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ORIGINAL ARTICLE

# Damage to Wistar rat livers after hypo-fractionated radiation and oxaliplatin

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## Abstract

**Objective** The purpose of this study was to clarify whether hypo-fractionated radiation therapy combined with oxaliplatin can aggravate liver damage, in order to determine its safety for clinical application.

**Methods** Eighty Wistar rats were randomly divided into four groups: the control group, the chemotherapy treatment group, the radiation treatment group, and the concurrent chemoradiotherapy group. The rats' liver tissues were then collected for histological evaluation at the first, second, fourth, sixth, and eight week after irradiation. The tissues were histologically evaluated using hematoxylin and eosin staining, and immunohistochemistry to analyze the expression of Bcl-2 and Bax.

**Results** Histological examination revealed swollen hepatocellular cells in the experimental groups, with visible liver degeneration and necrosis. Alanine aminotransferase and aspartate aminotransferase levels were significantly different between the groups ($F$ = 85.869 and 214.663; $P$ < 0.001). The intra-group expressions of Bcl-2 and Bax were also significantly different between each time point ($F$ = 6.047 and 43.344; $P$ < 0.05). Bax expression was significantly different between each group ($F$ = 8.122; $P$ < 0.05), although no inter-group differences were observed for Bcl-2 expression ($F$ = 0.808; $P$ > 0.05).

**Conclusion** Chemoradiotherapy may aggravate liver injury, possible via overexpression of Bcl-2 and reduced expression of Bax. Therefore, this treatment should be used carefully in the clinic.

**Key words:** radiation effects; concurrent chemoradiotherapy; rat liver; Bcl-2; Bax

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Concurrent chemoradiotherapy is a standard treatment for many malignant tumors, and can improve the curative effect and prolong the survival period [1]. However, compared to radiotherapy or chemotherapy alone, concurrent chemoradiotherapy can expand the injured area in the irradiated tissue, and even result in severe complications. Nevertheless, the development of precise radiotherapy technology [e.g., stereotactic body radiation therapy (SBRT)] has increased the clinical use of radiotherapy for liver tumors [2–3]. Furthermore, liver tissues can be irradiated by different degrees, with the stomach, pancreas, and other abdominal organs involved during radiotherapy. In this context, the liver is the largest substantive gland and an important metabolic organ, which has many important and complex physiological functions [4]. In addition, previous radiation experiments in animals have suggested that liver is a radiosensitive organ that can be easily injured [5]. However, these studies were mainly focused on the conventional fractionated radiotherapy, and the effects of concurrent chemoradiotherapy are not well understood. Therefore, hypo-fractionated radiation therapy and concurrent chemotherapy were used in this study to assess the resulting damage to rat livers.

## Materials and methods

### Animals and grouping

Eighty male Wistar rats (5 weeks old, weighing 200 ± 20 g) were obtained from the Qingdao Municipal Institute for Drug Control (China). All experiments were approved by the Animal Care and Use Committee of Qingdao University Medical College. The rats were housed five per cage, were provided food and water ad libitum, and were acclimatized for 10 days before the treatment. The animals then were randomly assigned into four groups (n = 20, each): the control group (Group A), the chemotherapy group (Group B), the radiotherapy group (Group C),

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Table 1 Mean alanine aminotransferase levels (μmol/L) according to group and week

| Groups | 1st week | 2nd week | 4th week | 6th week | 8th week |
|---|---|---|---|---|---|
| A | 46.92 ± 2.94 | 48.25 ± 2.06 | 50.00 ± 7.61 | 47.06 ± 2.83 | 48.95 ± 2.30 |
| B | 67.00 ± 10.65 | 71.80 ± 8.22 | 82.00 ± 11.89 | 79.80 ± 10.06 | 77.60 ± 11.40 |
| C | 75.01 ± 12.55 | 82.20 ± 11.92 | 83.20 ± 9.31 | 85.00 ± 10.49 | 97.00 ± 11.30 |
| D | 103.40 ± 15.64 | 110.80 ± 14.49 | 112.40 ± 14.39 | 108.40 ± 15.85 | 115.40 ± 13.68 |

