Effect of Total Body X-ray Irradiation on Lymph Node in Tibet Minipig

Shao-Jie WU1†, Yu-Jue WANG2†, Kun-Yuan GUO1†, Chi CHEN1†, Tong-Feng ZHAO1, Mao-Ben SUN1, Wei-Wang GU2, Ying Ying GAO1, Hui Juan HAN1 and Fei ZOU3*

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The purpose of this study was to determine the time-dose-effect of total body X-ray irradiation on lymphocytes in lymph nodes and peripheral blood in Tibet minipigs. Forty-eight Tibet minipigs were assigned into 6 groups including 5 experimental groups with 9 and the control group with 3. The minipigs in experimental groups were subjected to a total body X-ray irradiation of 2, 5, 8, 11, and 14 Gy respectively. Lymph nodes and peripheral blood samples were collected at 6, 24, and 72 hours after X-ray exposure and received histological microscopy examination and apoptosis analysis. Histology observation showed that the number of lymphocytes decreased within the lymph nodes with the increase of radiation doses and exposure time. The observation of transmission electron microscopy (TEM) showed typical apoptotic cells below 11 Gy while at 14 Gy necrotic cells were dominant. The apoptotic rate of lymphocytes in the lymph nodes was positively correlated with radiation dose in the range of 2–11 Gy, and reached the maximal level (39.4 ± 2.8) at 24 hours after 11 Gy irradiation, followed by a decrease in the apoptotic rate, but still higher than that of the control group. The number of lymphocytes in the peripheral blood samples was decreased significantly by increasing of the radiation dose and exposure time. We conclude that early damage of lymphocytes by total body X-ray irradiation is dose and time dependent below 11 Gy and before 24 hours post irradiation, and that the dosage of irradiation less than 11 Gy induced apoptosis, whereas the dose at 14 Gy resulted in necrosis in lymphocytes of the lymph nodes.

INTRODUCTION

At present, with wide utilization of unclear technique, radiation damage will happen at any time and anywhere. Whereas, it is impossible to study radiation damage in human body, so we should use biology models to understand the characteristics of the radiation damage. Compared with small animal models such as rodents, large animal models are superior in many aspects for the study of human diseases and pre-clinical therapies. The miniature pig is similar to human in anatomy, development, physiology, pathophysiology, and disease occurrence, etc. (see for a review Tumbleson, 1986). Since the development of the Minnesota miniature pig in 1949 at the Hormel Institute (USA), miniature pigs have been used as a large animal model in medical studies for scientific, economic, and ethical reasons.1–3) Although miniature pigs as animal models have been applied in several fields of study, it is rarely used as an animal model for acute radiation injury. The Tibet minipigs have a character of genetic stability, small size, early maturation, high fecundity. Therefore, the Tibetan minipigs could be a suitable animal model for the study of radiation diseases.

Human and experimental data have shown that severe injury of lymphatic organs and decrease of lymphocyte count are being explained as important reasons to the difficulties in the curing of acute radiation injury by large doses radiation. It is also known that lymphocytes are one of the most radiosensitive immunocompetent cells in lymphatic tissues and peripheral blood.4–6) Previous studies have shown that low doses of X-ray irradiation could cause cell apoptosis (programmed cell death)7,8) and high doses of X-ray irradiation cause cell necrosis9) in vitro or in vivo by local or fractional radiation. It is critical to understand the death
modes (apoptosis or necrosis) of the lymphocytes so as to evaluate the irradiation injury. A correlation of the radiation dose or post-irradiation time and the death modes of lymphocytes induced by irradiation have not been studied systematically. The aim of this study was to define the early-stage damages to lymphocytes in the lymph node and peripheral blood in Tibet minipigs after total body X-ray irradiation.

