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Effects of Different Doses of Doxorubicin on H9C2 Cells

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Abstract

Objective: To study the effect of different doses of doxorubicin on H9C2 cells and to provide a reference for the clinical study of doxorubicin. **Methods:** Doxorubicin (1, 2, 4, 6, 10 ug/ml) was co-cultured with H9C2 cells for 6, 12 and 24 hours. The morphological changes of cells were observed, and the cell inhibition rates of different time and drug concentration were calculated. **Results:** Doxorubicin could inhibit the activity of cardiomyocytes in a dose-dependent manner from 1 to 10 ug/ml. **Conclusion:** A certain dose of doxorubicin has a toxic effect on cardiomyocytes and can cause cardiomyocyte necrosis and apoptosis.

Keywords

H9C2 Cells, Doxorubicin, Cell Inhibition Rate

1. Introduction

Doxorubicin is an anthracycline widely used in chemotherapy of malignant tumors. However, it has obvious cardiotoxicity, which seriously restricts its clinical application. The study found that when the dose of doxorubicin *in vivo* reached 450 - 500 mg/m^2, the incidence of heart failure was 30%; When the dose reached 600 mg/m^2, the incidence of heart failure was as high as 70% [1], and reducing heart injury as much as possible has always been a concern. In this study, H9C2 cells derived from rat embryonic heart were used to establish an *in vitro* model of doxorubicin induced myocardial cytotoxicity, so as to provide a reference basis for the clinical study of doxorubicin.

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2. Experimental Reagent

Doxorubicin hydrochloride injection was purchased from Shanxi Pude Pharmaceutical Co., Ltd. Rat cardiomyocyte H9C2 was provided by the cell bank of Chinese Academy of Sciences. DMEM complete culture medium, fetal bovine serum and MTT were products of GIBCO.

3. Experimental Method

H9C2 cells were cultured in DMEM medium containing fetal bovine serum (10%), and cultured in a 37˚C and 5% CO_2 saturated humidity incubator. When the cells adhered to the wall and 80% fused, they were washed with PBS solution for 3 - 4 times, digested with 1 - 2 ml trypsin for about 0.5 - 1 min. Discarding the digestive fluid, adding DMEM culture medium with fetal bovine serum (10%), blowing with straw until the cells were completely dispersed, and inoculating them in 96 well culture plate. At this time, the cells were divided into control group and doxorubicin group. Doxorubicin group was given different concentrations of doxorubicin (1, 2, 4, 6, 10 ug/ml) and cultured in 37˚C and 5% CO_2 saturated humidity incubator for 6, 12 and 24 h respectively. After that, MTT solution (20 mg/ml) was added to each well and cultured with the cells for 4 h. Then the supernatant was discarded in each well. Dimethyl sulfoxide (DMSO) (150 ul) was added and shaked for 10 min to fully dissolve the crystals. Carry out quantitative optical density detection at the wavelength of 550 nm on the microplate reader to reflect the activity and quantity of cells. Cell inhibition rate (%) = (control group OD-doxorubicin group OD)/control group OD × 100%.

4. Statistical Methods

The experimental data were statistically analyzed by SPSS 20.0 software. The quantitative test data of each group were expressed as mean ± standard deviation ($x \pm s$). T test was used for the comparison between the two groups, and LSD test was used for the comparison between the two groups. The difference was statistically significant ($P < 0.05$).

5. Results

5.1. H9C2 Cells Inhibition in Different Concentrations of Doxorubicin and Different Action Time

The results showed that different concentrations of doxorubicin inhibited the activity of H9C2 cells. However, doxorubicin (6 ug/L and 10 ug/L) significantly inhibited the growth of H9C2 cells; The changes of growth inhibition after 12 - 24 hours were the more obvious. The experimental results were shown in **Table 1**.

5.2. Effects of Different Doses of Doxorubicin on Cardiomyocyte Morphology

The normal cardiomyocytes in the control group showed spindle shape, the

Table 1. Inhibition rates of doxorubicin at different concentrations and action time on cells (%).

