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Biological effects of radiation on subjects with nuclear medicine equipment SPECT exam

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Abstract. SPECT examination will bring unavoidable medical exposure to the subject, the amount of radiation dose and whether it will bring injury to the human body has become the focus of the public and professionals, and small doses of radiation on the biological effects of human body is still controversial. In this paper, red crucian carp was termed as the experimental object to study and analyze the radiation dose and biological effects of SPECT to provide experimental basis for clinical application. The experiment results show that the radiation dose of SPECT should be reduced as far as possible, but the irradiation dose of single examination will not lead to deterministic effect, and the stochastic effect of linear random effects produced by it is also challenged.

Key words. Nuclear medicine equipment, SPECT examination, Subjects, Radiation, Biological effects.

1. Introduction

SPECT is the main imaging equipment of nuclear medicine, and its imaging technology has developed rapidly [1-2] with unique molecular imaging and functional imaging advantages. SPECT examination will bring unavoidable medical exposure to the client, and the patient's exposure dose is closely related to the image quality and the radiation safety of the client. With the wide application of nuclear medical imaging technology, the public's concern on radiation safety is increasing. At present, there are two "extreme" problems: the public and the media are exaggerating the radiation dose and radiation hazard to the client, overestimating its carcinogenic risk, which thereby leading the public to fear and reject nuclear medical imaging, which seriously affects the diagnosis and development of nuclear medical image equipment,

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as well as SPECT. In addition, professionals who value high image quality and easily overlook the control of a patient's radiation dose can exacerbate the negative effects of rejecting nuclear medicine imaging. The present study investigated the radiation dose and biological effects of SPECT and other nuclear medical imaging subjects, and provided a reference for the study of the exposure dose of nuclear medical imaging client.

With the development of nuclear power and nuclear facilities, the radiation technology has become more and more widely used in medicine, industry and agriculture, and people have paid more and more attention to the damage effect of radiation on organism. And people have found that nuclear radiation is with great damage effect on the body's hematopoietic system, immune system, nervous systems, endocrine system, and so on. At the same time, with the application of nuclear facilities, the problem of nuclear pollution is becoming more and more serious. Therefore, the monitoring and control of nuclear pollution is imperative. However, in the study of radiation pollution monitoring, the main methods currently adopted are physical monitoring methods. For example, the German physicist Hans Geiger developed radioactive material measuring instrument in 1908, and the design of nuclear radiation and chemical harmful gas monitoring apparatus that invented by Xiong Jianping, and so on, were applied for detection. Although these gauges can accurately measure the content of radioactive substances, they do not reflect the damage effects of such substances on animal organism and the damage procedures. Compared with the physical methods, biological methods are more reliable and direct. At present, the most common biological method is to find a substance that reacts directly to the effects of pollution and damage, which we often call the biological marker. The study of biomarkers for early warning of radiation pollution is conducive to timely, safe and efficient monitoring of nuclear radiation pollution, while also accurately reflecting the extent of the damage to biological organisms caused by radiation pollution. The data show that biomarkers can not only evaluate the harmfulness of environmental pollutants, but also can be applied to the monitoring and management of ecological environment, early warning of ecological restoration, environmental intervention and early diagnosis of diseases that induced by environment pollution, and even the development of new types biological sensor. This paper will also study the physiological and biochemical changes of red crucian carps under the influence of certain nuclear radiation and the influence of biomarkers, so as to observe the biological effects of nuclear radiation.

Currently, studies for biomarkers for the detection of nuclear radiation by SPECT, are mainly consist of antioxidant enzymes, such as metallothioneins (MTs), superoxide dismutase (SOD), Glutathione (GSH), peripheral serum transaminase, immune marker and DNA additive. As to the study shows that nuclear radiation can be applied for the organism, so that the molecules in the body produce ionization and excitate of physical and chemical to change, therefore forming of free radicals of biomolecules, which also acts on the surrounding medium of biological molecules, to produce very strong oxidation activity of free radicals, and thus acting on the body to produce new secondary free radicals. When free radicals produce too much and can not be cleared in time, lipid peroxidation is induced, which leads to a series of

radiation damage. Radiation can also lead to the changes of body's DNA and other genetic materials, as well as hematopoietic stem cell apoptosis or aging. Because SOD and GSH-Px are two important enzymes in antioxidant enzymes, this paper chooses these two as the research contents of this paper, and explores whether the radiation of SPECT has the same effect on the experimental red crucian carps.