**MATERIALS AND METHODS**

**Experimental Design**

Adult uncastrated male Tibet minipigs (weighing 21.16 ± 5.54 kg, supplied by Center of Laboratory Animal, Southern Medical University, Guangzhou, china) were kept under standard laboratory conditions with a 12 hour light and 12 hour dark cycle, and were allowed free access to feed and water. This study was approved by the supervising state agency (license number SCXK Yue 2006-0015) and performed in full accordance with the state guidelines. Forty-eight anesthetized (0.15 ml/kg Sumianxin) Tibet minipigs were divided into one control and five experiment groups and placed in a phantom for fixation postures to subject to irradiation exposure by an 8 Mv X-ray (isocenter) linear accelerator (Precise System Treatment, ELEKTA, Sweden). The irradiation was carried at the Cancer Centers of Armed Police Hospital of Guangdong following the described elsewhere. Animals in control (n = 3) were not exposed to X-ray, and those in experiment group (n = 9 in each group) received 2, 5, 8, 11, and 14 Gy doses of total body X-ray radiation, respectively, in a single fraction irradiation. The dose rate was 255 cGy/min in all treatment groups. The animals were sacrificed by bloodletting after anestheticated at the time points of 6, 24, and 72 hours post-irradiation, and specimens were collected for histological observation and apoptosis analysis, peripheral blood samples were collected from veins for lymphocytes count.

**Histological Study**

The cervical lymph nodes were fixed in 10% PBS buffered formalin solution for 48 hours. The samples tissues were dehydrated in different grade methanol- alcohol and embedded in paraffin. Two-micrometer sections were cut and stained with hematoxylin–eosin. To determine the numbers of lymphocytes in the lymph nodes, we counted the lymphocytes with hematoxylin–eosin. The irradiation injury. A correlation of the radiation dose or post-irradiation time and the death modes of lymphocytes induced by irradiation have not been studied systematically. The aim of this study was to define the early-stage damages to lymphocytes in the lymph node and peripheral blood in Tibet minipigs after total body X-ray irradiation.

**RESULTS**

All animals have survived the irradiation procedures and been maintained during the observation period after X-ray irradiation.

**Pathological Changes of Lymph Nodes after X-ray Irradiation at Different Dosages and Time Points**

Anatomically, in the animals from the experimental groups, the lymph nodes have shown signs of shrinkage and apparent peripheral hemorrhage with increase of radiation doses. Under light microscope, the lymphocytes have shown nuclear condensation, fragmentation, and dissolution at 6 hours after irradiation on all dosages of X-ray radiation (Fig. 1B1). The most obvious changes were found in lymphatic nodules. The cellular debris was cleared at 24 hours postirradiation (Fig. 1B2), and the lymphatic nodules became empty with increase of dosages. Similarly changes were seen at 72 hours, except for reticular cell and plasma cells can be discernable, and no regeneration of lymphocytes can be seen at this time point after different doses of X-rays. The number of the lymphocytes decreased sharply in the cortex and the cortex was atrophied, so that the cortex and medulla
of the lymph nodes became hardly distinguishable at 14 Gy (Fig. 1C). The total number of lymphocytes decreased significantly compared with control group (0 Gy) after different doses and different time of X-ray, especially at 24 and 72 hours (Fig. 1D). But in the control group, the above changes were not found (Fig. 1A).

**Ultrastructural Change of Lymph Node after X-ray Irradiation**

Typically Morphological features of apoptotic cells were observed in the deep-staining cell (Fig. 2B), in the early stages, the cells showed marginal condensation of the chromatin, followed by the formation of typical crescent and ring...
chromatins and compaction of cytoplasmic organelles. In the late stage, the cells were showing shrinkage and apoptotic bodies were observable. \(^{12}\) These morphological changes of lymphocytes could be seen throughout the observation period from the animals in all experimental groups at all time points. At 6 hours after 11 Gy X-ray irradiation, spotty necrosis could be seen. More prominent necrotic lesions were shown at 14 Gy (Fig. 2C). Phagocytosis phenomenon of microphage, and cytoplasmic vacuolization, mitochondria expansion in the reticular cells were observable, but absent in the control group (Fig. 2A).

**The Apoptosis Lymphocytes of Lymph Node After X-ray Irradiation at Different Dosages and Time Points**

It was found that the numbers of the apoptotic cells were significantly increased with the increases of the radiation strength from 2 to 11 Gy at 6, 24, and 72 hours after irradiation, compared with that of the control group \((p < 0.05)\), Mann-Whitney U test (Fig. 3). However, at 72 hours after radiation there was no difference in the number of apoptotic cells with the irradiation from 11 Gy to 14 Gy (Fig. 3). When comparing the apoptotic cells at different time points in each treatment group, we found obvious differences in the group with 11 Gy, while there were no differences between 6 and 72 hours within others irradiation groups, except for 11 Gy group (Fig. 3).

