Full Length Research Paper

Laminaria japonica polysaccharides on the recovery of rats’ spermatogenic function of testis damaged by chronic local ionizing radiation

Luo Qiong¹*, Liu Jun¹,², Yan Jun¹ and Cui Xiaoyan¹

¹School of Public Health, Wuhan University, Wuhan, Hubei 430071, China.
²College of Life Science and Technology, Xinjiang University, Urumqi, Xinjiang 830046, China.

Accepted 11 June, 2010

To investigate the degree of recovery by Laminaria japonica polysaccharides (LJP) on the damage to the rat spermatogenic function caused by chronic local ionizing radiation, 56 wistar rats were randomly divided into seven groups: Control, three model and three LJP groups. Results showed that although indicators in LJP and model groups were found different from the control group (P < 0.05) when compared with the corresponding model groups, except in LJP 7d group the difference of SOD activities was not obvious compared with model 7d group (P > 0.05). In the other 7 and 14d LJP group, the SOD and GSH-PX activities increased while MDA content decreased. In addition, mitochondrion membrane potential enhanced in testis cells while DNA injury relieved; furthermore the sperm count and survival rate increased, the difference was significantly (P < 0.05) and the testicular tissue damage reduced and mitochondrion were relieved, which were more similar to those of the control group. Our results suggested that lavaged by LJP100 mg·kg⁻¹·d⁻¹ at the duration from 7 days before radiation till the end of radiation, LJP has promoting effects on the recovery of the damages to spermatogenic function induced by chronic local ionizing radiation.

Key words: Laminaria japonica polysaccharides, ionizing radiation, spermatogenic function, damage recovery.

INTRODUCTION

With the development of nuclear technique, human beings are faced with more dangerous of ionizing radiation than before (Alonzo et al., 2008). As a sensitive organ to ionizing radiation, the testicular injury became one of the most common causes of the dysfunction of spermatogenesis (Zeng et al., 2004; Xue, 2006). In order to prevent and relieve the hazard to human reproductive health, induced by ionizing radiation, avoid the toxicity and side effects and for the labilization of anti-radiation drugs (Jaetia and Baliga, 2003; Ding et al., 2007), therefore, the seeking for anti-radiation drugs, from traditional food and medicine plant sources, is considered to be a good alternative.

Laminaria japonica is macroscopic algae of plant, belonging to phaeophyta, the phaeosporeae, laminariales, laminariaceae of laminaria (Li et al., 2002), kind of large edible-medicinal seaweed with nutritional value and many health functions (Liu et al., 2007). It also has been used in traditional Chinese medicine for weight loss, detumescence and elimination of phlegm (Guo and Liu, 2008) for almost 1000 years (Li et al., 2005) and was called kelp in traditional Chinese medicine. Furthermore it was shown, in several studies, that LJP has not only anticoagulant, anti-arteriosclerosis, anti-tumor, anti-virus, could improve the quality of freezing spermatozoa and other biological properties, but also had anti-radiation effect (Kaeffer et
products. The extracted products were: crude sodium alginate, fucoidan and laminarin respectively. The purity was qualified by a specific rotary power (Li and Dou, 2007) and gel chromatography (Yang et al., 1997) then we mixed them uniformly and determined that the ingredient was single. These products were mixed and stored at 4°C in refrigerator for further use.

Experimental animals

Fifty six 4-week-old male Wistar rats were obtained from the CDC Animal Center in Hubei Province (Wuhan, China). These rats were housed in fourteen groups in rectangular cages (40 × 25 × 20 cm) with wire mesh lids under standardized animal room conditions (12 h light/dark photoperiod at 23°C and 60% humidity). Food in pellets and tap water were available ad libitum. Animal experiments were performed according to the guidelines for the care and use of laboratory animals established by Wuhan University (Wuhan, China). These guidelines are in accordance with the Declaration of Helsinki of the World Medical Association (Wang et al., 2002).

Experimental grouping and treatment

In order to explore the spontaneous recovery of rat’s spermatogenic function caused by chronic local ionizing radiation and the effect and potential mechanisms of recovery, which was exhibited by the use of L. japonica polysaccharides (LJP), of the damaged rat’s spermatogenic function caused by chronic local ionizing radiation, so after seven days of adaptive feeding, the male rats were randomly divided into seven groups: radial model 1d, 7d,14d groups and interfered with LJP radial model 1d,7d,14d groups which the rats were killed at (1, 7 and 14) days after the cessation of irradiation, and the normal control group that the rats were killed at the 14th day after the cessation of irradiation in the same manner though without irradiation, and there were eight rats in each group. Then, the groups were named as control group, model 1d group, model 7d group, model 14d group, LJP 1d group, LJP 7d group and LJP14d group, respectively. Because the rats in this study need to be lavaged from 5-week-old, and the weight was lighter at this time, so we adopted 100 mg·kg⁻¹·d⁻¹ as intervention dose according to the formerly results of our group (Luo et al., 2004). The rats in LJP 1d, 7d and 14d groups were lavaged with LJP dissolved by physiological saline; the other groups were lavaged with physiological saline. The model 1d, 7d, 14d groups and LJP1d, 7d, 14d groups were administrated with ⁶⁰Co partial irradiation on the lower abdomen while the places above the anterior superior iliac crest were covered with 3 mm thick grid except the control group. The radiation source-target distance was 80cm. The total dose was 2.3 Gy·rat⁻¹ and the absorbed dose rate was 0.925 Gy·min⁻¹ (GWJX80 ⁶⁰Co teletherapy machine, Nuclear Power Equipment Manufactory, China).

