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In-Vitro Determination of Biological and Anabolic Functions of Weak Androgen Dehydroepiandrosterone (DHEA) Using a Variety of Cell Lines

James L. Cox[1], Yingzi Chang[2], Pandurangan Ramaraj[1*]

[1]Department of Biochemistry, Kirksville College of Osteopathic Medicine, A.T. Still University of Health Sciences, Kirksville, MO, USA
[2]Department of Pharmacology, Kirksville College of Osteopathic Medicine, A. T. Still University of Health Sciences, Kirksville, MO, USA
Email: *Pramaraj@atsu.edu

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Abstract

Dehydroepiandrosterone (DHEA) is a weak androgen and is shown to have anti-cancer, anti-atherogenic, anti-adipogenic and anti-inflammatory effects on mouse, rat and rabbit models. However, human clinical trials data did not support animal findings and were inconclusive. These systemic differences in biological actions between rodents and humans were attributed to the low level of DHEA in rodents. In order to further understand the differences in biological functions between rodents and humans, we resorted to an *in-vitro* approach involving mouse, rat and human cell lines to assess DHEA biological and anabolic functions separately and independently without systemic influence. Results indicated that DHEA was effective on mouse and rat cell lines but not on human cell lines, as observed in *in-vivo* studies. In addition, our *in-vitro* study showed that DHEA was able to induce myogenesis in mouse mesenchymal cells revealing its anabolic function, even though DHEA was considered as a weak androgen. This observation lent credence to the ban on DHEA by IOC medical commission, citing DHEA as an anabolic steroid. These *in-vitro* experiments suggested that the differences in biological actions of DHEA between rodents and humans existed not only *in-vivo* at the systemic level, but also *in-vitro* at the cellular level and thus paving the way to study the mechanism responsible for these differences at the cellular level itself.

Keywords

Dehydroepiandrosterone, Various Cell Lines, Biological Functions, Anabolic Function

*Corresponding author.

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1. Introduction

DHEA is the most abundant androgen (male steroid hormone) secreted by the adrenal cortex [1] and to a lesser extent by testes [2] and ovaries [3]. DHEA is also synthesized in brain and skin [4]. DHEA is metabolized into and rostenedione in the body and may further be converted into either testosterone or estrogen [5] [6]. Eventhough DHEA is a weak androgen, it is present in high concentration both in males and females [7]. So, it raises the question whether it acts through androgen receptor or estrogen receptor or its own receptor [8]. Hence, DHEA's physiological function is still not clear [9]. DHEA circulates in the blood stream mainly as the sulfated form DHEAS [10] [11]. The half-life of DHEAS is 7 - 10 hrs, whereas the half-life of DHEA is only 15 - 30 min [10]. Adult blood levels of DHEAS are 100 - 500 times higher than testosterone and 1000 - 10,000 times higher than Estradiol [11] [12]. DHEAS is converted to DHEA and then to sex hormones in body tissues. Blood levels are highest in developing fetus, dropping sharply after birth, beginning climbing again at age 6 - 8 to a peak level at age 25 - 30 and then declining to about 10% of the peak level by age 80 [11] [12]. There is no change in DHEAS serum level before the age of 90 and men over 90 with the highest serum DHEAS level show the best functional status [10] [11].

Since circulating level of DHEA peaks at age 25 and then steadily declines with age, some researchers consider DHEA as a possible anti-aging hormone. Considerable interest in DHEA has been developed in recent years with reports that it may play a role in the aging process. DHEA deficiencies in older individuals have been associated with a number of medical conditions ranging from cancer, cardiovascular disease, impaired memory and mental function and osteoporosis [12]-[15]. DHEA levels are also low in persons who are obese and those who have muscle atrophy. Low level is present in male impotence [12]-[15]. Administration of DHEA to rodents suppressed weight gain without significantly affecting food intake, ameliorated the severity of diabetes in genetically diabetic mice and restrained immune processes [9] [16]. DHEA offered protection against spontaneous tumors and chemical carcinogenesis. Also it depressed the mitogenic effects of carcinogenesis, tumor promoters and blocked viral and carcinogen induced cell transformation in mouse [16]. In view of the above beneficial findings in rodents, clinical trials were carried out with DHEA, but without any positive or conclusive data [17]-[21]. The United States Food and Drug Administration removed DHEA supplement from the market in 1985 due to false claims about health benefits [13]. However, since the passing of the US dietary supplement health and education act of 1994 [13], DHEA has made its way back to the market (health food stores, pharmacies and groceries) and its popularity continues to grow.

DHEA's purported uses include 1) Addison's disease, 2) Alzheimer's disease, 3) Atherosclerosis, 4) Cancer treatment, 5) Depression, 6) Immunostimulation, 7) Memory loss, 8) Rheumatoid arthritis, 9) Schizophrenia, 10) Sexual performance, 11) Systemic Lupus Erythematoses (SLS) and 12) Weight gain and Weight loss [12]-[16]. Since, DHEA is a prohormone that can be converted by the body to other sex hormones such as testosterone and estrogen, it is important to find out whether DHEA has any toxic side effects like testosterone. Since, DHEA may increase the production of the male hormone testosterone, women should be aware of the risk of developing signs of masculinization (such as loss of hair on the head, deepening of the voice, hair growth on face, weight gain around waist or acne). Similarly, men should be aware of the risks of excess testosterone (such as shrinkage of testicles, aggressive tendencies including sexual aggression, male pattern baldness and high blood pressure). There are mixed reports on the receptor through which DHEA acts [8]. Research with genome-wide analysis of DHEA and DHT induced genes expression in mouse hypothalamus and hippocampus demonstrated that DHEA was intrinsically androgenic [22]. It competed with DHT for binding to androgen receptor and induced androgen receptor (AR) regulated reporter gene expression *in-vitro* [22].