Table 2 Mean aspartate aminotransferase levels (mmol/L) according to group and week

| Groups | 1st week | 2nd week | 4th week | 6th week | 8th week |
|---|---|---|---|---|---|
| A | 167.64 ± 20.39 | 178.18 ± 26.23 | 168.31 ± 23.47 | 176.52 ± 19.53 | 167.32 ± 20.44 |
| B | 252.85 ± 25.47 | 213.12 ± 26.33 | 216.48 ± 23.76 | 198.06 ± 19.40 | 198.39 ± 21.05 |
| C | 306.42 ± 30.61 | 298.46 ± 29.49 | 311.42 ± 30.48 | 323.33 ± 40.50 | 320.91 ± 31.04 |
| D | 351.70 ± 41.60 | 365.51 ± 39.76 | 377.74 ± 31.42 | 378.44 ± 36.17 | 373.32 ± 33.28 |

and the concurrent chemoradiotherapy treatment group (Group D).

### Experimental procedure

All the rats were anesthetized using an intraperitoneal injection of 10% chloral hydrate (350 mg/kg) before the treatment. We then placed the fixed rats on a plank and selected the radiation field (4.0 × 2.0 cm), which was defined as from the xiphoid process to the iliac crest. The radiation parameters were X-ray beam energy of 6 MV, a dose of 12 Gy, a source-surface distance of 100 cm, and a depth of 1.5 cm (using a 0.5-cm tissue compensator). The control group received an equal-volume intraperitoneal injection of saline and false irradiation. The chemotherapy group received oxaliplatin (15 mg/kg) via intraperitoneal injection. The radiotherapy group received 12 Gy of 6-MV X-ray radiation at the disposable abdomen. The concurrent chemoradiotherapy group received both treatments, and all data were available for the preliminary experiments.

### Sample collection

We randomly selected 4 rats from each group at the end of the first, second, fourth, sixth, and eighth weeks. A 3-mL venous blood sample was obtained for each rat, which was promptly taken to the analytical laboratory for testing of the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels via an automatic biochemical analyzer. In addition, the livers were immediately dissected and washed with a physiological saline solution for the subsequent experiments. The liver tissues were then fixed in 10% neutral buffered formalin, dehydrated conventionally, embedded in paraffin sections, and stained with hematoxylin and eosin. The slides were then examined via light microscopy to evaluate any histological changes. The expression of Bcl-2 and Bax was detected via enzyme-linked immunoassay using an anti-rat Bax kit. The cytoplasm of Bcl-2-positive cells exhibited uniform diffuse claybank expression, while Bax-positive cells stained yellow or claybank in the cytoplasm and some nuclei. We randomly selected five fields from each slice via microscopy (400 ×), counted 200 cells per field of vision, and then calculated the percentage of positive cells.

### Statistical analysis

All statistical analyses were performed using SPSS software (version 17.0, SPSS Inc., USA). All data were compared using analysis of variance. Differences with a $P$-value of $<0.05$ were considered statistically significant.

## Results

### The morphological and histological changes in rat livers

Visual inspection revealed that control group livers were pink, soft, and had a smooth surface, while the experimental group livers were dark red and exhibited poor elasticity. Microscopy revealed that the hepatic lobules in the control group exhibited a clear structure and rows of hepatic cords. However, the hepatocytes in the experimental groups were swollen, degenerated, and necrotic. Compared to the other experimental groups, the most serious liver injury was observed in the chemoradiotherapy group (Fig. 1).

### Liver function according to blood biochemistry findings

The liver function results were shown in Tables 1 and 2. Although no intra-group differences in ALT and AST levels were observed ($F = 0.115$ and 0.003; $P > 0.05$), the inter-group differences in liver function at the same time points were significantly different ($F = 85.869$ and 214.663; $P < 0.001$).

### Comparison of Bcl-2 and Bax expression

The cytoplasm of Bcl-2 positive cells exhibited uniform diffuse claybank expression, while the cytoplasm

Table 3 Comparison of Bcl-2 protein expression (%)

| Groups | 1st week | 2nd week | 4th week | 6th week | 8th week |
|---|---|---|---|---|---|
| A | 9.94 ± 0.79 | 10.06 ± 1.63 | 9.40 ± 3.04 | 9.47 ± 2.14 | 9.80 ± 1.63 |
| B | 12.00 ± 2.55 | 11.60 ± 1.82 | 10.80 ± 1.30 | 8.40 ± 1.81 | 7.20 ± 1.33 |
| C | 11.80 ± 2.39 | 10.51 ± 1.51 | 9.07 ± 1.87 | 7.00 ± 2.10 | 7.00 ± 1.48 |
| D | 15.60 ± 2.07 | 13.01 ± 1.41 | 11.31 ± 0.92 | 9.60 ± 1.12 | 6.60 ± 2.37 |