The study of spermatogenic cell and its mitochondrial structure and injury of spermatozoa on rat

Testicular tissue of 3 mm³ (1 mm × 1 mm × 3 mm) size was obtained rapidly and fixed with 2.5% glutaraldehyde phosphate solution then we prepared electron microscopic sections. The structure of mitochondrion in testicular cell was observed by means of transmission electron microscope (H-600, Hitachi Company, Japan). A 10% neutral formaldehyde solution was used to fix the testes which were then embedded in paraffin and sectioned into 4 μm sections.

We then performed HE staining and light microscopy on testicular...
microscope camera to take a photograph (Olympus Company, Japan).

The shredded epididymis was transported into 6 ml normal tube with saline, then put in water bath at 37°C for 10 - 15 min. It is dripped into blood count panels and count sperm amount (N) in 5 boxes under microscope (200X). The amount of the sperm was diluted to N × 5 × 10^5 times per milliliter. Sperm survival rate was determined by using one-step eosin-nigrosin staining technique (Byrdnahl et al., 2003). Sperm that were white or unstained were classified as live, while those that showed pink or red coloration in the head region were considered dead. At least 200 spermatozoa were assessed for each preparation. Sperm survival rate = unstained sperm count / (stained + unstained sperm count) × 100% (BX51 microscope, Olympus, Japan).

The study of oxidative damage of testicular tissue on rat

Testis was separated, taking 1/2 testicular tissue of one side, rinsed with 0.9% sodium chloride solution thoroughly. After measuring, it was made into 10% tissue homogenate with 4°C 0.9% sodium chloride solution, and then centrifuged at 1000 g for 10 min under 4°C (5702 centrifuge. Eppendorf Company, Germany) and the supernatant were packaged. Oxidative indicators, such as MDA, SOD and GSH-PX were determined according to the manual strictly by using the spectrophotometer (722 Spectrophotometer, Shanghai Precision Scientific Instrument Ltd, China).

The study of mitochondrial membrane potential of testicular cell and the injury of DNA on rat

The other 1/2 testis was put into -70°C ultra cold freezer (Haier Company, China) for further use; frozen sections were made according to the manual of JC-1 kit. Eight slides in each group were selected to take photograph by using laser scanning confocal microscope (LSCM) (TCS SP2-AOBS-MP, LEICA Company, Germany). We dealt with each slides by Image-Pro plus 6.0 software according to Muramatsu’s method (Muramatsu et al., 2007), collected the data, and indicated the level of mitochondrial membrane potential (ΔΨm) measured at 590 nm for J-aggregates (red fluorescence) and at 530 nm for J-monomer (green fluorescence). The ratio of 530/590 nm was considered as the relative ΔΨm value.

Single cell suspension was prepared in the remaining 1/2 testis after the capsule and blood vessel were removed, adjusted cell concentration for 10^5 - 10^6/ml; stored at 4°C in refrigerator for further use. The experiment determined cell survival rate by observing trypan blue stain effect (Zhang et al., 2001). We carried it out with basic alkaline single cell gel electrophoresis empirical method modified by Singh et al (1988), and observed the results by fluorescence microscope, took a photograph synchronism by BX51 fluorescence microscope attached to a solid-state camera (Olympus, Japan). Eight slides were prepared for each treatment. One hundred cells per slide were randomly counted for incidence of comet-like cells and 20 cells measured for tail length. Tailling rate (%) = (maximum total length between comet head and tail–head diameter) / (sum of all total length). Statistical significance in the difference between the means of the control group to model and LJP groups was evaluated by one-way analysis of variance (ANOVA), and between LJP groups and corresponding model groups was evaluated by independent-sample t-test. Significance was set as P < 0.05.