Since, there was no supporting clinical trial in humans to back-up the beneficial findings in small animals (mouse, rat and rabbit) and the fact that people consumed DHEA as a dietary supplement without knowing that DHEA was a weak androgen, our objective was to determine DHEA's biological and anabolic functions *in-vitro* using mouse, rat and human cell-lines.

2. Materials and Methods

2.1. Chemicals

DHEA (dehydroepiandrosterone), MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), iso-

propanol, paraformaldehyde, Oil red O stain were purchased from Sigma chemical company, St. Louis, MO. Fetal bovine serum (FBS), Trypsin-EDTA (1×) and PBS powder were purchased from Atlanta biologicals, Lawrenceville, GA. RPMI and antibiotic/antimycotic solution 100× (10,000 I.U/ml penicillin, 10 mg/ml streptomycin, 25 µg/ml amphotericin-B) were purchased from Fisher scientific, Houston, TX.

2.2. Growth Medium (GM)

All cancer cell culture works were carried out in RPMI 1640 medium containing 10% FBS +1× Pen/Strep/Ampho as described in references [23] [24].

2.2.1 Cell Growth Assay

MTT proliferation assay [25] was used to quantitate cell growth in treated (with DHEA) and in untreated (control) samples. In addition, microscopic pictures of cells treated with hormone were taken to document hormonal effects on cell lines.

2.2.2. MTT Proliferation Assay [25]

B16F10 cells, BLM cells and NUGC3 cells were suspended in growth medium (GM) and plated at a density of 1×10^4 cells/well in a 96 well plate. Cells were left overnight at 37˚C to attach to the plate. Following day growth medium was replaced by GM containing DHEA hormone at different concentrations and incubated for 48 hrs. After 48 hrs, medium was replaced by 100 µl of 1 in 10 diluted (in GM) MTT solution and incubated for another 4 hrs at 37˚C. After 4 hrs MTT solution was removed. MTT was reduced by metabolically viable cells to a colored (purple) water insoluble formazan salt. The purple color precipitate was solubilized by adding 100 µl of isopropanol and shaken for 20 - 30 min at room temperature. Intensity of resultant purple color was measured at 570 nm in a SLT spectra plate reader.

2.3. Rat Vascular Smooth Muscle Cell Culture

Procedure for isolation of rat aortic vascular smooth muscle cells was described in reference [26]. A petri dish containing cells at 6/7 passage was obtained from a colleague. Cells were harvested from the plate using trypsin-EDTA and were re-plated in a 96 well plate in DMEM/F12 medium. Cells were left for 2 - 3 days to become 70% to 80% confluent. Regular medium was replaced by medium containing 10 and 100 µM concentrations of DHEA and incubated for 48 hrs. At the end of 48 hrs, MTT assay was carried out to quantitate cell growth.

2.4. Mouse 3T3-L1 Preadipocyte Culture

Mouse 3T3-L1 preadipocyte (American Type culture collection, Manassas, VA) was maintained in growth medium (GM) containing DMEM supplemented with 10% FBS as described previously [27]. Differentiation was induced in 100% confluent 3T3-L1 cells by incubating in adipogenic medium (AM) (GM with 0.5 mM isobutylmethylxanthine, 1 µM dexamethasone and 10 µg/ml insulin) for three days with or without androgens. This was followed by changing the medium to GM containing 10 µg/ml insulin with or without androgens at various concentrations for another 2 days and then to GM plus treatments for up to 10 days.

2.5. Oil Red O Staining

Cells were fixed in 2% paraformaldehyde after treatments and stained with 0.3% Oil Red O for 15 min as described [28]. For quantitative analysis of Oil red O retention in these cells, stained adipocytes were extracted with 1 ml of 4% Igepal CA-630 (Sigma) in isopropanol and absorbance was measured by spectrophotometer at 520 nm.

2.6. Mouse Mesenchymal Multipotent Cell Culture

Mouse 10T1/2 cells grown at 37˚C in DMEM with 10% FBS, 4 mM glutamine, and 1× antibiotic-antimycotic (GM), were treated with 20 µM 5-azacytidine (aza) in GM. After 3 days cells were split 1:2 and were allowed to recover in GM for 2 days. Cells were seeded at 70% confluence in 6-well plates or chamber-slides and were grown with DHEA for 0 to14 days [27].

2.7. Immunohistochemistry

For immunochemical analyses, cells grown in chamber slides were fixed in 2% paraformaldehyde for 20 min, quenched with H_2O_2, blocked with normal horse serum and incubated with anti-myosin heavy chain II (MHCII) antibody [27]. Detection was based on a secondary biotinylated antibody, followed by the addition of streptavidin-horseradish peroxidase ABC complex (1:100) (Vectastain Elite ABC System, Novocastra Laboratories, Newcastle upon Tyne, UK) and 3,3-diaminobenzidine. The cells were counter-stained with Meyer's hematoxyline.

