Effects of Low-Intensity Swimming on Radiation-induced Leg Contracture in Mice

Xing Min QU[1], Gang LI[1], Zhi Min LI[1], Kang Tao MA[2], Ping CHEN[2], Chun Yan ZHOU[2], Xu Chen MA[1]

Objective: *To evaluate the effects of low-intensity swimming on radiation-induced leg contracture.*
Methods: *Forty mice were randomly and equally divided into four groups: 1) irradiation; 2) swimming before irradiation; 3) swimming after irradiation; 4) swimming after contracture, and their left hind legs were exposed to gamma irradiation of 60 Gy. The mice were allowed to swim freely for 10 minutes, three times per day. For group 2, the mice were allowed to swim for only 1 week before irradiation. For group 3, the mice were allowed to swim immediately after irradiation until day 130, post-irradiation. For group 4, the mice were allowed to swim after leg contracture happened (on day 30 post-irradiation) until day 130, post-irradiation. The leg lengths and knee joint angles were measured. Leg contracture was defined as the decrease in the hind leg lengths and the knee joint angles of each animal. The ultrastructural changes of gastrocnemius muscles were observed using transmission electron microscopy.*
Results: *The radiation could result in leg contracture and mitochondrial injury of the muscles. However, the group of swimming immediately after irradiation had less leg contracture and no vacuolar degeneration in the mitochondria, compared with the other groups.*
Conclusion: *Low-intensity swimming that began immediately after the mice were irradiated could effectively prevent the irradiated legs from contracture. Patients with irradiated mastication muscles were recommended to begin mouth-opening exercises immediately after radiotherapy.*

Key words: *exercise therapy, radiation injuries, radiotherapy, trismus*

Radiotherapy to treat oral and maxillofacial cancer inevitably involves an exposure of normal tissues[1]. When the masticatory muscles are irradiated, mouth opening limitation or even trismus will happen[2]. The limitation of mouth opening will impact patients' nutrition, dental hygiene, swallowing, and phonation[3]. Radiation-induced trismus develops as a result of damage of the temporomandibular and the skeletal muscles of mastication, especially the pterygoid muscles[4,5]. Unfortunately, so far few effective methods have been introduced to deal with radiation-induced trismus.

Exercise training has the potential to promote muscle mass in patients with muscle wasting disorders[6]. Some exercise regimen was proposed to prevent mandibular hypomobility in head and neck cancer patients[7]. Contemporary exercise training that provides strong anabolic effects for muscle and bone may have an impact on counteracting some of the side effects of cancer management[8]. In the present study, an animal model of radiation-induced leg contracture[9] was employed to simulate mouth-opening limitation. The effects of low-intensity swimming on this contracture were evaluated and the ultrastructural changes of gastrocnemius muscles were observed.

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1 Department of Oral and Maxillofacial Radiology, Peking University School and Hospital of Stomatology, Beijing, P.R.China.
2 Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Peking University, Beijing, P.R.China.

Corresponding author: Dr Xu Chen MA or Dr Gang LI, Department of Oral and Maxillofacial Radiology, Peking University School and Hospital of Stomatology, #22 Zhongguancun Nandajie, Haidian District, Beijing 100081, P.R.China. Tel: 86-10-62179977 ext 5345; Fax: 86-10-62173402; E-mail: kqxcma@bjmu.edu.cn or kqgang@bjmu.edu.cn

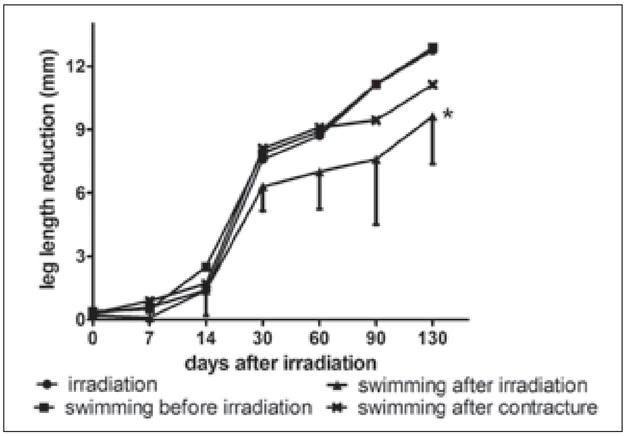

Fig 1 The time course of leg length reduction after radiation exposure with different swimming interventions (*$P < 0.05$ versus the other three groups).