2. Materials and methods

2.1. Experimental Materials

Neutral formaldehyde (10%), gradient alcohol, xylene, hydrochloric acid (1%), alcohol, paraffin, hematoxylin-eosin, neutral gum, slides, cover glasses, embedding machine, rotary slicing machine, automatic dehydrator, electric hot plate, exhibition table, baking box, staining jar, OLYMPUS microscope, HITACHI7600-020 automatic blood biochemical analyzer.

There are 420 aged 2 C1HD series experimental red crucian carps, which was all trained and provided by Prof. Wu Duansheng et al. in Laboratory Animal Division, University of South China. Among which, 360 experiment red crucian carps that belong to C1HD system were used for morphological measurement; and 140 experiment red crucian carps that belong to C1HD serious were used to determine blood biochemical indexes; also, there are 3 experiment red crucian carps that from C1HD series were used to study the main organ histology. The experimental red crucian carps were raised in a 3 meters diameter round cement pool, and were daily fed with the full grains, the used water was aerated tap water and changed every half a month.

This study uses GE's Discovery NM/CT 670 model SPECT system for scanning. The selection of scanning parameters is consistent with the clinical scanning parameters. The rotating radius of the SPECT probe is fixed to 17cm, the peak is 140 KeV, the window width is 20%, each 6 ° gathers as 1 frames, and each frame acquisition time is 10s. The CT Scanning tube voltage is the 120 kV; the tube current is 20 mA; the reconstruction image layer thick chooses 2.5 mm. The Xeleris software in SPECT system is adopted to reconstruct image. The four reconstruction methods used are: filtered-projection (FBP), ordered subset expectation maximization (OSEM), OSEM + AC, OSEM +AC + SC. OSEM + AC indicates that attenuation correction (AC) is turned on in OSEM reconstruction, OSEM + AC + SC indicates that attenuation correction and scattering correction (SC) are also enabled in correction reconstruction.

To eliminate the effect of the non-linear relationship between the counting rate and activity caused by the dead time and other factors, the relationship between the counting rate and the activity in the activity range used in this research was designed. Four times of SPECT scans were carried out for small cylindrical body, the bottom surface of small cylinder was perpendicular to the bed surface, and the activity degree was 76.22, 188.70, 300.07, 432.90 MBq respectively. The scan results of each activity were reconstructed using the above 4 reconstruction methods, and then the total number in the reconstructed image was computed. Finally, the linear

regression analysis of the reconstructed count rate and the activity of the same reconstruction method was carried out.

2.2. Toxicological test of nuclear radiation in experimental red crucian carps

Before the formal test, the pre-experiment was carried out, that is, the HXFS-IA biological irradiation apparatus was used to irradiate the experimental red crucian carp, and a total of 10 groups of 10-100Gy were set up to observe the death of the experimental red crucian carp and to obtain the maximum dose and the minimum dose of 30d death. Then, the gap between the maximum dose and the minimum dose was set to 6 experimental groups by taking 30d as radiation dosage, namely 20Gy, 26.3Gy, 34.7Gy, 45.7Gy, 60.2Gy, 80Gy, and the experimental red crucian carp in each experiment group were 10. The rearing method and environment of the experiment red crucian carps were the same as earlier experiments. Finally, the mortality rate of each group was calculated according to the deaths among the different groups. The C1HD series experimental red crucian carp was calculated to obtain a lethal dose of 30d of nuclear radiation (LD_{50}).

By taking half-lethal dose of experimental red crucian carp after 30d nuclear irradiation as the standard, namely 1/16 LD50 (1.94Gy) dosage Group, 1/8 LD50 (3.88Gy) dosage Group, 1/4 LD50 (7.76Gy) dosage Group, 1/2 LD50 (15.53Gy) dosage Group, and another 1 group of control group. In each experimental group, the experimental red crucian carps are with 20 similar size, good growth condition and healthy vigor in each group, except for the irradiation, all was given in the same radiation doses. After irradiation of 24, 48, 72 and 96 hs, the fish were executed according to normal anatomical methods, and the gonad tissues were used to determine the GSH-Px and SOD activity of the gonad tissue. After irradiation 7 and 14 ds, the fish were executed and dissected; their heart, liver, kidney and spleen were removed, and was wrapped in foil paper for the weight measuring, which was also used for the expression analysis of Hsp70 protein. Among which, part of 14 d radiated tissues were used for histopathological examination.

2.3. Sample Pretreatment

① Preparation of 10% homogenate

A. Firstly, experiment red crucian carps are treated with radiation then with MS-222 for anesthesia, and then sacrificed for anatomy. Taking out of the gonads, put it into the early prepared cold saline, and cleaned with normal saline (as far as possible to remove the blood and adipose tissue of gonads), the filter paper will be used to absorb the saline, and weighted with electronic balance then put in a small beaker.