2.8. Reverse Transcription-PCR

Total RNA was extracted from cells by using Trizol reagent (Invitrogen, Carlsbad, CA) and was purified and characterized by measuring at A_{260}. Two micrograms aliquot of total RNA was reverse transcribed and the resulting cDNA was amplified for 35 PCR cycles by melting at 94˚C for 30 sec, primer annealing at 58˚C for 30 sec and extension at 72˚C for 1 min. PCR products were analyzed by electrophoresis in 1.5% agarose gel using ethidium bromide staining [27]. The locations of DNA sequences for the forward/reverse PCR primers are as follows: C/EBPα (225 bp), 843 - 864/1067 - 1047 on NM_007678; and GAPDH (152 bp) 606 - 626/758 - 738 on BC023196.

2.9. Statistical Analysis

Data are presented as mean ± SEM. The experiments were repeated a minimum of three times for consistency and representative cell pictures from experiments were shown. Pair-wise comparison between groups was performed using Student's t-test. P value less than 0.05 was considered as statistically significant.

3. Results

3.1. DHEA Biological Actions

3.1.1. Effect of DHEA on Mouse Melanoma (B16F10) Cell Growth

Addition of DHEA to mouse melanoma cells showed a significant decrease in cell growth between 10 μM and 100 μM concentrations. DHEA decreased mouse melanoma cell growth to 93.4% at 10 μM and 46.2% at 100 μM concentrations. Actually, cell morphology was slightly different between control (untreated) and treated cells as shown in **Figure 1**. The change in cell morphology was due to differentiation of cells as reported earlier [29].

3.1.2. Effect of DHEA on Human Melanoma (BLM) Cell Growth

Though addition of DHEA to human melanoma cells showed difference in cell growth between untreated control and treated cells, there was no significant dose-dependent decrease in cell growth between DHEA 10 μM and 100 μM concentrations. Ten μM DHEA treated cell growth was 86%, whereas 100 μM DHEA treated cell growth was 82.7% as shown in **Figure 2**. DHEA affected only marginally human melanoma cell growth. The reason for this muffled response of human melanoma cells to DHEA treatment was not known. However, this observation supported clinical trial data [17].

3.1.3. Effect of DHEA on Human Gastric Cancer (NUGC3) Cell Growth

Since DHEA was essential for healthy skin and was also synthesized in the skin, effects of DHEA on mouse and human melanoma cancer cell lines (target cancer) were checked. Later, we extended our investigation to non-target cancer cells to check whether DHEA had any effect on other cancer. As human gastric cancer (NUGC3) cell was earlier used to study the effect of Orlistat on cell growth and was found to inhibit gastric cancer cell growth [30], we used gastric cancer cell line to study the effect of DHEA on cell growth. There was no effect of DHEA on gastric cancer cell growth. There was no difference between untreated and treated cells even at 100 μM concentration of DHEA as shown in **Figure 3**. The two experiment results, first one with human melanoma cell line and second one with human gastric cancer cell line supported the clinical trial outcome for DHEA.

3.1.4. Effect of DHEA on Normal Rat Vascular Smooth Muscle Cells

In order to understand the role of DHEA as an anti-atherogenic agent, we used normal rat vascular smooth muscle

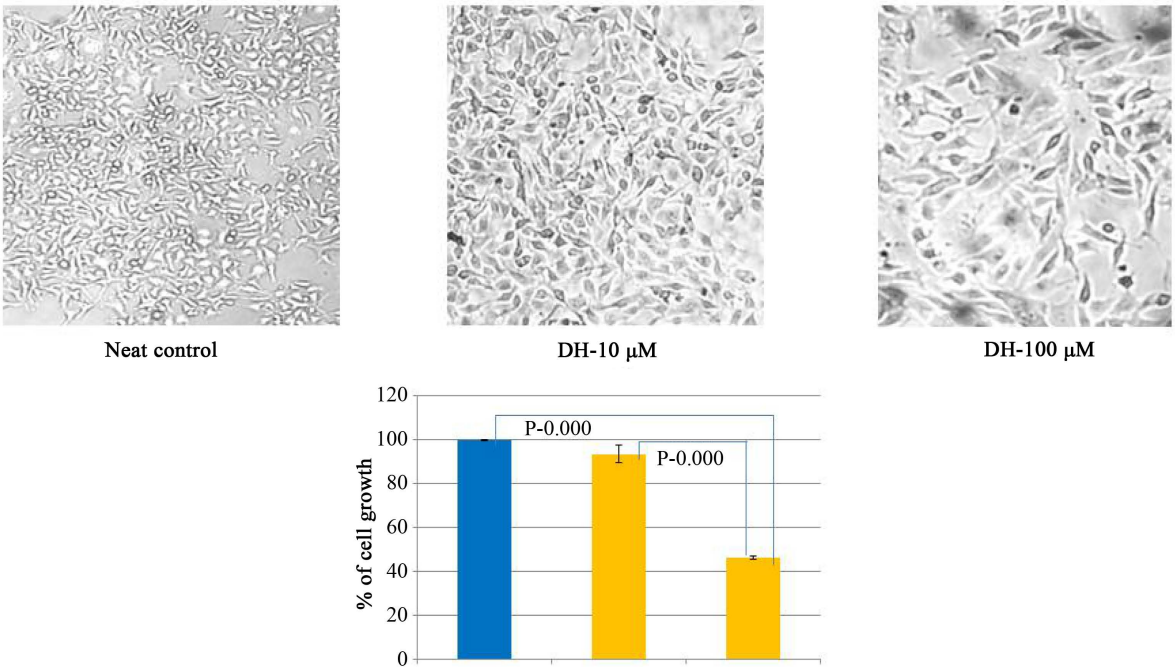

Figure 1. Treatment of mouse melanoma (B16F10) cells with DHEA: DHEA treatment showed a significant decrease in cell growth between untreated control and DHEA-100 μM treated cells (P value of 0.000) and also differentiation of mouse melanoma cells (10× magnification). Similarly a significant decrease in cell growth was noticed between DHEA-10 μM and DHEA-100 μM treatment as shown by a P-value of 0.000.

