

Effects of Gamma-Irradiation on Microbial Contamination and on Histological Changes of Muscle in *Poecilia reticulata*

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Abstract

This study describes the effect of gamma-irradiation on the microbial skin and organ contamination and on the histological changes of fish skeletal musculature. The aquarium fish *Poecilia reticulata* were exposed to the radiation effect of ⁶⁰Co at the doses of 20 and 30 Gy (11.36 Gy/min). Frequent intravital haemorrhages were observed from day 8 after the exposure. A general long-term body fading appeared from day 10 to day 30 of the experiment. Microbiological and histological examinations were done on days 7, 14, 21 and 28. Individual muscle cells were bound together into fascicles surrounded by connective tissue. No significant morphological changes were observed on skeletal muscle cells, except for more watery connective tissue.

The presence of *Escherichia coli* and *Aeromonas hydrophila* was proved in the body surface samples and in aquarium water based on the microbiological test. A moderate increase of the number of *E. coli* and *A. hydrophila* was observed in the intestines in both exposed groups (20 and 30 Gy) depending on the time that elapsed from the exposure (from 1×10^1 to 6×10^3 CFU). However, the heart, liver and musculature remained sterile. No microbial contamination of musculature was found for the intravital ionizing radiation dose of 30 Gy even for the single specimen killed on day 28 from the exposure.

Ionizing radiation, foodstuff safety, radiation sickness, model fish

An extensive introduction of nuclear energy in practical fish culture is called alternative radiation protective methods of fish health. For this reason, detailed knowledge of biological effects of ionizing radiation for pathogenesis of radiation sickness is very important (Albaum 1960; Be ová et al. 2006). At present, abundant data on different aspects of ionizing radiation and its impacts on the living organisms have been gathered (Flory and Neuhaus 1976; Falis et al. 2004). However, a unified theory of the biological effects of this mechanism on the organisms has not been published yet. In many studies, little attention has been paid to the serious biological effects such as impaired immunity and possible auto-infections in relation to food safety (Puzová et al. 1962; Be ová et al. 2002).

Unlike in mammals and birds (Kunstý and Pospíšil 1962; Kalina et al. 1994), the gamma-irradiation effect in fish has been investigated in a relatively small group so far. However, the meat of fish treated by gamma radiation can represent a remarkable risk for the consumers. Additionally to non-pathogenic organisms, fish and other water organisms may contain microorganisms that have a negative effect on human health. Especially, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* (Pipová et al. 2006) are the most important pathogens.

Higher radiation doses may cause the gastrointestinal syndrome that leads to defects of the intestinal mucosa barrier with successive contamination of musculature.

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From the experimental point of view, aquarium fish such as *Poecilia reticulata* and *Danio rerio* present a suitable experimental model to study the above-mentioned questions (Lešník and Jurina 1994).

Materials and Methods

We used *Poecilia reticulata* from our own laboratory breeding colony. The body weight of the fish varied from 4 g (males) to 6 g (females). The breeding was maintained under constant ambient conditions, i.e. water with a temperature of 24 °C, Ca + Mg components of 0.7 mmol/l, pH-factor of 7.04, artificial aeration, water filtration, photo-period of 12:12 h L:D (OECD 1992).

The fish were fed living organisms (*A. franciscana*) and dry and granular fodder (AQUAEXOTIC, Kapušany, Slovakia). Fish specimens used for the experiments were randomly selected, divided into groups and kept under standardized conditions and fed only the granular highly nutritious fodder specified for this species (Easy fish). Two groups of fish were formed for the test of two gamma radiation doses at the beginning of the experiment. There were thirty fish specimens (a total of 90 specimens in three replicates) in each experimental group. Twenty-litre aquaria were used to keep the fish of both groups during the experiment.

The fish were exposed to the gamma-radiation doses of 20 and 30 Gy using Chisostat (⁶⁰Co source and the dose rate of 11.36 Gy/min). They were exposed to radiation in Petri dishes in aquarium water, with the water column of 1 cm. The control samples were sham-exposed, i.e. the control samples were subjected to the complete procedure except for gamma radiation exposure.

For 30 days from the exposure every day we observed and recorded data on all the changes manifested on the body surface. On days 7, 14, 21 and 28 after the radiation exposure, we took sterile samples of the intestine, heart, liver and muscle from six fish specimens (6 × 4 + 6 control), and performed microbiological tests. The body surface samples and water samples also were subjected to the microbiological test. The results were completed with the histological examination of muscle.

The samples intended for histological examinations were processed using standard methods. All pieces of fish were fixed in 3% neutral formalin and covered with paraffin. The histological sections with a thickness of 7 µm were stained with haematoxylin-eosin. They were viewed by means of the JENAMED optical microscope with a micro-camera.

Blood agar, McConkey agar, End agar and Brain Heart Infusion Broth substrate (Mikula et al. 1997) were used in the microbiological tests. The samples were shaken out and diluted in saline by the quantitative method for determining the number of bacteria in 1 ml. Specific kinds of bacteria were detected by commercial testing products (ENTEROTest 16, LACHEMA Brno and NEFERMtest 24).

Results

Haemorrhages were observed on the exposed fish surface based on the changes that were manifested during days 8 to 12 after exposure in both experimental groups. The most significant haemorrhages were observed in the area of liver and the lower part of the body cavity (Plate XIII, Fig. 1). Fading of the body colouration was detected from day 10 and it persisted till day 30 from the exposure day. We observed a reduced food intake in both experimental groups.

Microbiological investigations of the body surface sample and the aquarium water sample yielded the same results. *E. coli* and *A. hydrophila* were present in both cases.