B. According to the weight of the gonad tissue, a certain amount of cold saline was taken by a cylinder, then taking the ratio of the normal saline (ml) and the tissue weight (g) as 9:1 adding saline; firstly, adding half of the normal saline, the gonad tissue was cut with a small eye shear (in hot days, the small glass beakers with gonad tissue shall be kept into the ice water to prevent high temperatures affecting

GSH-Px activity).

C. the dissected gonad tissue was placed into the glass homogenizer, the remaining saline was used to flush the residue gonad tissue in the beaker, all were kept into the glass homogenizer for homogenating. In homogenating, the homogenate was palced into the ice water (to prevent friction generated heat affecting the activity of GSH-Px), the rod was vertically inserted into the cannula and rotate up and down for grinding, the tissues were fully grinded to homogenate.

D. The homogenized tissue was pipetted into the EP tube and placed in a high-speed refrigerated centrifuge for centrifugation at 3000 r/min for 10 min. The centrifugal tissue homogenate was prepared and the supernatant liquid was taken for the determination of GSH-Px activity.

② Acquisition of optimum sampling concentration and sampling quantity of tissue homogenate. According to the test kit provided by the determination method, the 10% normal group of the gonad homogenate supernatant was diluted into 1% homogenate, then 1% of the homogenate diluted into 1%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.05%, and other different concentrations of gonad tissue homogenate. When the inhibitory rate of tissue homogenate concentration was between 45% and 55%, it is the best, the concentration of tissue homogenate was selected to determine the activity of gonad GSH-Px.

2.4. Determination of gonad GSH-Px activity

The determination of GSH-Px activity of gonad was carried out according to the method provided by Nanjing Jiancheng biological company. The test was carried out in 2 parts.

The first part of the experiment was enzymatic reaction. That is, adding 0.2ml of 1mmol/L GSH into the enzyme tube and the non-enzyme tube, then adding 0.2ml of the homogenate to be tested in the enzyme tube, adding nothing to the non-enzyme tube, then all were co-bathed for 5min at 37 °C, then adding 0.1ml reagent-application solution, and water bathing at 37 °C for 5min, the enzyme tube was added 2ml reagent two application solution, while non-enzyme tube was added 2ml reagent two, application solution and 0.2ml of gonadal homogenate, after mixing, it was centrifuged in a high-speed refrigerated centrifuge with the speed of 4000r/min for 10min, then taking 1ml supernatant for coloring.

The second part of the experiment is color-rendering reaction. Namely, in the enzyme tube and the non-enzyme tube, a 1ml of enzymatic reaction supernatant will be added, 1ml reagent, tri-applied liquid, 0.25ml reagent, four-applied liquid, 0.05ml reagent, five-applied liquid, and the control tube and the standard tube were also added the same reagent added in enzyme tube and the non enzyme tube, while the control tube added the 1ml GSH standard substances, and the standard tube added 20 umol/L's GSH standard substances. The solution was mixed, and kept at room temperature for 15min, the absorbance (OD value) was measured at wavelength of 412nm. The activity of the GSH-Px in the tissues is calculated by stipulating that the effect of the non enzymatic reaction is deducted per mg protein, and the GSH concentration in the reaction system reduced 1umol/L as an enzyme activity unit.

3. Results Analysis

3.1. Toxicological characteristics of experimental red crucian carp

The experimental red crucian carp showed different living status after treatment with nuclear irradiation at different doses at different time. In the 1d nuclear irradiation, experimental red crucian carp in 26.3 Gy irradiation group and irradiation group larger than 26.3 Gy dose showed adverse reaction, and with the phenomenon of fast swimming speed and excessive activity appeared. At the same time, the head surfaced and the operculum enlarged, while the operculum became weaker as the exposure time extended, after which the fish unbalanced, sunk into the bottom, and finally died. With the increase of radiation doses, the number of experimental red crucian carps with death symptoms is more. In 5d and 10d of nuclear irradiation, the experimental red crucian carp died from 26.3 Gy irradiation group to 80 Gy dose irradiation group, but the death rate was relatively slow. When nuclear irradiation was in 15d, the experimental red crucian carp in the 20 Gy dose group began to die. At the 20d nuclear irradiation, the experimental red crucian carp were all dead in the 80 Gy dose irradiation group. In addition, the body color of the experimental red crucian carp irradiated by nuclear irradiation was also changed from red to black. Initially from the abdomen, a part of the scale black will be seen, and over time the surface of most of the fish scales black, and the morning and evening with the irradiation dose has a certain relationship, the higher the radiation dose its body color changes in the earlier time. After nuclear irradiation, the mortality rate of the experimental red crucian carp is shown in table 1.

