

4 [9] [11] [14] [16] reported the influence of colostrums intervention on the nosocomial infection rate of premature infants, and the combined results showed that the inter-study heterogeneity was relatively small ($P = 0.17$, $I^2 = 40\%$). Therefore, the fixed model was selected, and the results showed that colostrum intervention could effectively reduce the nosocomial infection rate of premature infants [RR = 0.42, 95% CI (0.25, 0.73), P = 0.002]. As shown in **Figure 3**.

3.4.3. Effect of Colostrums Oral Intervention on Immune Function

SlgA and lactoferrin were used as evaluation indicators in 2 papers [9] [11],

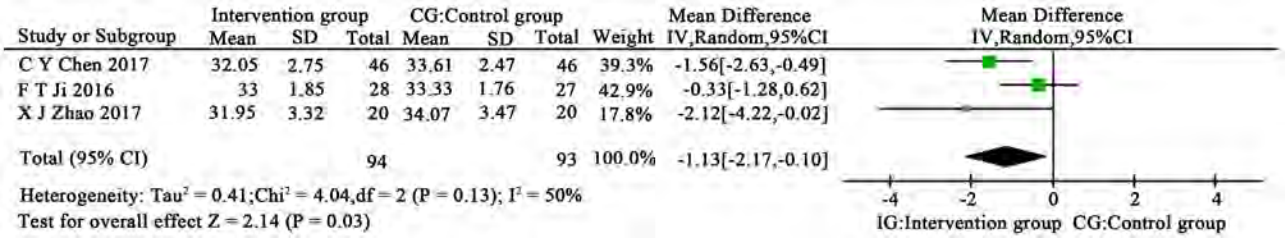

Figure 2. Effect on gestational age of premature infants with complete non-retention.

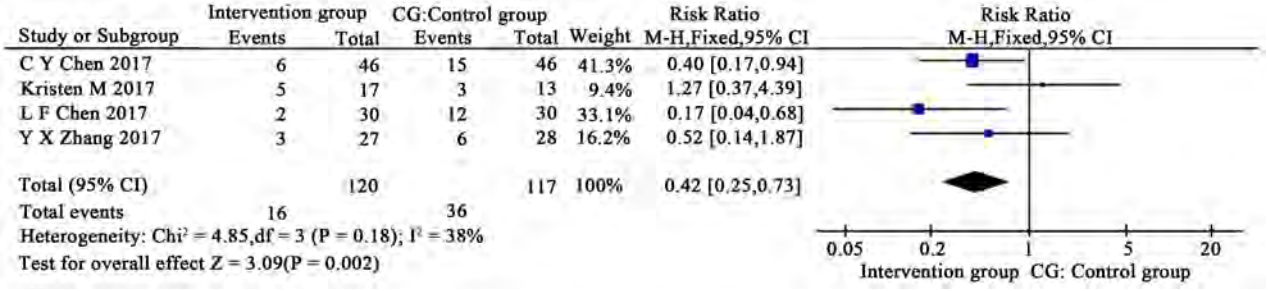

Figure 3. Effect of colostrums oral intervention on nosocomial infection.

However, because of the obvious clinical heterogeneity [17] of the measure method. Including venous blood, urine, saliva, and tracheal secretion, only descriptive analysis was conducted. Glass K found lactoferrin will significant increased after the colostrum intervention, but the change of the sIgA is not statistically significant, the main reason is that sIgA in colostrums is highest on the first day after delivery, and then it goes down. However, the timing of oral intervention of colostrums was mostly 4 - 5 days after delivery, it was in the decline stage, so sIgA was not significantly improved.

3.4.4. Other Effects of Colostrum Oral Intervention

4 articles [12] [14] [15] [16] reported feeding intolerance, merge the result shows: the intervention group and control group there was no significant difference results [RR = 0.58, 95% CI (0.30, 0.31), P = 0.10), namely the colostrum oral intervention cannot reduce the incidence of preterm infant feeding intolerance, 5 articles [9] [11] [14] [15] [16] reported necrotizing enter colitis, data merging results showed that colostrum oral intervention can effectively reduce the incidence of necrotizing enter colitis, there was no statistically significant difference in the two groups [14] [16] (RR = 0.71, 95% CI (0.40, 0.40), P = 0.25). Two articles reported the effect of colostrum oral instillation on hospitalization time of children, and the combined results showed that colostrum oral intervention could not effectively reduce the hospitalization time of children [MD = −8.06, 95% CI (−21.00, 4.88), P = 0.22].

3.5. Sensitivity Analysis

Sensitivity analysis was performed for all the combined results. When sensitivity analysis was performed on the complete non-retention gestational age, the larg-

est sample size Chen Caiyun was removed and the results changed, it is suggested that the result is not stable. As fewer than 10 articles were included, the funnel plot analysis was not done to evaluate the publication bias.