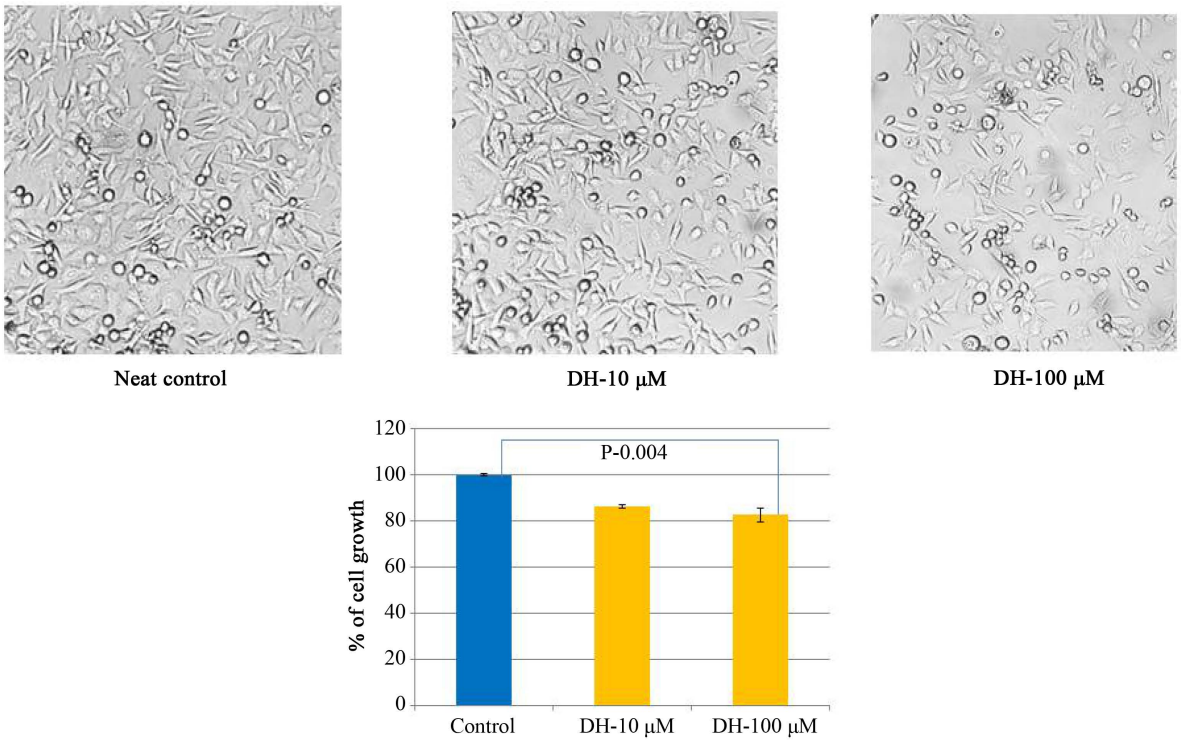

Figure 2. Treatment of human melanoma (BLM) cells with DHEA: There was no significant inhibition of human melanoma cell growth at 10 and 100 μM concentrations of DHEA (P-value 0.299) as shown in the picture. Though comparison of cell growth between untreated control and DHEA 100 μM treated cells showed a marginal effect on cell growth, yet it was statistically significant with a P-value of 0.04 by MTT assay.

cells to study the effect of DHEA on cell growth and morphology. Vascular smooth muscle cells were isolated from normal rat carotid artery and incubated with DHEA at 10 and 100 μM concentrations. After 48 hrs of incubation, DHEA decreased rat vascular smooth muscle cell growth slightly (86.6% at 100 μM conc.) as shown in **Figure 4**. Visually there were fewer cells in 100 μM DHEA treated group, but cells were larger in size. The larger size could be due to association of cells or differentiation of cells as reported earlier [31] [32]. Yet, there was no significant dose-dependent decrease in cell growth in DHEA treated cells, as shown by the quantitative MTT assay (**Figure 4**).

3.1.5. Effect of DHEA on 3T3-L1 Preadipocyte Cells

DHEA was anti-adipogenic and decreased fat synthesis [33] [34]. So, 3T3-L1 preadipocyte cells were treated with DHT 10 nM (as positive control) and DHEA as test hormone at 10 nM, 100 nM, 1 μM and 10 μM concentrations. After 10 - 12 days cells were stained with Oil red O stain and extracted with isopropanol for quantitation. Quantitation of adipogenesis showed a gradual decrease in adipogenesis from 10 nM to 10 μM as shown in **Figure 5**. DHEA decreased adipogenesis in 3T3-L1 preadipocytes to 79% at 10 μM conc. Decreases in adipogenesis were seen at 1 μM and 10 μM concentrations of DHEA compared to untreated control cells.