Table 1. Mortality of experimental red crucian carp after nuclear radiation treatment

Radiation dose (Gy)	Experimental red crucian carp mortality rate (%)					
	1d	5d	10d	15d	25d	30d
Control group	0	0	0	0	0	0
20.0	0	0	0	10	20	30
26.3	10	10	20	20	30	40
34.7	10	20	40	40	50	60
45.7	30	40	50	60	60	70
60.2	40	60	70	80	80	90
80.0	50	70	90	90	100	100

Calculated from table 1, the experimental red crucian carp irradiation in 1d, 5d, 10d, 15d, 20d, 25d and 30d were 61.94Gy, 52.48Gy, 43.25Gy, 39.81Gy, 35.65Gy, 31.05Gy (Table 2).

Table 2. The half lethal dose and confidence interval of experimental red crucian carp after nuclear radiation treatment

Radiation source	Irradiation Time (d)	LD50(Gy)	95% Confidence Interval (Gy)
CS137	1	61.94	49.48∼69.42
	5	52.48	44.05∼62.52
	10	43.25	36.27∼51.57
	15	39.81	33.30∼47.60
	25	35.65	29.71∼42.77
	30	31.05	25.94∼37.15

3.2. SOD activity of gonad of experimental red crucian carp

Experimental red crucian carps were irradiated with 1/16LD50 (1.94Gy) dose for 24h, 48h, 72h and 96h, respectively with gonad SOD activity:336.50U/mgprot, 292.75U/mgprot, 283.50U/mgprot and290.44U/mgprot; The experimental red crucian carps were irradiated with 1/8LD50 (3.88Gy) dose for 24h, 48h, 72h and 96h, respectively with gonad SOD activity: 310.64 U/mgprot, 263.37U/mgprot, 260.37U/mgprot and 246.79U/mgprot; The experimental red crucian carps were irradiated with 1/4 (3.88Gy) dose for 24h, 48h, 72h and 96h, respectively with gonad SOD activity: 328.19U/mgprot, 246.13U/mgprot, 249.13U/mgprot and 239.83 U/mgprot; The experimental red crucian carps were irradiated with 1/2 (15.53Gy) dose for 24h, 48h, 72h and 96h, respectively with gonad SOD activity: 268.92 U/mgprot, 224.99U/mgprot, 211.33U/mgprot and 216.71U/mgprot;

Table 1. Gonad SOD activity of the experimental red crucian carps after different doses of nuclear radiation (Unit: U/mgprot)

Irradiation Group (Gy)	SOD Activity (U/mgprot)			
	24h	48h	72h	96h
Control Group (0Gy)	417.00±35.12	416.43±32.79	408.96±51.64	392.58±27.70
1/16LD50(1.94Gy)	336.50±27.74*	292.75±16.44#	283.50±27.33#	290.44±23.36#
1/8LD50(3.88Gy)	310.64±23.47*	263.37±18.99#	260.37±31.26#	246.79±33.89#
1/4LD50(7.76Gy)	328.19±34.17*	246.13±22.00#	249.23±37.37#	239.83±36.68#
1/2LD50(15.53Gy)	268.92±21.80#	224.99±12.66#	211.33±9.01#	216.71±19.98#

NOTE: * stands for when comparing with the control group, the P <0.05; # stands for when comparing with the control group, P <0.01;

It is shown from the Table 3 that in irradiation doses, the SOD activity of the experimental red crucian carps in each irradiation group were decreasing with significant difference when compared with the control group. In irradiation doses, the SOD activity of the experimental red crucian carps in each irradiation group were decreasing with significant difference when compared with the control group.

From Fig. 1, we directly see that after radiation, and after the same time, SOD activity in the gonads of experimental red crucian carp was decreased, and there

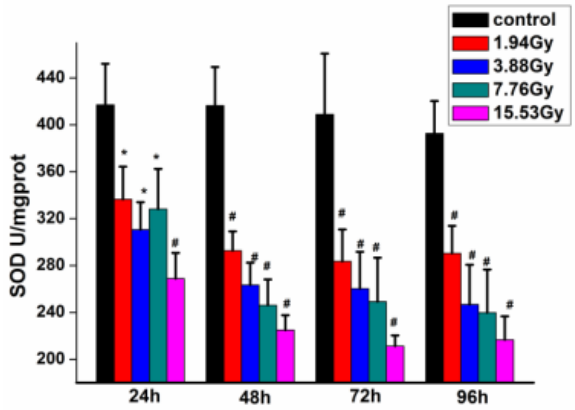

Fig. 1. Gonad SOD activity of the experimental red crucian carps after different doses of nuclear radiation (Unit: U/mgprot)

was a significant difference compared with the control group.

