Experimental animal study of docetaxel combined with carboplatin in the treatment of retinoblastoma

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Abstract. The synergistic effects of docetaxel (DTX) combined with carboplatin in the treatment of retinoblastoma (RB) was explored in mouse RB xenografts compared with carboplatin alone and DTX alone groups. Retinoblastoma Y-79 cells ($4.0x10^7$/ml) were injected into the vitreous body of the right eye of mice to establish the mouse model of RB xenografts. Then the mice were randomly divided into 4 groups (n=30): DTX combined with carboplatin group (group A), carboplatin group (group B), DTX group (group C) and blank control group (group D). The changes in tumors, the survival time of mice, and the synergistic effects of DTX combined with carboplatin were observed and analyzed. The diameters and weight of the right eyeballs of the Institute for Cancer Research (ICR) mice were significantly larger and higher than those of the left eyeballs in each group, respectively (P<0.05). The diameters and weight in group A were significantly shorter and lighter than those in the other three groups, respectively (P<0.05), and there was no significant difference compared with that of normal eyeballs (P>0.05). There was no difference in diameter and weight between group B and group C (P>0.05), but the diameters and weight were shorter and lighter than those in group D, respectively (P<0.05). The survival time of ICR mice in groups A, B and C was significantly longer than that in group D (P<0.05). The survival time in group A was significantly longer than that in groups B and C (P<0.05). There was no significant difference in the survival time between the group B and group C (P>0.05). DTX, carboplatin and the combination of the two have significant inhibitory effects on RB; however, DTX combined with carboplatin has a better therapeutic effect on RB.

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Introduction

Retinoblastoma (RB) is a common cancer in ophthalmology caused by the mutation of the *RB1* gene, and it has been widely investigated in recent years (1). RB is more common in children, and the incidence rate is 7-25%. Approximately 70% of the patients develop a unilateral eye tumor. It is the first tumor found to have a genetic basis (2), and approximately 40% of RB is hereditary (3). Although the survival rate of RB patients is very high, its mortality cannot be ignored, because RB is easily complicated with other malignant tumors. Therefore, radiotherapy is often avoided in the treatment (4).

Drug treatment is a good direction. Docetaxel (DTX) is a second-line therapy for some tumors, and it can effectively prolong the survival period of patients, with less side effects (5). Platinum drugs, such as carboplatin, can destroy cancer cells by inducing double-stranded deoxyribonucleic acid (DNA) breaks (6).

In this study, effects of DTX combined with carboplatin treatment on the survival of RB mice were explored by establishing RB mouse models.

Materials and methods

Research objects. ICR mice, grade CL were purchased from Better Biotechnology Co., Ltd. (Nanjing, China). Shuke and Beita rat feed of specific-pathogen-free (SPF) grade was purchased from Jiangsu Xietong Organism Co., Ltd. (Nanjing, China) for feeding. ICR mice were aged 9-11 weeks and weighing 15-25 g. The animals had free access to food and water at room temperature of 21±2˚C and humidity of 30-70%, with fluorescent lighting; the feeding box was replaced weekly 1-2 times, and the bottle was replaced weekly 1-2 times. DTX was purchased from Shanghai Shifeng Biological Technology Co., Ltd. (Shanghai, China); carboplatin was purchased from Shenzhen Simeiquan Biotechnology Co., Ltd. (Shenzhen, China); and retinoblastoma Y-79 cell lines were purchased from the Institute of Basic Medicine, Chinese Academy of Medical Sciences.

Establishment of ICR mouse models. Establishment of ICR mouse models referred to the modeling methods of Corson *et al* (7). ICR mice received intraperitoneal anesthesia

Table I. Basic conditions of the four groups of mice.

	Group A	Group B	Group C	Group D	F value	P-value
Age (weeks old)	8.1±0.4	8.5±0.2	8.4±0.3	8.6±0.1	0.632	0.899
Body weight before the modeling (g)	19.4±1.3	16.5±1.8	14.2±1.5	17.1±1.2	0.512	0.645
Body weight after the modeling (g)	29.8±1.2	22.4±1.3	27.6±2.5	26.1±1.6	0.589	0.785

with 50 mg/kg pentobarbital sodium; retinoblastoma Y-79 cell lines were prepared into a cell suspension at a concentration of $4.0x10^7$/ml and injected into the vitreous body of the right eyes of ICR mice to establish mouse models of RB xenografts; lincomycin hydrochloride and erythromycin were used to reduce inflammation after operation; and observation was conducted for 7 days. The intraocular conditions of mice were recorded daily, and the success of models was determined through pathological diagnosis.