3.2. DHEA Anabolic Action

Effect of DHEA on myogenesis: Eventhough DHEA was shown to have anti-cancer, anti-adipogenic and anti-atherogenic actions on mouse and rat models, the underlying fact was DHEA was a weak androgen and an intermediate in testosterone biosynthesis. Also DHEA was an immediate precursor of androstenedione, another weak androgen, which was shown to promote myogenesis [35]. AD was recently banned as over the counter supplement because of its toxic side effects. So, the anabolic action of DHEA was checked by using *in-vitro*

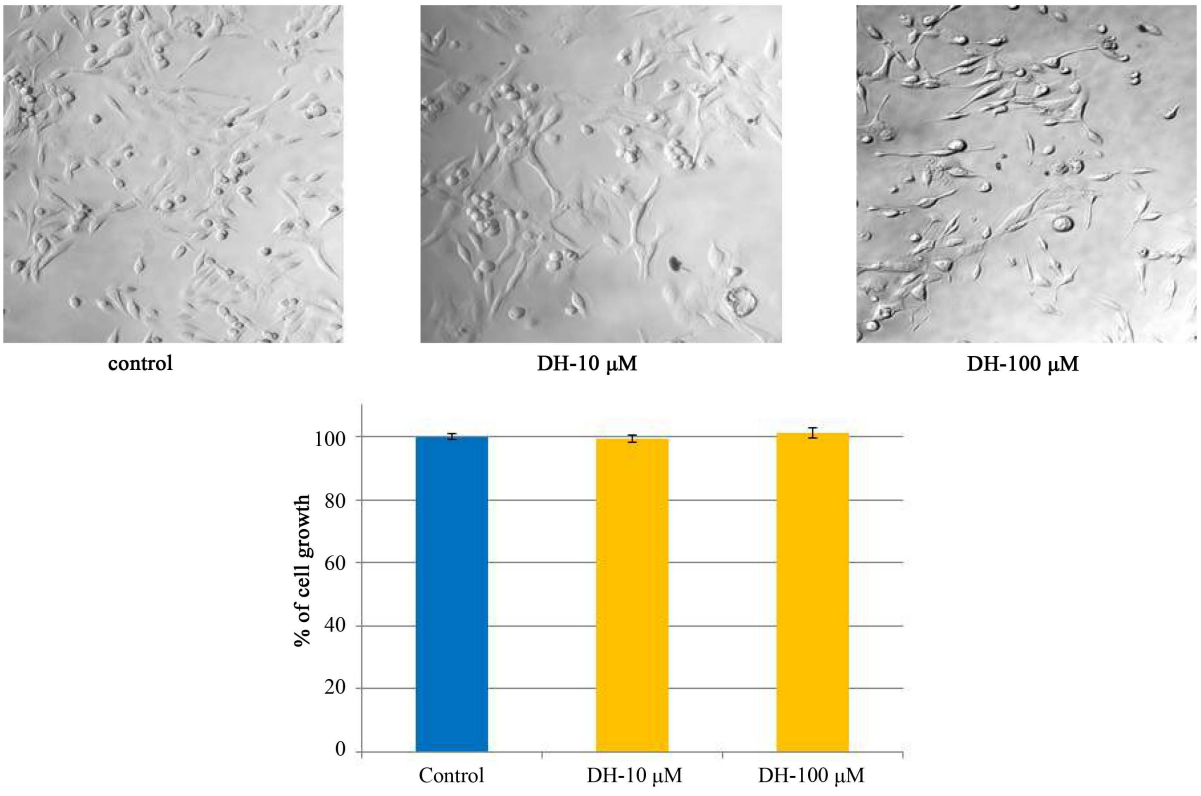

Figure 3. Treatment of human gastric cancer (NUGC3) cells with DHEA: In order to check the effect of DHEA on other cancer, we used human gastric cancer (NUGC3) cell line. DHEA did not show any effect on the growth of human gastric cancer cells even at 100 μM concentration. There was no difference in cell growth between control (untreated) and DHEA (10 and 100 μM) treated cells, as shown by the MTT assay.