Table 4 Comparison of Bax protein expression (%)

| Groups | 1st week | 2nd week | 4th week | 6th week | 8th week |
|---|---|---|---|---|---|
| A | 3.42 ± 0.64 | 3.60 ± 1.14 | 4.40 ± 0.17 | 3.40 ± 1.67 | 4.20 ± 1.07 |
| B | 5.01 ± 1.55 | 5.97 ± 0.72 | 8.00 ± 1.58 | 10.60 ± 2.04 | 13.80 ± 1.77 |
| C | 5.40 ± 1.14 | 6.10 ± 0.41 | 7.80 ± 1.59 | 8.60 ± 2.17 | 10.20 ± 1.92 |
| D | 7.80 ± 1.19 | 9.1 ± 1.05 | 13.15 ± 1.54 | 15.00 ± 1.51 | 16.00 ± 1.30 |

and some nuclei of Bax-positive cells were stained yellow or claybank. The Bcl-2 expression declined over time in each experimental group ($F = 6.047$; $P < 0.05$), although there were no inter-group differences at the same time points ($F = 0.808$; $P > 0.05$). In contrast, Bax expression was highly upregulated, with significant inter-group differences at the same time points ($F = 8.122$; $P < 0.05$), and significant intra-group differences over time ($F = 43.344$; $P < 0.001$; Tables 3 and 4).

## Discussion

Concurrent chemoradiotherapy is a form of systematic treatment for malignant tumors, and is the basic principle for cancer treatment [6]. Furthermore, concurrent chemoradiotherapy can improve the tumor control rate and improve the prognosis, and so it has become one of the most common cancer treatment modalities [7–8]. In this context, the hypo-fractionated radiation therapy pattern can be used to increase the split single dose and shorten the overall treatment period [9], whereby each dose of hypo-fractionated radiation is > 2 Gy and is administered < 5 times per week (typically 2–3 times per week). This pattern can kill cancer and anaerobic cells, thereby reducing tumor cell repopulation and improving the local tumor control rate [10–11]. However, this pattern also inevitably increases the incidence of normal tissue damage, and it is clinically important to limit normal tissues damage [12]. Therefore, the resulting effects on normal tissue have limited the widespread use of this radiation therapy.

Hepatic carcinoma is one of the most common malignant tumors in China [13], and this tumor has a particularly poor prognosis, with a 5-year survival rate of only approximately 7% and the second highest mortality rate among all tumors. Personalized comprehensive treatment has become the standard method for managing liver cancer, and advances in radiation physics, radiation biology, and computer and medical imaging technology have increased the clinical use of SBRT and other advanced precision radiotherapy techniques [14]. Furthermore, Bujold *et al* [15] have reported that SBRT and other low-intensity radiotherapies are a promising treatment modality, because they are relatively safe, with good patient tolerance. In addition, the third generation of platinum-based anticancer drugs (cisplatin, carboplatin, and oxaliplatin) [16] have resulted in significant progress in gastrointestinal cancer chemotherapy, and they have significantly improved patients' prognosis. The EACH study [17] was the first to demonstrate that oxaliplatin-based systemic chemotherapy was safe and effective for patients with liver cancer, with good tolerance and fewer side effects. Interestingly, the liver is the organ that limits the therapeutic intensity of radiation, due to the related adverse effects, and the liver's tolerance of hypo-fractionated radiation therapy is considered a topic that is worthy of additional research [9]. However, very little current research has examined liver dosage during concurrent chemoradiotherapy. Therefore, we conducted this study using Wistar rats to examine the liver's morphological and pathological changes after abdominal X-ray hypo-fraction irradiation and oxaliplatin. Furthermore, we investigated the expression of Bcl-2 and Bax in these rats, in order to evaluate the related effects on the rats' livers, and to provide a suitable reference for the clinical use of concurrent chemoradiotherapy.