Materials and methods

Mice

Forty female mice of 12 weeks inbred specific pathogen-free C3H/He were used in this study. The animals were randomly divided into four groups: 1) irradiation; 2) swimming before irradiation; 3) swimming after irradiation; 4) swimming after contracture. All animals were housed in rooms with lights from 8 am to 8 pm and received food pellets and drinking water *ad libitum*. All experiments were approved by the Animal Care and Welfare Committee of Peking University.

Irradiation

To produce a significant leg contracture, the left hind leg of each mouse was irradiated with a single 60 Gy gamma ray. The radiation method was introduced by Stone[9]. Briefly, the left leg of each mouse was irradiated using a 60-Co source with a 50 cm focus-to-skin distance and a dose rate of 4.2 to 4.5 Gy/min. The field was about 3 cm in diameter, while the rest of its body was shielded with 3 cm thick lead brick. The right leg was used as an unirradiated control.

Swimming protocols

Mice were put into a barrel (five mice per barrel) with water filled to a depth of 30 cm. The water temperature was maintained at 35°C. The mice were allowed to swim three times per day (at 9 am, 1 pm and 5 pm), each time for 10 min. For the group 2, the mice were allowed to swim for only 1 week before irradiation. For group 3, the mice were allowed to swim immediately after irradiation until day 130 post-irradiation. For group 4, the mice were allowed to swim after leg contracture happened (on day 30 post-irradiation) until day 130 post-irradiation.

Leg length assay

The method of measurement was similar to that described by Stone[9]. Extensibility of both the irradiated and controlled hind legs in each mouse was made using a jig. Each leg was extended along a millimetre ruler, and the length was measured at the heel. All measurements were performed by the same clinician. The leg lengths were measured periodically.

Knee joint angle assay

Mice were destroyed on day 130 post-irradiation. Both legs were removed by cutting at the hip joint from the body. To obtain the knee joint angles under constant conditions without artifacts, the legs were gently stretched to their reversible maximum length, pinned on thick paper and fixed in 10% formalin[10]. Radiographs of the legs were acquired by the REGIUS PureView System with the femur and tibia in close parallel contact to the receptor in the lateral view (25 kV, 26 mAs) at a working length of 30 cm. Posterior angle between the femur and tibia was measured as the knee joint angle.

Leg contracture was defined in the present study as significant reduction of the hind leg length and knee joint angel of the irradiated leg compared with the contralateral leg on day 30 post-irradiation[9,10].

Transmission electron microscopy

Gastrocnemius muscles were dissected unilaterally and fixed in 2.5% glutaraldehyde, post fixed in 1% osmium tetroxide at 4°C for 1.5 hours. Samples were dehydrated in ascending grades of ethyl alcohol and embedded in epoxy resin. Thick sections were stained with toluidine blue to identify the best longitudinal block for each animal. Ultrathin sections cut for transmission electron microscopy survey were double-stained with uranyl acetate and lead citrate and examined using a Jeol JEM 1230 transmission electron microscope.

Statistical analysis

Calculations were performed with the software package SPSS 11.0 for Windows (SPSS, Chicago, IL). Difference in leg contracture was analysed for significance by one-way ANOVA followed by Tukey's test. Statistical significance was recognised for P values of 0.05 or less.

Results

Effects of swimming on leg length

The irradiation method employed in this study was able to induce obvious mice leg length reduction from day 30 post-irradiation (Fig 1). On day 130 post-irradiation, the reduction in the hind leg length for groups 1 to 4 was 12.8, 12.9, 9.6 and 11.1 mm, respectively, with the reduction of group 3 significantly less than that of other three groups ($P = 0.002$).

Effects of swimming on knee joint angle

Radiographs of the legs taken on day 130 post-irradiation are shown in Figure 2. The reduction in knee joint angle for groups 1 to 4 was 76.3, 73, 26.9 and 48.6 degrees, respectively, with the reduction of group 3 significantly less than that of other three groups ($P < 0.001$) (Fig 3).