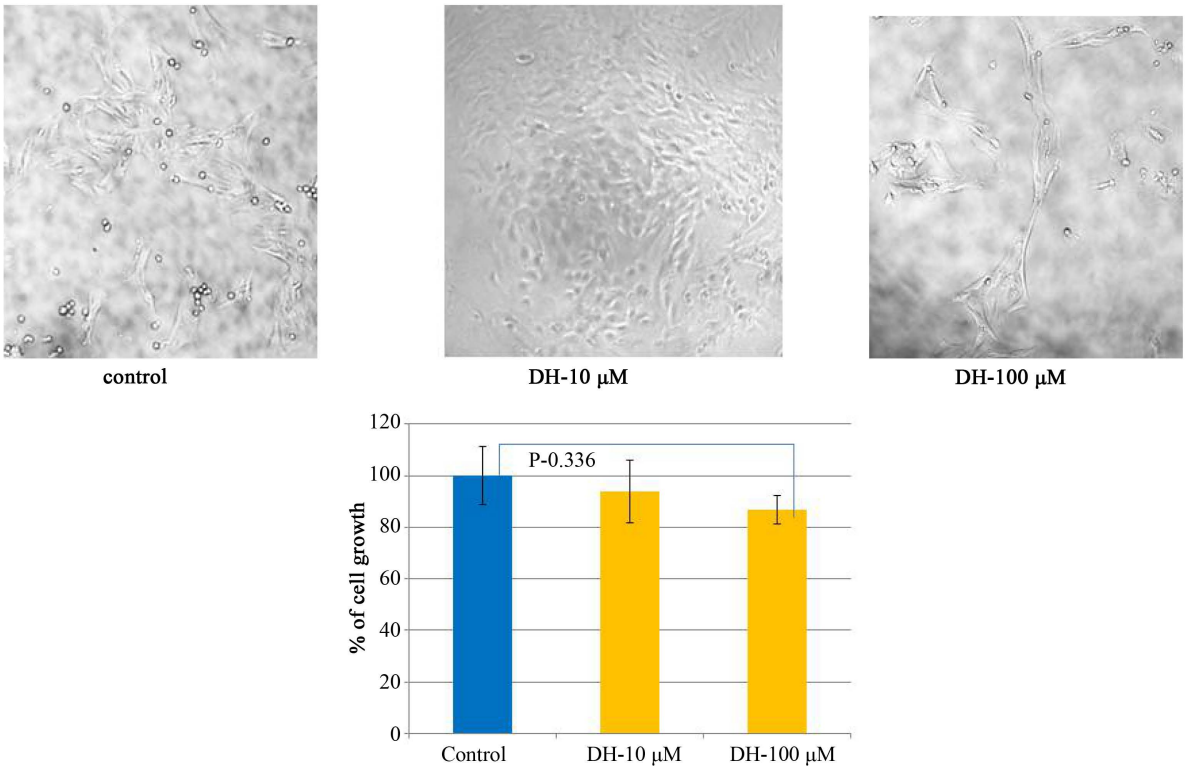

Figure 4. Treatment of rat vascular smooth muscle (VSMC) cells with DHEA: DHEA was shown to be anti-atherogenic by virtue of its action on vascular smooth muscle cells. So, we used normal rat sub-cultured vascular smooth muscle cells to check the effect of DHEA on smooth muscle cell growth. As reported, DHEA induced differentiation of smooth muscle cells and thereby caused a decrease in cell growth compared to the control cells. However, the difference in cell growth between control and DHEA-100 µM was not significant as shown by the P-value (0.336). Similarly, the difference in cell growth between DHEA-10 µM and DHEA-100 µM was not significant as given by the P-value of 0.621 by MTT assay.

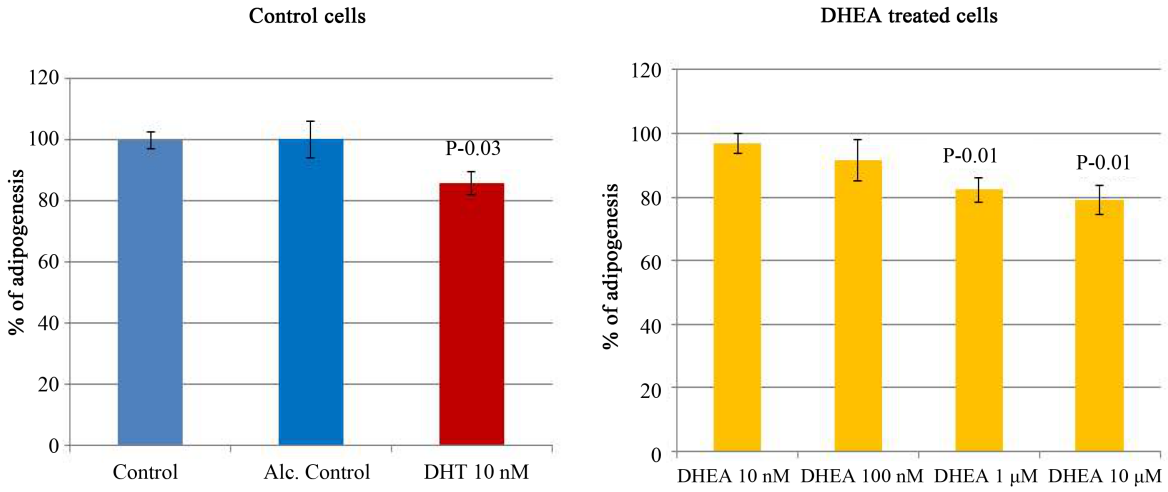

Figure 5. Colorimetric quantitation of adipogenesis in control and DHEA treated pre-adipocyte (3T3-L1) cells: DHEA effect on adipogenesis was checked using 3T3-L1 preadipcytes. Control panel cells showed no difference in growth between untreated and alcohol treated cells. However, with powerful androgen DHT (10 nM), adipogenesis was decreased when compared to control cell as shown by a P-value of 0.03. Though DHEA showed a dose-dependent decrease in adipogeneis, the decrease was not significant at DHEA-10 nM and DHEA-100 nM concentrations when compared to the control, as determined by the P-value (0.49 and 0.30 respectively). But, DHEA1 µM and DHEA-10 µM concentrations showed a significant decrease in adipogenesis when compared to the control as shown by the P-value of 0.01 and 0.01 respectively.

mesenchymal differentiation assay. This assay was used to demonstrate the anabolic action of powerful androgens T and DHT [35]. Since, DHEA was used in conjunction with T and DHT, where T concentrations were 30 and 100 nM and DHT concentration was 10 nM, we kept the maximum concentration of DHEA to 10 μM and did not try 100 μM concentration of DHEA in the anabolic and the adipogenic experiments. Mouse mesenchymal (10T1/2) cells were treated with DHEA 100 nM (low) and 10 μM (high) concentrations. After 10 - 12 days of treatment, cells were checked for myotube formation by carrying out immunohistochemical staining for myosin heavy chain IIb (MHCIIb) protein, terminal myogenic marker. As shown byimmunohistochmical staining for MHCIIb protein (**Figure 6(a)**), there were myogenesis in 100 nM and 10 μM DHEA treated cells. So DHEA stimulated myogenesis at low and high concentrations, just like the other weak androgen AD [35]. Biochemically myogenesis was confirmed by RT-PCR for MHCIIb RNA expression (**Figure 6(b)**). Reverse transcription PCR for androgen receptor (AR), showed the expression of AR (**Figure 6(b)**) following treatment with DHEA. AR expression in 10T1/2 cells was also shown in previous studies [35]-[37] following treatments with AD, DHT and T.