**The Effect of Irradiation on the Peripheral Blood Lymphocytes Count**

The average peripheral blood lymphocyte counts was
decreasing with the increases of the irradiation doses from 2 to 14 Gy at every time points after irradiation (Fig. 4). With the extension of observation period to 72 hours after radiation, the decrease of peripheral blood lymphocyte counts became more significant, when compared with the control group ($p < 0.05$, post hoc Turkey test) (Fig. 4).

**DISCUSSION**

Pigs share many advantages for experimental studies. They are large enough to allow varied surgical techniques to be carried out and are suitable for numerous experimental protocols. Pigs have particular advantages for radiobiological studies. The pigs have been successfully used in studies of skin and renal and lung and intestinal effects of radiation exposure.\(^{13}\) Results from studies in non-irradiated animals are in agreement with reported data for different breeds of pig\(^{13, 14}\) and these data have shown similarities to those in man, particularly the data related to the concentration of white blood cells, percentage of lymphocytes and neutrophils.

This study using Tibetan minipigs has demonstrated that the lymph node was one of the radiosensitive lymphatic tissues. We observed cellular debrises and hyperemia and apoptosis cells in the lymph nodes examined at 6 hours after exposure to different dosages, that is why we did not find difference between irradiated groups through lymphocytes counting in this time point; and the cellular debrise was almost cleared at 24 hours, when lymph nodes appeared almost hollow. We did not see lymphocytes regeneration at 72 hours after 2 Gy X-ray exposure, except that reticular cells and plasma cells were observable, meanwhile, Kvacheva IuE results have shown that the regeneration of bone marrow begin in weeks after X-ray.\(^{15}\) Similar results were observed in animals in all irradiated groups and the pathological changes were more prominent when radiation doses increased. Also, the apoptotic lymphocytes with typically morphological features were discernable under transmission electron microscopy (TEM). These observations were in accordance with the results of previous studies.\(^{16, 17}\)

It was found that the count of peripheral blood lymphocytes decreased rapidly in the Tibetan minipigs. The lymphocytes count reduced to a minimal level ($0.065 \pm 0.02$) at 72 hours after 14 Gy X-rays exposure, which was also noted by other researchers.\(^{18, 19}\) Whereas, Azizova TV et al. observed lymphocyte count decrease nearly to zero over 6 Gy at 72 hours in human,\(^{18}\) possibly due to the high radiosensitivity of these cells.

In this study, we found that the apoptosis rate of lymphocytes in the lymph nodes increased to a maximal level ($3.9.4 \pm 2.8$) at 24 h after 11 Gy X-ray exposure, and the apoptosis rate was positively correlated with the irradiation dosages in the range of 2–11 Gy. However, such a correlation was not found after 14 Gy irradiation, where the apoptosis analysis has shown a reduced apoptotic response to irradiation, compared with 11 Gy at 24 hours ($P < 0.05$), that because the apoptotic rate decreased and the necrotic rate rose; and necrotic foci could be observed under both light and electron microscopy at 14 Gy dosage of irradiation. Transmission Electron Microscopy (TEM) was, therefore, used as a gold standard to distinguish adequately two morphologically distinct modes of cell death: apoptosis and necrosis. These results are consistent to other reported results, Payne et al.\(^{20}\) observed the peak cell loss occurred after high-dose irradiation (10–20 Gy), but the peak level of apoptosis occurred after low-dose Irradiation. H. Louagie et al.\(^{21}\) found that the primary necrosis occurred at 20 Gy of gamma radiation, but no details were given in terms of the dosages. These results suggested that apoptosis is the main mode of death for lymphocytes following the X-ray radiation exposure below 11 Gy.

In conclusion, in Tibetan minipig model, the early injury of lymphocytes by the whole body X-ray irradiation is dose and time dependent below the dosage of 11 Gy and within 24 hours following the irradiation. The dosage of irradiation less than 11 Gy induces dominantly apoptosis in the lymphocytes of the lymph nodes, followed by necrosis when the dosage increased to 14 Gy. Also, the apoptotic rate of the lymphocytes in lymph node is positively correlated with the radiation doses at 2–11 Gy X-ray irradiation, suggesting that apoptosis is the major way of lymph node lymphocyte death after ≤ 11 Gy radiation.
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REFERENCES