Ultrastructural changes

Compared to the non-irradiated muscle in group 1, scattered organising myofibrils were observed in the irradiated muscle, with some mitochondria swollen and crista broken. Vacuolar degeneration was apparent on day 130 post-irradiation (Fig 4a). This is consistent with the previous studies on radiation-induced ultrastructural changes in skeletal muscle[11,12]. Similar ultrastructural changes were observed in groups 2 and 4 (Fig 4b and d). However, in group 3, when swimming began immediately after irradiation, few ultrastructural changes were observed in the irradiated muscles (Fig 4c).

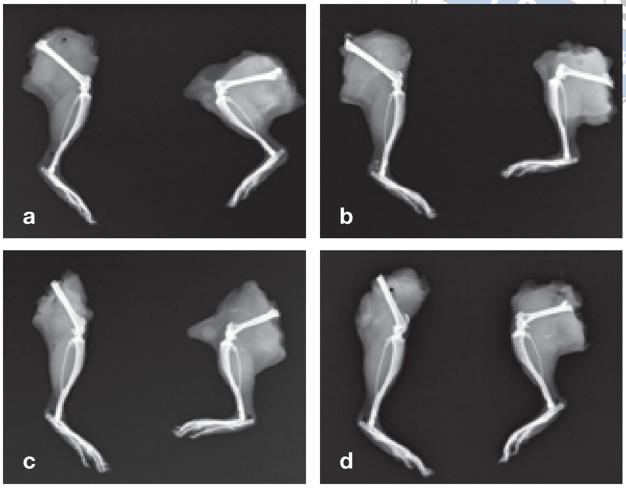

Fig 2 Radiographs of representative pairs of disarticulated hind legs from mice. The radiation was limited to the left leg (right side in the figures). (a): irradiation; (b): swimming before irradiation; (c): swimming after irradiation; (d): swimming after contracture.

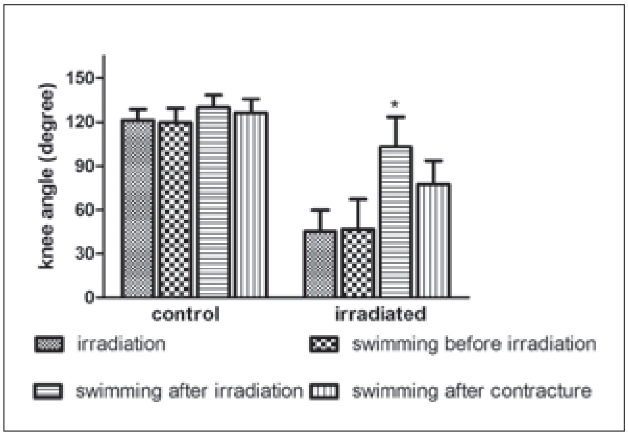

Fig 3 Comparison of knee joint angles among different groups. (*$P < 0.05$ versus the other three groups).

Discussion

Normal tissues involved in radiotherapy for cancers may result in symptomatic injury[1]. Radiation-induced mouse hind leg contracture was often employed to simulate normal tissue injury, including skin, muscle, connective tissues and bone[9,13–16]. Radiation exposure shortened leg length and lessened the knee joint angle[10]. The present study demonstrated the results were similar to those of the previous.

Several drugs have been studied on their radioprotective effect against radiation-induced leg contracture[10,16,17]. These drugs have been proved to be able to protect against late radiation damage to normal tissues, but the drugs need to be injected and may be accompanied by some side effects.

Exercise (eg, swimming, walking) is a non-invasive rehabilitation therapy without complications. The present results clearly indicated that swimming that began immediately after the mice were irradiated could effectively prevent the hind legs from length shortening and knee joints from angle lessening. The reason