RESULTS

LJP on recovery of rats’ spermatogenic cells and sperm damaged by radiations

In the control group, we could see spermatogonia, spermatocytes, spermatids arranged compactly and regularly in turn from basal lamina to cavosurface in seminiferous tubules of testis, and there were piles of mature sperm in lumens. Compared with control group, the model groups, stratifications and numbers of spermatogenic cells in rats’ seminiferous tubules of testis decreased gradually. The spermatogenic cells were arranged crumbly, and mature sperms in lumens decrease, but there were more cells cast-off; the basilar membrane of seminiferous tubules in model groups crimp intensify, and the cell layer of spermatogenic cells is indiscriminate in model 14 d group, and cavity was formed; the change of LJG groups is similar to the corresponding model groups, but the arrange of the cell layer of spermatogenic cells, cells cast-off in lumens and the number of mature sperm were better than that in the corresponding model groups. The results are shown in Figures 1A-C.

As is shown in Figures 2A-C, from the electron microscope, we could see that there was more mitochondrion in cytoplasm of the control group, and the size of it was almost the same. The structure of spinal meninges was sharp. In eukaryotic cells, oxidation phosphorylation was proceeding mainly on spinal meninges of mitochondrion, and it was the central link of energy metabolism. When radiation injuries was occurred, the mitochondrial matrix would expanse, the spinal meninges would decrease, and the oxidation phosphorylation in mitochondrion would be inhibited, so the normal energy metabolism in cells was affected (Xia et al., 1998). But in model groups, electron-dense lipid granule could be seen in cytoplasm, mitochondrion in cytoplasm which has differed in size has swelling. Spinal meninges have decreased, cavities have formed and parts of mitochondrion have degenerated; and the mitochondrion has damaged seriously. In LJP groups, there were lipid granules in cytoplasm, the size of mitochondrion almost the same, the structure of spinal meninges was sharp than that of model groups, some of the mitochondrion has swelling, but the injury was slighter than that of the corresponding model groups.

From Table 1 we could see that, compared to the control group, the sperm count has decreased obviously
in model groups and LJP groups, the difference was significant (P< 0.05); compared with corresponding model group, and the sperm count of the LJP groups was higher than Qiong et al. 1403

Figure 1. LJP on recovery of rats’ testicular seminiferous tubules damaged by radiations. (A) The control group, (H&E staining, x200). (B) Model 14 d group, (H&E staining, x200). (C) LJP 14 d group, (H&E staining, x200).

Figure 2. LJP on recovery of rats’ testis cells mitochondrion damaged by radiations. (A) The control group, (TEM×4000). (B) Model 14 d group, (TEM×4000). (C) LJP 14 d group (TEM×4000).

Table 1. Effect of LJP on sperm count and survival rate in irradiated rat.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sperm count (×10⁶/ml)</th>
<th>Sperm survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.60 ± 1.22</td>
<td>74.50 ± 1.25</td>
</tr>
<tr>
<td>Model 1d</td>
<td>15.15 ± 0.70*</td>
<td>57.19 ± 3.48*</td>
</tr>
<tr>
<td>Model 7d</td>
<td>14.25 ± 0.36*</td>
<td>55.25 ± 3.15*</td>
</tr>
<tr>
<td>Model 14d</td>
<td>13.73 ± 0.50*</td>
<td>55.19 ± 3.12*</td>
</tr>
<tr>
<td>LJP 1d</td>
<td>20.48 ± 0.50†</td>
<td>61.97 ± 2.65†</td>
</tr>
<tr>
<td>LJP 7d</td>
<td>19.28 ± 0.50†</td>
<td>64.75 ± 4.12†</td>
</tr>
<tr>
<td>LJP 14d</td>
<td>18.75 ± 0.48†</td>
<td>69.75 ± 2.70†</td>
</tr>
</tbody>
</table>

Note: n = 8 (in each group); (mean ± S.D); *: P<0.05 compared with control group; †: P < 0.05 compared with corresponding model group.

As shown in Table 2, the content of MDA in model groups and LJP groups was obviously higher than the control group (P < 0.05); and it obviously has decreased more in LJP groups than in corresponding model groups (P < 0.05). Compared with control group, the activity of SOD in model groups have decreased obviously (P < 0.05), but in LJP 1d group, the activities of SOD have increased (P<0.05), and in LJP 7d, 14d groups, the activities of SOD have decreased obviously (P < 0.05); The activities of

the corresponding model groups (P < 0.05). The tendency of survival rate of sperm and its count were identical in model groups, but in LJP groups, it was opposite. The differences between the model groups, LJP groups and the control group were all statistically significant (P < 0.05).

LJP on recovery of rats’ testicular tissue oxidation damaged by radiations

As shown in Table 2, the content of MDA in model groups and LJP groups was obviously higher than the control group (P < 0.05); and it obviously has decreased more in LJP groups than in corresponding model groups (P < 0.05). Compared with control group, the activity of SOD in model groups have decreased obviously (P < 0.05), but in LJP 1d group, the activities of SOD have increased (P<0.05), and in LJP 7d, 14d groups, the activities of SOD have decreased obviously (P < 0.05); The activities of
SOD were increased activity in LJP 1d, 14d groups compared to corresponding model groups (P < 0.05). The activity of GSH-PX in the model groups and LJP groups have significantly decreased compared with control group (P < 0.05); but were obviously higher in LJP groups compared to the corresponding model groups (P < 0.05).