In this study, visual inspection revealed that the control rats' livers were pink and soft, while the experimental rats' livers were dark red and exhibited poor elasticity. Furthermore, microscopy revealed that the control groups' hepatic lobules exhibited clear structure and rows of hepatic cords, while the experimental groups' hepatic lobules exhibited varying degrees of cellular swelling, necrosis, and degeneration. Moreover, liver injury was significantly greater in the chemoradiotherapy group, compared to that in the other two experimental groups, which indicates that concurrent chemoradiotherapy increases liver injury.

In addition to our microscopic evaluation, we selected specific serological markers of liver function (ALT and

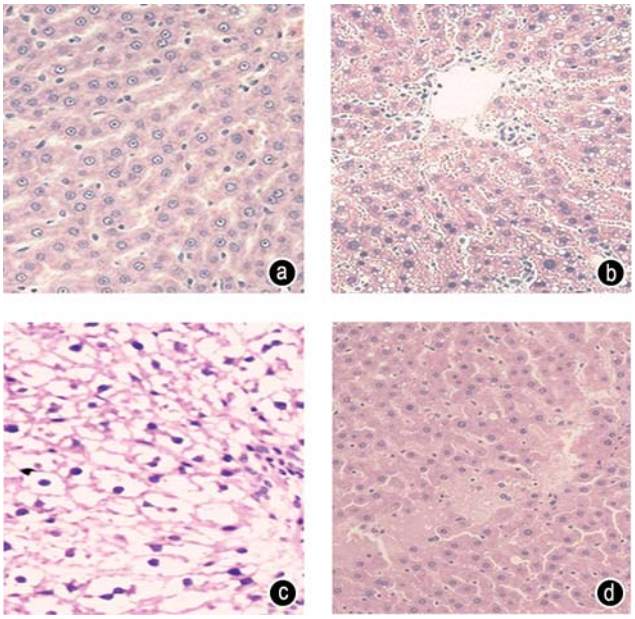


Fig. 1 The histopathological changes of liver tissue at the 1, 2, 4, 6, 8 w after treatment in all groups (HE staining × 400). (a) The normal tissue; (b) Liver cell swelling; (c) Liver cell swelling and central venous congestion; (d) A wide range of liver cell necrosis

AST) to evaluate the rat livers' response to concurrent chemoradiotherapy. In this context, hepatocyte inflammation and necrosis impairs liver function, and results in obvious increases in ALT and AST levels. Our results revealed significant inter-group differences at the same time points ($F$ = 85.869 and 214.663; $P$ < 0.001), and that the levels in the experimental group was higher than that in the control group. Furthermore, the changes in liver function were consistent with the changes in liver morphology, although no significant intra-group differences in ALT and AST levels were observed over time ($F$ = 0.115 and 0.003, $P$ > 0.05). This finding may be related to the fact that we administered a single large dose of radiation at the beginning of the experiment, and did not repeat the radiation treatment at each time point.

The role of Bcl-2 and Bax in the development of apoptosis has been extensively studied [18]. In this context, the *bcl-2* gene family is one of the major regulators of apoptosis in mammalian cells (via a strong anti-apoptotic effect), while the *bax* gene also regulates apoptosis via a pro-apoptotic effect. In addition, Lu *et al* have demonstrated that Bcl-2 and Bax were involved in mediating irradiation damage to normal cells [19]. Similarly, we found that Bcl-2 expression decreased in the experimental group, while Bax expression increased in the experimental group. Furthermore, these differences in expression were sustained throughout the course of disease progression, and were statistically significant ($P$ < 0.05). Moreover, Bax expression was significantly higher in the concurrent chemoradiotherapy group, compared to the other groups ($P$ < 0.05). This finding suggests that oxaliplatin can aggravate radiation-induced liver injury. In addition, although Bcl-2 expression was the highest in the concurrent chemoradiotherapy group, this increase was not significantly different; similar results have been reported by Zhuang *et al* [20]. Thus, it appears that hepatocyte apoptosis may be related to Bcl-2 and Bax expression.

In conclusion, concurrent chemoradiotherapy resulted in more severe liver damage, compared to that observed in the groups that received radiotherapy or chemotherapy alone. This finding may be related to the low expression of Bcl-2 (an anti-apoptotic protein), and the high expression of Bax (a pro-apoptotic protein). Therefore, concurrent chemoradiotherapy may aggravate liver injury, and we recommend that its clinical application should be carefully evaluated.

### Conflicts of interest

The authors indicated no potential conflicts of interest.

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