	Doxorubicin concentration (ug/L)	Doxorubicin action time (h)		
		6	12	24
Control group	0	0	0	0
Doxorubicin group	1	1.59 ± 2.26	5.89 ± 3.65	11.25 ± 2.69
	2	3.1 ± 1.61	11.45 ± 3.12	15.67 ± 5.1
	4	4.58 ± 2.65	12.19 ± 2.69	19.61 ± 2.14
	6	8.12 ± 2.51*	21.45 ± 2.78*	31.71 ± 2.56*
	10	12.58 ± 1.41*	25.61 ± 3.12*	41.25 ± 5.56*

Compared with the control group *P < 0.05.

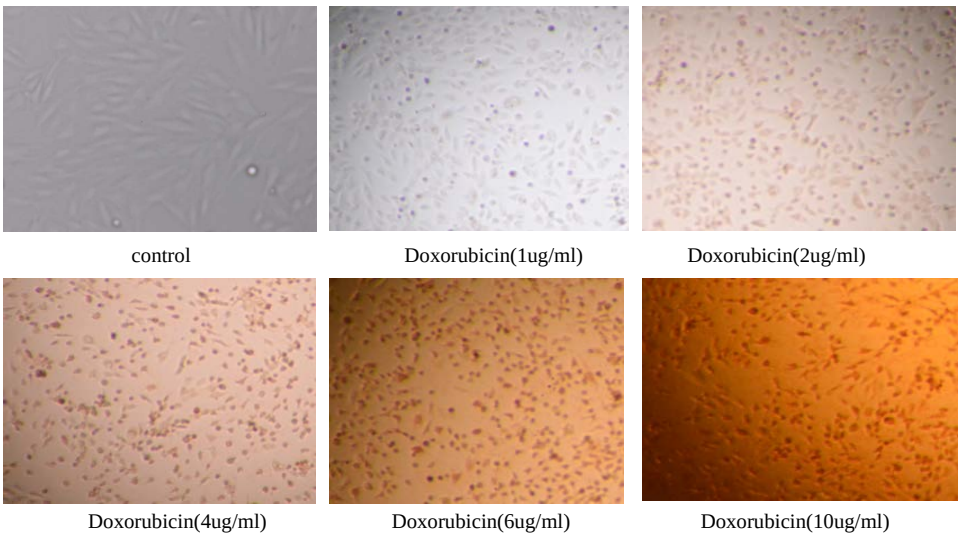

Figure 1. Morphological changes of cardiomyocytes after 12 hours of doxorubicin (×100).