LJP on recovery of rats' testis cells mitochondrial transmembrane potential and DNA damaged by radiations

Detected by JC-1 staining, as it was shown in Figure 3, the Δψm was higher than the corresponding model groups, and was obviously higher in LJP groups compared to the control group (P < 0.05).
LJP 7, 14d groups than that in model 7, 14d groups (P<0.05). We used fluorescence microscope to observe single cell gel electrophoresis, the results were shown in Figure 4(A-E), in control group, we could see tadpole shape sperm head, and other testis cells were all with round fluorescent head, which indicating no DNA breakage. In model groups and LJP groups, we could see the breakage of DNA in some Qiong et al. (2005).

![Figure 4](image_url)

**Figure 4.** LJP on recovery of rats’ testis cells DNA damaged by radiations. (A) The control group, (EB staining ×400). (B) Model 14 d group, (EB staining ×400). (C) LJP 14 d group, (EB staining ×400). (D) Comparison of comet tail length in each group; (E) Comparison of comet tailing rate in each group; n = 8 (in each group), (mean ± S.D); *: P < 0.05 compared with control group; †: P < 0.05 compared with corresponding model group.

of testis cells, the fragment moved to anode, the middle part of the head showed dense but the periphery light, and there was spindle-shaped or radiated Comet tailing phenomenon, symptomatizing DNA damage. With the cessation of radiation time prolonging, the number and length of the tailing testis cells were all increasing obviously in model groups and LJP groups compared to control group (P<0.05), but were decreasing in LJP groups compared to model groups (P<0.05).

**DISCUSSION**

As is known to all, *L. japonica* is a plants resource for both food and medicine. LJP is an important functionality component, extracted from *L. japonica*; although Skoryna, Harrison, Fan, Li et al studied the anti-radiation function of it (Fan and Chen, 1988; Zhang and Zhang, 1999; Li et al., 1999, 2002), but there were scarce reports on LJP on recovery of damage by chronic local of ionizing radiation. Our study is showing that in 14 spontaneous recovery days of rat, after the cessation of radiation, the anti-oxidant system of testis has been destroyed and the recovery was slow in the model groups, however, with the recovery time prolonging, in LJP groups, slowed down the lipid peroxidation of testis tissue produced by chronic radiation effectively, enhance the activity of antioxidase, improve the recovery of anti-oxidant defense system, and it was accordance with previous reports that LJP had the effect of anti-lipid peroxidative (Yan et al., 2003; Zhu et al. 2008), and it was also concerned with protecting the spermatozoa quality by reducing the oxidative damage of spermatogenic organ (Chitra et al., 2003).

Our study is also showing that, at the recovery stage of radiation injury in each model group, the mitochondrial membrane permeability of testicular cell on rat
testis cells, the injury of seminiferous tubules became more serious and finally the number and survival rate of spermatozoa decreased. Although the decrease in the mitochondrial membrane potential, aggravation of the injury of DNA, seminiferous tubules and mitochondrial with time prolonging in LJP groups; but still less than those in the corresponding control groups (Figures 1 - 4). which showed that LJP could amend the mitochondrial membrane potential, release the injury of mitochondrion, what followed next, it promoted the recovery of DNA in testis spermatogenic cells of the rat, slowed down the damage of spermatogenic cells in convoluted tubule, maintained the spermatogenic function of testis, and raise the number and survival rate of sperm (Table 1).

Conclusions

From above we can draw a conclusion that in the recovery stage, at the cessation of radiation for 14 days, the damage of rat testicular tissue continued, the spontaneous recovery of spermatogenic function unobvious, lavaged with LJP100mg·kg$^{-1}$·d$^{-1}$ to rat from 7 days before irradiation to the end of irradiation could release the injury of testicular tissue on rat s and promote the recovery of spermatogenic function in rat s testis. The mechanism concerned not only with that LJP could enhance the ability of antioxidase, release the DNA damage of spermatogenic cells and the oxidative injury in testis tissue, improve the recovery of anti-oxidant defense system, but also that LJP could promote the upswing of mitochondrial membrane potential, release the injury of mitochondrion, ensure the normal energy metabolism of testis cells, and promote the recovery of DNA injury in spermatogenic cells.

ACKNOWLEDGEMENTS

This work was supported by the Food Nutrition and Education Fund of DANONE Institute (DIC2007-06, China). We are grateful to Dr. You Lu and Chen Chuang (Wuhan University) for their revision and comments on the manuscript.

REFERENCES