Treatment methods. A total of 120 RB ICR mouse models were successfully established and divided into four groups, A, B, C and D (n=30) by random number table method. Group A received DTX combined with carboplatin treatment, group B received DTX alone treatment, group C received carboplatin alone treatment, and group D was given normal saline. DTX, carboplatin and normal saline, all with a dose of 10 mg/kg, were injected into the caudal vein, once a day for one week.

Observation indexes. Among 150 ICR mice, a total of 120 ICR mice were pathologically diagnosed with positive RB. The survival time of ICR mice was observed. After a 1-week administration, the ICR mice were sacrificed with carbon dioxide (CO_2), both eyes were collected, and the size and weight of tumor were observed.

Statistical analysis. Statistical Product and Service Solutions (SPSS) 19.0 Asia Analytics (formerly SPSS China) was used for statistical analysis. The χ^2 test was used for comparison of the rates. Measurement data were expressed as mean ± SD, and t-test was used for pairwise comparisons. Analysis of variance and Dunnett's post-hoc test was used for comparisons among multiple groups. P<0.05 indicated that the difference was statistically significant.

Results

General data. The selected mice were healthy ICR mice of grade CL, and they were uniformly fed with Shuke and Beita rat feed of SPF grade. Among the 150 ICR mice, 120 mouse models were successfully established, the mortality rate was 20%. The 120 ICR mice with successful models had no difference in body weight (F=1.225, P=0.48) before and after the modeling. The average age of ICR mice was 10.5±0.4 weeks (Table I).

Examination of eyeball diameter of ICR mice. After injection of retinoblastoma-79 cells for 2 days, the right eyeballs of ICR mice were slightly protuberant compared with the left eyeballs; 4 days after the modeling, the right eyeballs of ICR mice became more protuberant; and 7 days later, the right eyeballs were protruding outside the eye sockets. Seven days after the modeling, ICR mice were sacrificed with CO_2, and both eyes were collected. Diameters of the right eyeballs in ICR mice were significantly larger than those of the left eyeballs (P<0.05), diameters of the affected eyeballs in group A were significantly shorter than those in the other three groups (P<0.05), and there was no significant difference compared with normal left eyeballs (P>0.05). Diameters in group B and group C were not different from each other (P>0.05), but shorter than those in group D (P<0.05) (Fig. 1).

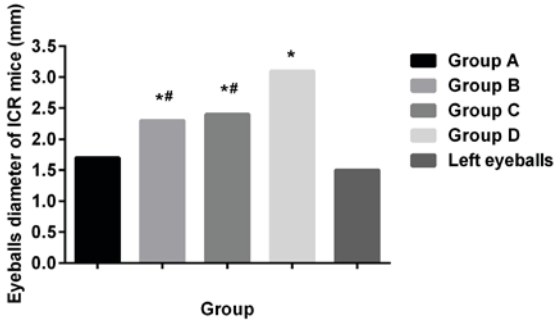

Figure 1. Examination of eyeball diameter of ICR mice. Seven days after the modeling, ICR mice were sacrificed with CO_2, and both eyes of the mice were collected. Diameters of the right eyeballs in ICR mice were found significantly larger than those of the left eyeballs (P<0.05), diameters of the affected eyeballs in group A were significantly shorter than those in the other three groups (*P<0.05), and there was no significant difference compared with normal left eyeballs (P>0.05); diameters in group B and group C were not different from each other ($^\#$P>0.05), but shorter than those in group D (P<0.05).

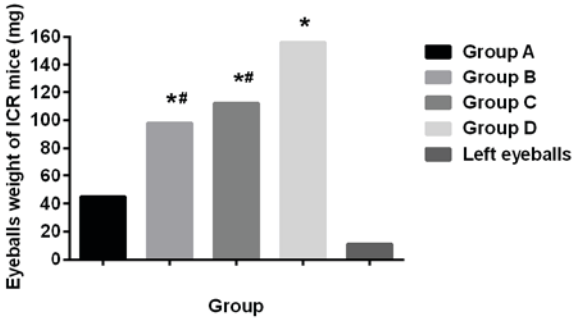

Figure 2. Comparisons of eyeball weight of ICR mice. The weight in group A was less than that in the other three groups (*P<0.05), and there was no significant difference compared with normal eyeball weight (P>0.05). There was no difference in weight between groups B and C (P>0.05), but the weight was less compared with that in group D ($^\#$P<0.05).