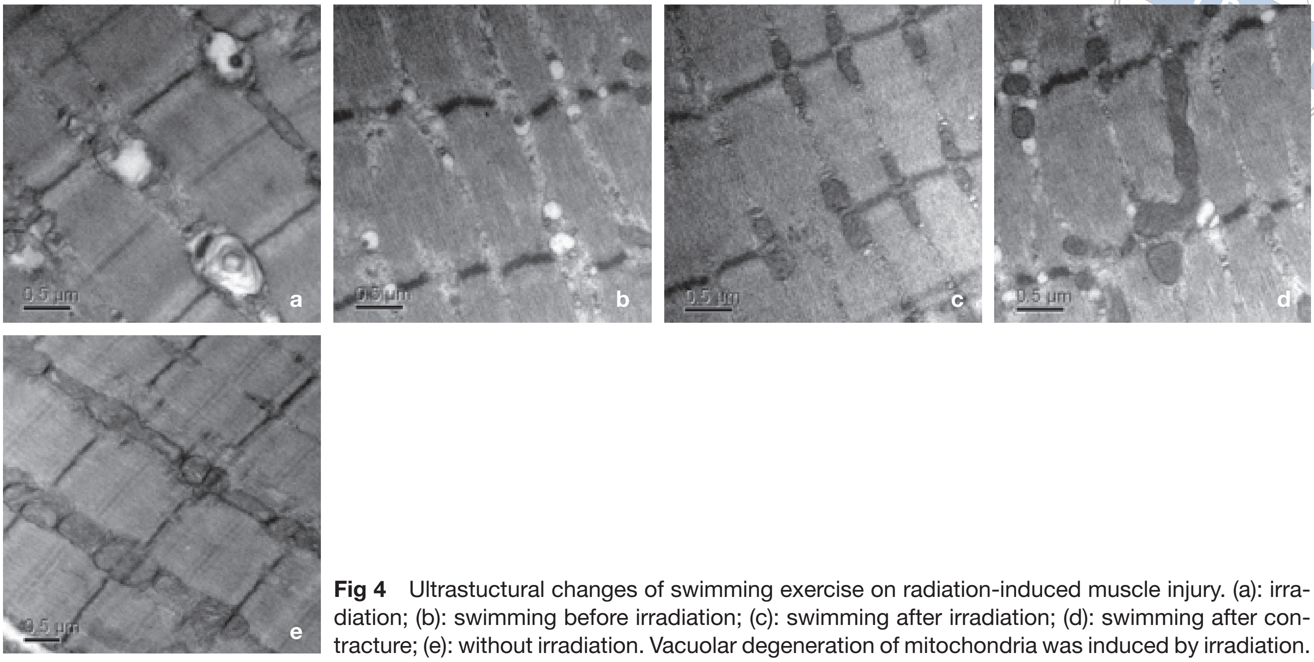

Fig 4 Ultrastuctural changes of swimming exercise on radiation-induced muscle injury. (a): irradiation; (b): swimming before irradiation; (c): swimming after irradiation; (d): swimming after contracture; (e): without irradiation. Vacuolar degeneration of mitochondria was induced by irradiation.

for radiation-induced leg contracture was mostly due to skin shrinkage and fibrosis of subcutaneous tissues[9,13]. Skeletal muscle atrophy might appear secondary to these changes. Swimming that began immediately after irradiation can sustain the leg tissues at a relatively normal condition. However, when leg contracture happened, the employed swimming protocol could little help. Unfortunately, many clinical exercise interventions were not carried out until symptoms appeared[8]. It might be one of reasons for the effectiveness of these interventions. With regard to group 2, no effects were observed when mice just swam for 1 week before irradiation. In the opinion of the authors, 1 week of swimming exercise might not be long enough to prevent legs from radiation injury. It is also not possible in a clinical situation to make cancer patients do more exercises before receiving radiotherapy.

Transmission electron microscopic observation demonstrated that radiation could lead to mitochondrial injury of some skeletal muscles. The mitochondria could be prevented from vacuolar degeneration by swimming that began immediately after the mice were irradiated. These ultrastructural changes of some mitochondria might not be significant enough to impact on the muscle function of contraction and relaxation because there are much more mitochondria in skeletal muscle than other tissues[11]. However, these changes should draw attention because the pathological process of radiation injury begins immediately after radiotherapy, while the clinical features may not become apparent until for weeks, months, or even years[1]. Further studies need to focus on the cellular and molecular changes of normal tissues included in the radiation field.

The reason for the single radiation dose employed in the present study was to get an experimental model for radiation-induced leg contracture[9,13]. Since this method differs from the clinical application of fractionated radiation and might result in a severe biological response, the present results of radiation injury to skeletal muscle could not explain the clinical observing complications of radiotherapy. However, the effects of swimming on radiation-induced leg contracture might be far more significant when a smaller radiation dose is delivered. Further studies with fractioned doses of radiation, especially with clinical-range doses, are necessary for the assessment of the radioprotective potential of swimming.

In conclusion, a low-intensity swimming that began immediately after the mice were irradiated could effectively prevent the irradiated legs from contracture. Mouth opening exercise intervention is recommended to begin as soon as possible for the head and neck cancer patients who receive radiotherapy.

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