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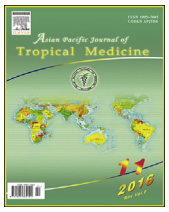

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Protective effect of antioxidant on renal damage caused by doxorubicin chemotherapy in mice with hepatic cancer

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ABSTRACT

Objectives: To investigate the protective effects and mechanism of antioxidant TBHQ on renal damage caused by doxorubicin chemotherapy in mice with hepatic cancer.

Methods: Cell H22 of mice with hepatic cancer which was subcultured for three times was subcutaneously transplanted to the groin of right lower limb of 45 SPF Kunming mice to establish the transplanted tumor model. The doxorubicin chemotherapy group and antioxidant intervention group received intraperitoneal injection of ADM (1 mg/kg·0.2 mL/2 d). The model control group received normal saline (NS) of the same volume at the same time. 1% TBHQ was added into the diet of mice of the antioxidant intervention group. Seven weeks later, morning urines and peripheral blood were randomly collected to detect UAlb, UCr, BUN, Scr and UAlb/Cr levels. All mice were beheaded. The renal tissues were made into homogenate, and SOD, T-AOC and MDA content in tissues were detected followed by cell lysis. All data were processed using SPSS19.0.

Results: The UAlb/Cr, BUN, Scr and MDA of doxorubicin chemotherapy group were significantly higher those of model control group and the activities of SOD, T-AOC in doxorubicin chemotherapy group were lower than those of model control group ($P < 0.01$). The UAlb/Cr, BUN, Scr and MDA of antioxidant intervention group were lower than those of doxorubicin chemotherapy group and the activities of SOD, T-AOC of antioxidant intervention group were higher than those of doxorubicin chemotherapy group doxorubicin chemotherapy group ($P < 0.05$). The BUN of model control group was higher than that of blank group, and T-AOC was lower than that of blank group, and difference was statistically significant ($P < 0.05$).

Conclusions: Doxorubicin chemotherapy could lead to abnormal antioxidant capacity and renal function of tumor-bearing mice with hepatic cancer. TBHQ antioxidant intervention could effectively improve the antioxidant capacity of renal tissue and reduce the renal damage caused by doxorubicin to some extent.

1. Introduction

Liver cancer, with mortality rate ranging from 20 per 100 000 to 30 per 100 000 in China, is one of the common malignant tumors in digestive system, only second to lung cancer among malignant tumors [1]. Doxorubicin chemotherapy serves as one of the routines in treating liver cancer clinically. Doxorubicin, killing cancer cells via inhibiting the replication of RNA and DNA to play its anti-tumor effect, is widely used as chemotherapeutics in the treatment of malignant tumors, such as liver cancer, breast cancer, ovarian cancer, gastric cancer, non-small cell lung cancer, prostate cancer, etc. [2–7]. However, the severe toxic and side effects of doxorubicin on human body have restricted its clinical application. Researches have shown that doxorubicin chemotherapy could generate severe tissue injury in heart and kidney [8,9], and the symptoms of renal

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damage like hematuresis and proteinuria are especially evident. Therefore, seeking clinical treatment to lower the side effects of doxorubicin chemotherapy and protect renal damage has been one of the priorities in recent researches.

One of the mechanisms of renal tissue injury caused by doxorubicin was the free radical it produced could cause lipid peroxidation of glomerulus which affected normal physiological function of renal tissues, thus generating metabolic disorders [10,11]. Therefore, one of the methods to reduce renal damage is to inhibit peroxidation. Tertiary butylhydroquinone (TBHQ) is a edible antioxidant with a safer non-toxic property compared with other antioxidants. Studies have indicated that the protective effect of TBHQ was realized through inhibition of peroxidatic reaction, inflammatory response and apoptosis [12]; however, the researches concerning its clinical applications are still immature. To provide theoretical reference and experimental bases for new type adjuvant chemotherapy, the study explored the effects of TBHQ prevention on renal damage caused by doxorubicin chemotherapy through establishing transplanted tumor model of H22 mice with hepatic cancer and conducting the experiments of doxorubicin chemotherapy and antioxidant prevention.