3.3. GSH-Px activity of gonad of experimental red crucian carps

The experimental red crucian carps were irradiated with 1/16LD50 (1.94Gy) dose for 24h, 48h, 72h and 96h, respectively with gonad GSH-Px activity: 2.41U/mgprot, 2.41U/mgprot, 2.39U/mgprot and 2.38U/mgprot. The experimental red crucian carps were irradiated with 1/8 (3.88Gy) dose for 24h, 48h, 72h and 96h, respectively with gonad GSH-Px activity: 2.38U/mgprot, 2.37U/mgprot, 2.35U/mgprot and 2.35U/mgprot. The experimental red crucian carps were irradiated with 1/4 (7.76Gy) dose for 24h, 48h, 72h and 96h, respectively with gonad GSH-Px activity: 2.36U/mgprot, 2.35U/mgprot, 2.33U/mgprot and 2.34u/mgprot. The experimental red crucian carps were irradiated with 1/2 (15.53Gy) dose for 24h, 48h, 72h and 96h, respectively with gonad GSH-Px activity: 2.33U/mgprot, 2.301U/mgprot, 2.29U/mgprot and 2.31U/mgprot.(Fig. 4 & Table 2)

Table 5. Gonad GSH-Px activity of the experimental red crucian carp after different doses of nuclear radiation (Unit: U/mgprot)

Irradiation Group (Gy)	GSH-Px Activity (U/mgprot)			
	24h	48h	72h	96h
Control Group (0Gy)	2.44±0.02	2.44±0.04	2.45±0.04	2.43±0.05
1/16LD50(1.94Gy)	2.41±0.02	2.41±0.02	2.39±0.03	2.38±0.04
1/8LD50(3.88Gy)	2.38±0.03*	2.37±0.04	2.35±0.03*	2.35±0.03
1/4LD50(7.76Gy)	2.36±0.04#	2.35±0.06*	2.33±0.07#	2.34±0.06
1/2LD50(15.53Gy)	2.33±0.03#	2.30±0.03#	2.29±0.02#	2.31±0.06*

NOTE: * stands for when comparing with the control group, the P <0.05; # stands for when comparing with the control group, P <0.01

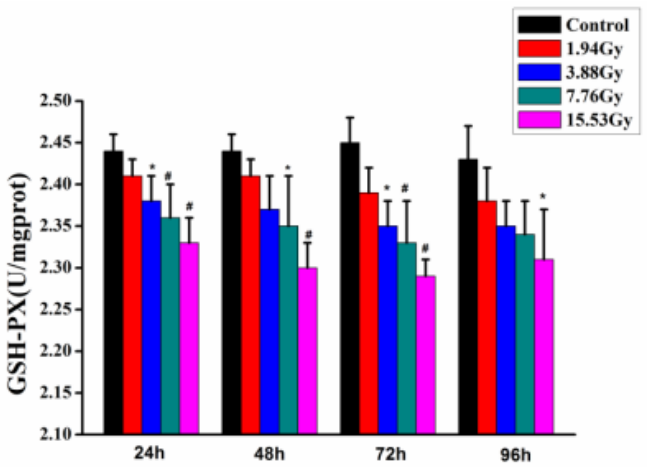

Fig. 2. Gonad GSH-PX activity of the experimental red crucian carps after different doses of nuclear radiation (Unit: U/mgprot)

From Table 4 and Fig. 2, we know that from the irradiation time, after 24h radiation treatment, the GSH-Px activity of the experimental red crucian carps in each irradiation group were decreasing when compared with the control group, and there are significant differences. In irradiation doses, the GSH-Px activity of the experimental red crucian carps in each irradiation group were decreasing with significant difference when compared with the control group.

4. Concluding Remarks

The introduction of CT based attenuation correction in SPECT significantly improved the quantitative accuracy of the activity of SPECT, and in the results of this study, the minimal activity quantitative error was obtained by the OSEM+AC reconstruction method. In this paper, red crucian carp was used as the experimental object to study the radiation dose and biological effect of SPECT examination, which aiming at providing experimental basis for clinical application. The experiment results show that the radiation dose of SPECT should be reduced as far as possible, but the irradiation dose of single examination will not lead to deterministic effect, and the stochastic effect of linear random effects produced by it is also challenged.

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