4. Discussion

4.1. Methodological Quality Evaluation

A total of 7 [9] [11]-[16] articles were included in this study, among which 5 [9] [11] [13] [14] [15] used random methods, 4 (57.1%) [9] [11] [14] [15] explicitly stated the use of random numbers **Table 1** [9] (14.2%) reported the method of allocation concealment, and 1 [12] (14.2%) only mentioned allocation concealment but did not specify the specific method. Seven (100%) results use the blind method. Six [9] [11] [13] [14] [15] [16] (85.7%) reported the loss of data, and 1 [9] (14.2%) reported increased sample loss rate. All 6 [9] [11] [12] [13] [14] [16] studies (85.7%) compared baseline levels of premature infants, and baseline consistency ($P > 0.05$).

4.2. Methods and Frequency of Colostrum Oral Intervention

Colostrums oral intervention includes two forms, one is the colostrum oral drip, namely after removing the oropharyngeal secretions of premature infants, 0.2 ml of normal temperature colostrum was extracted using 1ml syringe, along the side of the mouth, pointing to children with throat, uniform slowly push 0.1 ml, then moved to contralateral injection of 0.1 ml. The second is the colostrum smear, that is, after removing the oral secretions, use cotton swab to apply the colostrum restored to room temperature to the buccal mucosa of premature infants. 5 [9] [11] [12] [13] [14] used colostrum oral instillation, and 2 [15] [16] used colostrum oral smear. 4 [9] [12] [13] [14] performed on premature infants every 4 hours. 3 [15] [16] [18] performed every 3 hours, and one article [15] performed every 6 hours. 5 articles [9] [11] [12] [13] [14] indicated that each use of 0.2 ml of colostrum, 4 articles [9] [12] [13] [14] lasted for 7 days and 1 article [16] lasted for two weeks.

4.3. Colostrums Oral Intervention Can Accelerate the Process of Nutrition

Premature infants often suffer from stomach retention, abdominal distension and vomiting due to immature digestive system [19]. Gastric retention, also known as gastric emptying delay, the results showed that the residual gastric fluid extracted from the stomach before feeding was more than 50% of the previous feeding. Complete non-retention is an indicator of complete recovery of gastrointestinal function and the amount of gastric residue in the normal range. The combined results of this study showed that the colostrum oral intervention can reduce the gestational age at the time of complete non-retention enhance the digestive function and promote the process of entreat nutrition. However, the stability of the combined results was poor, which may be attributed to the lack of

a clear definition of feeding intolerance in some studies, and the inclusion of abdominal distension, vomiting and other factors into the tolerance range, which had an impact on the results.

4.4. Early Colostrum Intervention Can Reduce Hospital Infection in Premature Infants

Premature infants need to face the external environment alone after birth, but the each system is not mature, and the antibodies in breast milk cannot be obtained through mouth to resist external pathogenic bacteria, so the risk of infection is high. Studies have shown that the mother's colostrum of premature infants contains more cytokines and enzymes than that of full-term infant [18] [20] [21] [22]. These substances can be directly absorbed in the buccal mucosa to help premature infants resist external bacteria and development of premature infants. Therefore, the oral intervention of the colostomy of premature infants is more important than that of full-term infants. The combined results of the study also showed that the colostrum oral intervention can effectively reduce the incidence of nonsocial infection and promote the normal growth of premature infants [5] [11] [23].

4.5. Effect on Immune Function

Lactoferrin in colostrum has strong immunomodulatory, bactericidal, anti-infection and anti-virus effects [24], sIgA and lactoferrin can be distributed on the oral mucosa surface and intervene oral colonization in the mouth, have the effect of local immunity, Lactoferrin and sIgA are rarely used as evaluation indicator in domestic studies. But in foreign studies that are used as evaluation indexes, their measurement methods are too heterogeneous to be combined with data, According to specific research data, colostrum oral intervention can promote the increase of lactoferrin, but it has no significant advantage for sIgA growth [5] [25]. The main reason is that sIgA content is the highest one day after giving birth and gradually decreases after that. However, the primary oral cavity intervention is mostly in the 4 - 5 days after giving birth, which is in the decline stage of sIgA. Therefore, the primary oral cavity intervention has no significant impact on sIgA of premature infants.

4.6. Limitations

1) Only searched published Chinese and English articles, and did not search gray literatures. 2) Less than 10 studies were included without funnel plot analysis. 3) The domestic research in enteral feeding process for ending index, study abroad in the urine, saliva sIgA and lactoferrin as the ending index, there is obvious heterogeneity, descriptive analysis only.

5. Conclusion

The methods, frequency, dosage and time of colostrum intervention were dif-

ferent, so there are different views on the effects of colostrum intervention on gastrointestinal function and related diseases of premature infants. Now the homogeneous research is conducted for meta-analysis. The combined results showed that colostrum oral intervention can reduce the incidence of nosocomial infection; however, there was no advantage in necrotizing enters colitis, length of hospital stay, and feeding intolerance. The results showed that the gestational age of premature infants with complete non-retention could be reduced by colostrums intervention, but the stability of the results was poor, which needs further demonstration. Because sIgA and lactoferrin were measured in different ways, only descriptive analysis was performed. It is suggested that the future study should be based on the urine sIgA and lactoferrin, which do not cause damage to premature infants, and have comparability between studies. We should further explore the start time, frequency and duration of colostrums intervention in premature infants to form a standardized pattern of neonatal oral intervention.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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