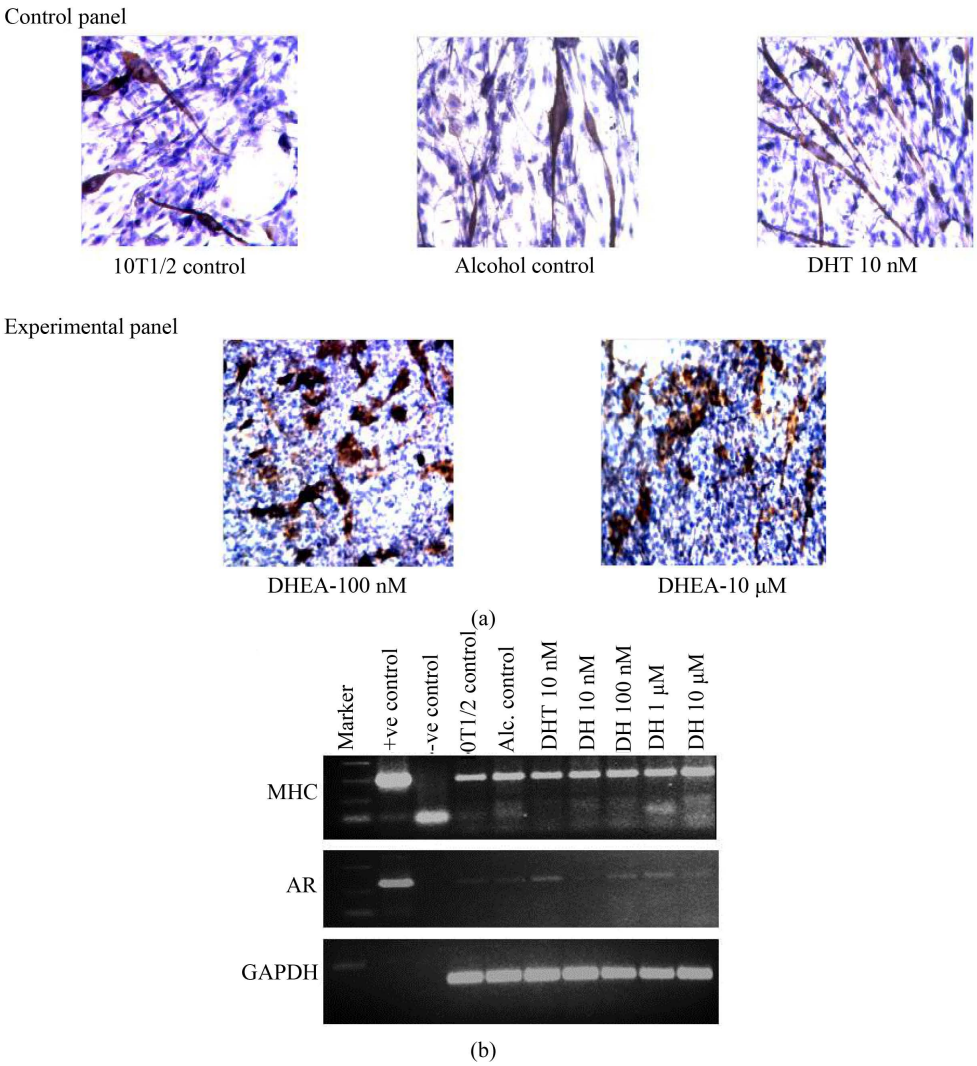

Figure 6. (a) Immunohistochemical staining for MHC-II protein after treatment of mouse mesenchymal (10T1/2) cells with DHEA: The anabolic effect of DHEA was checked using mouse mesenchymal multipotent (10T1/2) cells. The control panel showed an increase in myogenesis with powerful androgen DHT (10 nM). DHEA also stimulatedmyogenesis at low (100 nM) and at high (10 μM) concentrations, as indicated by the brown immunohistochemical staining. (b) RT-PCR for myogenic marker expression: Expression of biochemical marker for myogenesis, MHC-IIbRNA was shown by RT-PCR. In addition AR was also shown to be expressed on DHEA treated cells. GAPDH was used as control gene expression.

4. Discussion

Based on the beneficial effects of DHEA on small animals, people started taking DHEA as a nutritional supplement. But mouse was not a good model system to study the effect of DHEA, because in mouse very little DHEA was produced compared to human. DHEA was also a weak androgen and hence suspected to have anabolic action naturally associated with androgens. So our aim was to find out the *in-vitro* effect of DHEA on mouse, rat and human cell lines on its purported benefits and also to assess the anabolic effect of DHEA on myogenesis using an *in-vitro* mouse mesenchymal differentiation assay.