2. Materials and methods

2.1. Materials

A total of 60 SPF Kunming mice (half male and half female), aged 6–8 weeks and weighed 18–23 g, were purchased from the Laboratory Animal Center, Zhengzhou University. The study was performed after one-week adaptive feeding and the room temperature of feeding environment was kept between 21 °C and 25 °C and relative humidity between 35% and 50%. Hepatoma cell line H22 of abdominally transplanted mice was purchased from Shanghai Hanbo Biotechnology Co., Ltd., doxorubicin from Melone Pharmaceutical Co., Ltd., TBHQ, SOD, and xanthine oxidase from Sigma Co., T-AOC kit from Nanjing Jiancheng Bioengineering Institute. Beckmann fully automatic biochemical analyser (AU5821 version) was used as experimental instrument.

2.2. Methods

Cryopreserved cell H22 was transplanted into abdominal cavity of mice after thawing with 37 °C aqueous bath. After 5–7 days, substantial fluids were developed in the abdominal cavity. Cell H22 was subcultured for three times before collecting abdominal dropsy of hepatoma mice, which was diluted into cell suspension under the microscope with a cell population of (1.0–1.5) × 10^7 per mL. 0.2 mL cell suspension from each mouse among 45 randomly selected mice was subcutaneously transplanted to the groin of right lower limb. The tumor block grown subcutaneously after 1–2 days was regarded as a successful modeling.

Forty-eight hours after modeling, 45 tumor-bearing mice were randomly divided into three groups: model control group, doxorubicin chemotherapy group and antioxidant intervention group, with 15 mice in each group. The doxorubicin chemotherapy group and antioxidant intervention group received intraperitoneal injection of ADM (1 mg/kg·0.2 mL/2 d); the model control group received intraperitoneal injection of normal saline (NS) of the same volume at the same time. 1% TBHQ was added into the diet of mice of antioxidant intervention group. A total of 15 normal mice served as blank group. Seven weeks later, urines in the morning and peripheral blood (supernatant was collected after plasma centrifugation) were randomly collected and UAlb, UCr, BUN, Scr and UAlb/Cr were detected afterwards. All mice were beheaded. The renal tissues were made into homogenate, and SOD, T-AOC and MDA content in tissues were detected followed by cell lysis. MDA was detected using TBA, other indicators were operated in strict accordance with kit instructions. All data were processed using SPSS19.0 and demonstrated as $\bar{x} \pm \sigma$. *T*-test was conducted between two data sets, and difference was statistically significant when $P < 0.05$.

All experiments in this study are conducted in accordance with animal healthcare and user guide of Laboratory Animal Center of Zhengzhou University, Henan province and under the approval of local ethics committee, meeting criteria for laboratory animal care and application guidelines of US National Institutes of Health (Publication. 85-23, revised in 1985).

3. Results

Renal function indexes (BUN, Scr and UAlb/Cr) in mice of each group were shown in Table 1. BUN in model control group increased compared to blank group and the difference is significant, differences in other indexes showed no difference. BUN, Scr and UAlb/Cr of tumor-bearing mice underwent doxorubicin chemotherapy elevated significantly, indicating that doxorubicin chemotherapy would damage renal function. These indexes in mice receiving antioxidant intervention all decreased, indicating that antioxidant intervention to some extent could protect renal damage in mice with heptic cancer.

Oxide and antioxidant indexes (SOD, T-AOC and MDA) in mice of each group were shown in Table 2. As shown in Table 2, T-AOC in model control group reduced compared to blank group showing significant difference. The activity of SOD and T-AOC of tumor-bearing mice receiving doxorubicin chemotherapy declined while MDA content increased, demonstrating that doxorubicin chemotherapy would lower antioxidant capacity of mice and strengthen peroxidatic reaction. The activity of SOD and T-AOC increased after antioxidant intervention during chemotherapy and MDA content decreased or even reached to

Table 1
Comparisons of renal function indexes of mice among groups.