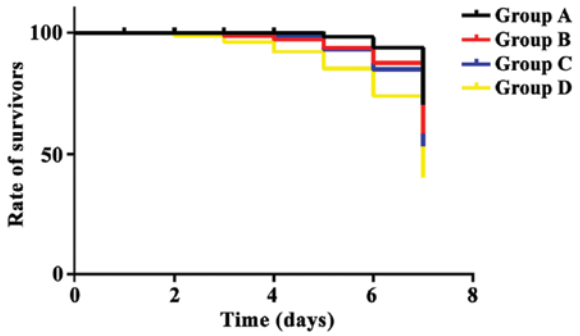

Figure 3. Comparisons of the survival time in ICR mice after treatment by Kaplan-Meier survival analysis and a log rank test. The survival time of ICR mice in group D was shorter than that in the other three groups (P<0.05), the survival time of ICR mice in group A was significantly longer than that in groups B and C (P<0.05). There was no significant difference in the survival time of mice between groups B and C (P>0.05).

Comparison of eyeball weight of ICR mice. The weight of the right eyeballs of ICR mice was significantly heavier than that of the left eyeballs (P<0.05). The weight of the affected eyes of ICR mice the groups A, B, C and D was different, the weight in group A was lighter than that in the other three groups (P<0.05), and there was no significant difference compared with normal eyeball weight (P>0.05); there was no difference in weight between group B and group C (P>0.05), but the weight was lighter compared with that in group D (P<0.05) (Fig. 2).

Comparison of the survival time in ICR mice after treatment. The survival time of ICR mice in groups A, B and C was significantly longer than that in group D (P<0.05); the survival time of ICR mice in group A was significantly longer than that in group B and group C (P<0.05). There was no significant difference in the survival time of mice between groups B and C (P>0.05) (Fig. 3).

Discussion

RB is a very common primary malignant tumor among minors in ophthalmology (8). With the extension of the course of disease, the risks of bone tumors, soft tissue sarcoma and melanoma are also increasing (9,10). RB also becomes more harmful, so how to control and even cure RB is a very important issue.

In this study, RB ICR mouse models were established to explore the effects of DTX combined with carboplatin in the treatment of RB. All the mice selected in this study were of grade CL. Its advantages are that it avoids ethical disputes, has a wide source of materials, and avoids drug damage to patients.

In this study, ICR mice were given DTX treatment, carboplatin treatment, and combined treatment of the two, respectively. It was found that both DTX and carboplatin could prolong the survival time of ICR mice; however, DTX combined with carboplatin had a better effect on the prolongation of the survival time of mice. DTX has a significant antitumor activity. It can inhibit expression of cyclin dependent kinase 4 (CDK4), cyclin D1 and cyclin E1, induce low phosphorylation of RB, and block the transformation of cells in G0/G1 to S phase (11). Carboplatin is a common second-generation platinum chemotherapy drugs, and it is also a non-specific antitumor drug applied by injection. It destroys the cytotoxicity of DNA and hinders the development of tumors (12). Our results show that the weight and diameters of the affected eyes were all improved after treatment with DTX and carboplatin, which was more obvious in the mice treated by DTX combined with carboplatin, indicating that the efficacy of DTX, carboplatin and the combination of the two in RB are confirmed.

In recent years, there are some reports on the therapeutic effects of DTX in RB. The study of DTX in non-small cell lung (13), breast (14) and prostate cancer (15) is widely reported, similarly to Carboplatin (16-18). In a study by Li and his colleagues (19), it was found that although DTX combined with cisplatin is more effective than monotherapy in the treatment of non-small cell lung cancer, combination therapy produces more frequent side effects, such as anemia, thrombocytopenia, nausea and vomiting. This problem was also found in patients with prostate cancer in the treatment with DTX combined with carboplatin by Bouman-Wammes and his colleagues (20). Changes in blood parameters of mice were not collected in this study, so they could not be compared. It will be the direction of our research in the next study, and we also need large clinical data to support us. Therefore, although DTX combined with carboplatin is more effective in the treatment of RB, its safety remains to be studied.

The therapeutic effects of DTX, carboplatin and the combination of the two in the treatment of RB are worthy of recognition. They can effectively prolong the survival time of mice, but their safety remains to be studied.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

CS conceived and designed the study, and drafted the manuscript. CS and QZ established ICR mouse models, and analyzed and interpreted observation indexes. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Weihai Municipal Hospital (Shandong, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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