As DHEA was shown to be present in normal skin, its role in skin cancer (melanoma) was checked. DHEA anti-cancer action was checked on mouse and human melanoma cell lines. As expected DHEA showed a significant effect on mouse melanoma cell line at 100 μM concentration, but DHEA did not show any significant effect on human melanoma cell growth even at 100 μM concentration. This muffled effect of DHEA on target cancer cell line, prompted us to check the effect of DHEA on non-target gastric cancer cell line and also to check the prowess of DHEA in affecting cancer in general. DHEA did not show any effect on human gastric cancer cell line even at 100 μM concentration of DHEA. So far DHEA showed either muffled or no effect on human cancer cell lines.

DHEA was shown to have anti-atherogenic action by virtue of its effect on vascular smooth muscle cells and endothelial cells. So, we decided to study the effect of DHEA on normal rat vascular smooth muscle cells. As reported earlier [31] [32], DHEA was anti-proliferative and induced differentiation of smooth muscle cells. This was confirmed by the appearance of larger and branched cells at 100 μM concentration of DHEA. But, again it was another rodent (rat) cell, so DHEA effect was seen.

DHEA was shown to be anti-adipogenic based on its effect on 3T3-L1 preadipocytes, where DHEA showed a dose-dependent decrease in adipogenesis. DHEA anti-adipogenic effect was again seen on a mouse cell line.

So far the *in-vitro* experiments results supported human clinical trials data by showing a muffled or no effect on human cell lines (**Table 1**). As DHEA is a precursor of androstenedione, which was shown to have effect on muscle growth by using *in-vitro* mouse mesenchymal differentiation assay [35], it was important to check the anabolic action of DHEA also. Using this assay, we showed myogenesis upon addition of DHEA to 10T1/2 cells. Moreover, myogenesis was confirmed by the expression of terminal myogenic marker MHCIIb at the RNA and protein levels. Based on previous 10T1/2 studies [35]-[37], where expression of AR in 10T1/2 cells was correlated with the actions

Table 1. Summarizes anti-cancer, anti-atherogenic, anti-adipogenic and anabolic actions of DHEA on a variety of cell lines.

DHEA Experiments	Cell Lines		
	Mouse cell lines	Rat sub-cultured cells	Human cell lines
Effect on melanoma cell growth	Decreased mouse melanoma cell growth significantly		No significant decrease on human melanoma cell growth
Effect on gastric cancer cell growth			No effect at all on human gastric cancer cell growth
Effect on vascular smooth muscle cell growth		Modeerate decrease in cell growth due to differentiation of cells	
Effect on adipogenesis	Decreased 3T3-L1 preadiposcyte differentiation to fat cell and hence decreased adipogenesis		
Anabolic action	Promoted differentiation of mouse mesenchymal stem cell line 10T1/2 to myotubes		

of androgens (AD, DHT, T) mediated through AR, expression of AR after treatment with DHEA could be mediated through AR. Since, DHEA stimulation of myogenesis through AR and its structural similarity to testosterone, DHEA was anabolic in nature. Moreover, report of an earlier experiment with DHEA showed androgen-specific gene expression in the hypothalamus [22], indicating it was an androgen and also had anabolic action. Recently IOC, medical commission included DHEA in the list of banned chemicals and prevented athletes from using DHEA as a supplement [38] [39].

So to summarize, DHEA decreased mouse melanoma cell growth, whereas DHEA affected only marginally human melanoma cell growth. In addition, DHEA did not affect human gastric cancer cell growth even at 100 μM concentration. DHEA induced differentiation in rat vascular smooth muscle cell and decreased fat cell differentiation in 3T3-L1 pre-adiopcytes. DHEA also induced differentiation of mouse mesenchymal multipotent (C3H10T1/2) cells to myotubes. In our *in-vitro* studies DHEA was effective on rodents (mouse, rat) cell lines. However, DHEA was either marginally effective or not effective on human cell lines (**Table 1**). The reason for this difference could be due to the difference in the processing or metabolizing of DHEA inside the cells, because our unpublished data showed that atleast in mouse and human melanoma cell lines, DHEA actions were mediated through AR. Eventhough DHEA was a weak androgen, it still exhibited anabolic action as shown by the differentiation of mouse mesenchymal multipotent cells to myotubes. Further recent banning of DHEA use by athletes by the International Olympic Committee's (IOC) medical commission, citing DHEA as an anabolic steroid [38] [39], added credence to the above findings. Our *in-vitro* results supported the clinical trial data [17], which did not show any beneficial effect on humans. Thus we were able to show the differential biological effects of DHEA between rodents (mouse, rat) and humans at the cellular level itself.

5. Conclusion

In conclusion, *in-vitro* experiments not only supported human clinical trial data, but also showed that the differences in biological actions of DHEA between rodents and humans existed even at the cellular level. The ban on DHEA by IOC medical commission lent credence to the anabolic nature of DHEA, as demonstrated by the myogenic experiment. These *in-vitro* experiments using cells, thus paved the way for future study to check the mechanism of differences in biological actions of DHEA between rodents and humans at the cellular level itself. This *in-vitro* approach was emphasized in a recently published biotechniques newsletter [40], where the author Kristie Nybo wrote that dishes of cultured cells could provide new insights into the mechanisms of human diseases (citing advances in *in-vitro* approach to find a cure for Lou Gehrig disease).

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Abbreviation

DHEA—dehydroepiandrosterone
DHEAS—dehydroepiandrosterone-sulfate
DHT—dihydrotestosterone
T—testosterone
IOC—International Olympic Committee