Group	BUN (mmol/L)	Scr (μmol/L)	UAlb/Cr (μg/μmol)
Blank group	9.86 ± 2.37	65.21 ± 5.98	1.74 ± 0.21
Model control group	10.21 ± 2.95[b]	67.85 ± 6.13	1.81 ± 0.35
Doxorubicin chemotherapy group	21.36 ± 5.32**	91.65 ± 10.35**	5.62 ± 0.93**
Antioxidant intervention group	15.21 ± 3.64**[a]	76.32 ± 8.64*[a]	2.42 ± 0.45*[a]

*$P < 0.05$ compared with model control group; **$P < 0.01$ for the comparisons with model control group, Statistically significant difference.
[a] Compared with doxorubicin chemotherapy group. [b] Compared with blank group.

Table 2
Comparisons of oxide and antioxidant indexes of mice among groups.

Group	SOD (U/mL)	T-AOC (U/mL)	MDA (nmol/g)
Blank group	216.52 ± 19.22	12.61 ± 4.13	4.98 ± 1.27
Model control group	212.61 ± 20.54	10.82 ± 3.56[b]	5.41 ± 1.68
Doxorubicin chemotherapy group	148.63 ± 12.35**	6.39 ± 2.16**	7.69 ± 2.12**
Antioxidant intervention group	186.76 ± 17.62**[a]	9.24 ± 2.85*[a]	6.13 ± 2.01[a]

*$P < 0.05$ compared with model control group, statistical difference; **$P < 0.01$ compared with model control group.
[a] Compared with doxorubicin chemotherapy group. [b] Compared with blank group.

normal level, demonstrating that TBHQ antioxidant could effectively improve antioxidant capacity and reduce peroxidatic reaction.

4. Discussion

Peroxidatic reaction was considered as one of the primary mechanisms of renal damage caused by doxorubicin chemotherapy [13]. In this study, transplanted tumor model of H22 mice with hepatic cancer was established, doxorubicin chemotherapy plus TBHQ prevention were conducted, antioxidant capacity indexes and renal function indexes were measured after treatment and prevention and the protective effect and mechanism of TBHQ prevention on renal damage caused by doxorubicin chemotherapy were explored. Results showed that BUN, Scr and UAlb/Cr in mice after receiving doxorubicin chemotherapy increased, with renal function damage present; the activity of T-AOC and SOD declined, MDA content elevated, antioxidant capacity decreased, Peroxidatic reaction strengthened. BUN, Scr and UAlb/Cr in mice underwent doxorubicin chemotherapy combined with TBHQ antioxidant intervention were lower than those of doxorubicin chemotherapy group, while antioxidant capacity and SOD activity were higher, indicating antioxidant capacity of tumor-bearing mice was improved and its renal damage was protected to some degree.

The mechanisms of renal damage caused by doxorubicin chemotherapy mainly consists of two aspects: one is direct damage. The general toxicity of doxorubicin on cells directly damaged renal cells, which produced inflammatory response in renal tissues and released such inflammatory factors as TNF-α and interleukin, hence strengthening the growth of renal matrix and glomerular sclerosis [14]. The other is indirect damage. Under the action of multiple reductase, doxorubicin produced free radical, induced lipid peroxidation in glomerular epithelial cell and caused abnormal glucose and protein metabolism; moreover, it brought about elevated urinary albumin through altering and breaking the structure and filterability of renal filtration membrane [15,16]. Most researches come to the conclusion that indirect damage predominates. Irregular glomerular epithelial cell, inflammatory response in renal matrix and nephrotic syndrome (urine protein, hematuresis, etc.) were present followed by renal damage which was caused by doxorubicin, according to the study of Carron PL *et al.* [17]. THBQ, as an antioxidant, is frequently used in the food industry. Researches had shown that TBHQ could activate Nrf2 and then activate the expressions of its mediated NQO1 and SOD before playing a protective role in cellular antioxidant reaction and inhibiting peroxidatic reaction, which played a part in tissue protection under the conditions of heart toxic damage, renal damage, stroke, brain trauma [18,19]. Therefore, it can be inferred that TBHQ inhibits lipid peroxidation as well as enhances antioxidant capacity of renal cells via activating the expressions of Nrf2 mediated SOD, thus reducing renal damage caused by doxorubicin chemotherapy to some degree. The application of TBHQ antioxidant in clinic and medicine at present are still immature, however, with gradually carried out researches and increasingly progress, solving this medical challenge can be expected soon.

Conflict of interest statement

We declare that we have no conflict of interest.

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