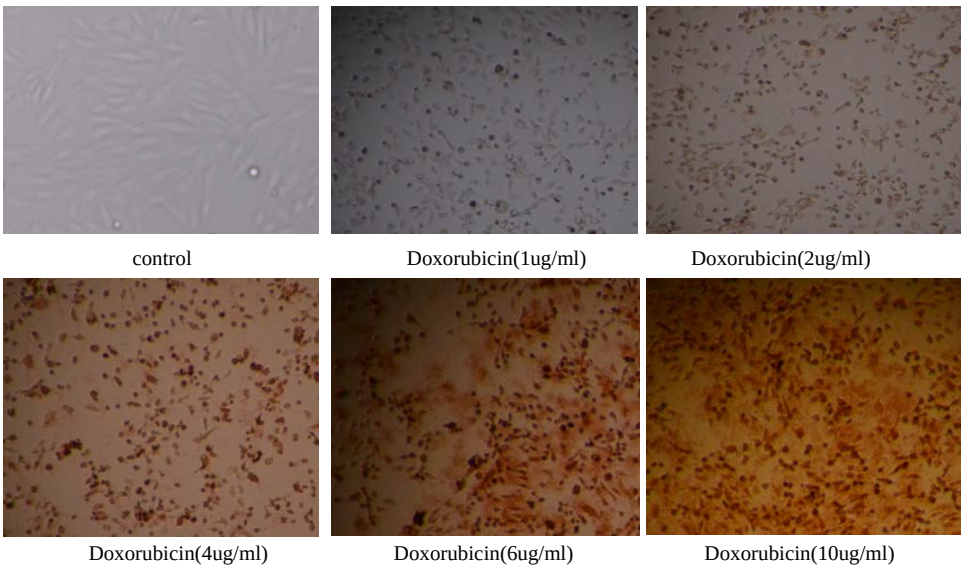

Figure 2. Morphological changes of cardiomyocytes after 24 hours of doxorubicin (×100).

cardiomyocyte structure was normal, the cell membrane was complete, and the cells connected with each other. In the doxorubicin group, cardiomyocytes showed round shrinkage, cell junctions decreased, pseudopodia disappeared, cells swelled and even died. With the extension of action time and the increasing doxorubicin concentration, the number of death cells increased (**Figure 1**, **Figure 2**).

6. Discussion

Doxorubicin which had a wide anti-tumor spectrum, high clinical efficacy and the same effect on hypoxic cells, was widely used as a first-line anticancer drug in the treatment of solid tumors such as breast cancer, liver cancer and prostate cancer malignancies [2]. But it was toxic to the heart. At present, the exact mechanism of doxorubicin induced cardiotoxicity was not clear. There were traditional views such as free radical injury, mitochondrial injury, abnormal energy metabolism, calcium overload, apoptosis, cell atrophy and so on [3]. However, it was generally believed that it was related to the production of excess oxygen free radicals. Inhibiting or eliminating excess oxygen free radicals would help to reduce the cardiotoxicity of doxorubicin [4].

MTT assay was the preferred method for detecting the sensitivity of anticancer drugs by NCI. The *in vitro* results of this study were consistent with the reported *in vivo*: a certain concentration of doxorubicin had obvious inhibitory effect on cardiomyocytes, and the change of growth inhibition was more obvious in 12 - 24 hours than others. This showed that doxorubicin induced myocardial injury was time-dependent, and the longer the time, the more serious the injury. The data provided by this experiment showed that doxorubicin had obvious dose-dependent damage to myocardium. These studies provided a reference for clinical medication. Doxorubicin induced cardiomyocyte necrosis or apoptosis was irreversible. Therefore, it was particularly important to use doxorubicin safely. This study also laid an experimental foundation for finding drugs that inhibit doxorubicin induced myocardial injury in clinic.

Conflicts of Interest

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

References

[1] Chen, Z.C., Chen, L.J. and Cheng, J.T. (2013) Doxorubicin-Induced Cardiac Toxicity Is Mediated by Lowering of Peroxisome Proliferator-Activated Receptor Delta Expression in Rats. *PPAR Research*, **4**, 456-461. https://doi.org/10.1155/2013/456042

[2] Oliveira, M.S., Carvalho, J.I., Campos, A.C., *et al.* (2014) Doxorubicin Has *In Vivo* Toxicological Effects on *Ex Vivo* Cultured Mesenchymalstem Ceils. *Toxicology Letters*, **3**, 380-386. https://doi.org/10.1016/j.toxlet.2013.11.023

[3] Raj, S., Francc, V.I. and Lipshultz, S.E. (2014) Anthracycline-Induced Cardiotoxicity: A Review of Pathophysiology, Diagnosis, and Treatment. *Current Treatment*

Options in Cardiovascular Medicine, ***16***, 315.
https://doi.org/10.1007/s11936-014-0315-4

[4] Pereira, G.C., Silva, A.M., Diogo, C.V., Carvalho, F.S., Monteiro, P. and Oliveira, P.J. (2011) Drug Induced Cardiac Mitochondrial Toxicity and Protection: from Doxorubicin to Carvedil. *Current Pharmaceutical Design*, **17**, 2113-2129.
https://doi.org/10.2174/138161211796904812