A moderate increase of the number of *E. coli* and *A. hydrophila* (Table 1) was observed in the intestine in both exposed groups (20 and 30 Gy). Heart, liver and muscles remained sterile in all the monitored periods after the exposure.

Skeletal muscle cells (Plate XIII, Fig. 2) were not damaged, and additionally their surface was covered by a continuous membrane - sarcolemma. In the sarcoplasm they contained multiple oval nuclei, located peripherally or centrally within the cell. When viewed in the longitudinal section, transverse striations were present as alternating light

Table 1. The changes of microflora (CFU) in the fish intestine depending on the exposure time

		Control	Day 7		Day 14		Day 21		Day 28	
			20 Gy	30 Gy	20 Gy	30 Gy	20 Gy	30 Gy	20 Gy	30 Gy
Intestine	<i>E. coli</i> in CFU·g ⁻¹	2 × 10 ¹	1 × 10 ¹	2 × 10 ³	3 × 10 ³	2 × 10 ³	3 × 10 ³	3 × 10 ³	5 × 10 ³	3 × 10 ³
	<i>A. hydrophila</i> in CFU·g ⁻¹	3 × 10 ¹	3 × 10 ¹	3 × 10 ¹	2 × 10 ³	1 × 10 ³	3 × 10 ³	4 × 10 ³	6 × 10 ³	5 × 10 ³

and dark bands. Each muscle cell contained myofibrils composed of thick and thin myofibrils, responsible for contraction. Thick and thin myofibrils overlapped in darker A band (anisotropic), whereas only thin myofibrils were present in the lighter I band (isotropic). Individual muscle cells were bound together into fascicles surrounded by connective tissue. No significant morphological changes were observed on the skeletal musculature, only the tissue seemed more watery than in the control specimens.

Discussion

In general, as a consequence of high radiosensitivity of the dividing enterocytes, their production in crypts is interrupted; and their movement to the villus peak and replacement of necrotic enterocytes is observed. Complete recovery process of the intestinal epithelium, and moreover, the intestinal mucosa loses its functional capability. From the clinical point of view, this status is characterized by diarrhoea, nausea and successive cachexia. Damage of the intestinal barrier is typical during irradiation sickness in mammals and it related to high radiosensitivity of endothelial cells (Procházka and Dvořák 2002; Beová et al. 2002).

In our study, an increase of blood capillary penetrability led to the development of haemorrhagic diathesis which was manifested by haemorrhage in different parts of the body of the monitored pieces of fsh. Haemorrhages were similar to the changes described by Hršel (1950) as inflammation processes on the skin of the exposed pieces of fsh. The body colour fading often occurs in fsh during changes of life conditions. This change of body colour is usually of only short-term nature (Samochvalová 1935; Hršel 1950). In our experiment, the fading persisted approximately from day 10 till the completion of the experiment, that is, 30 days after the exposure.

Lack of appetite observed in our experiment in *Poecilia reticulata* exposed to X-rays was similar to that described in mammals and birds after exposure as reported by Seidelová (1986).

Penetration of the existing bacteria commonly present in the digestive tract of the exposed organism through the mucosa and the damaged blood capillaries of the intestinal wall into the blood circulation under physiological conditions was described in mammals. The found bacteria penetrate other organs and skeletal muscles through blood (Kunstý and Pospíšil 1962; Puzová et al. 1962). In our trial, a moderate increase of the number of bacteria in the digestive tract was observed in the monitored fsh. Organs and musculature remained sterile.

Microflora of the living fsh depends on the microbial water contamination in which the individuals live (Frazier 1967). The following are the most often occurring genera: *Alcaligenes*, *Acinetobacter*, *Bacillus*, *Enterobacteriaceae*, *Flavobacterium*, *Pseudomonas* and *Vibrio*. Besides non-pathogenic organisms, fsh and water animals may contain even microorganisms that can endanger human health. Of the most important pathogens we can mention the following: *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* (Pipová et al. 2006).

Frazier (1967), Grieger and Vařejka (1991) found that the number of microorganisms on the fsh surface ranged from hundreds to many million per square centimetre. The number of microorganisms in the digestive tract ranged from 10^3 to 10^8 CFU·g⁻¹. The total numbers of microorganisms observed in fsh muscle were from 0 to 10^3 CFU·g⁻¹, which corresponds to our results. The great differences in the species spectra and number of microorganisms in fsh skin and fsh muscles led us to the conclusion that the fsh skin (when not damaged) formed an effective barrier.

We did not observe any significant morphological changes in fsh musculature. The changes on skin due to the exposure did not damage the barrier, and the musculature was

contaminated neither by the microorganisms present in the aquarium water nor by auto-infection by the bacteria present in the digestive tract. *Aeromonas* bacteria are ubiquitous suspected pathogenic organisms of the aquarium fish. They induce a pathological response particularly under unsuitable living conditions. However, the symptoms of such infections are different from those resulting from radiation sickness. The infection of *P. reticulata* was proved in the grey spots on skin, resulting in the erythema symptom with protrusions of the affected zones above the skin surface. This process was best illustrated in *P. reticulata* by Bassleer (2003). However, the observed erythema was not of the haemorrhagic character considered as a symptom of radiation sickness. No other changes of skin or gills were observed.

Alternative model systems are used in biomedical research (Dvořák and Beová 2002; Sklenář et al. 2006; Nováková et al. 2007) in order to protect higher vertebrates. Our experiments have not proved any microbial contamination of fish musculature even 28 days after the intravital exposure of specimens to the ionizing radiation dose of 30 Gy.

Vplyv žiarenia gama na mikrobiálnu kontamináciu a na histologické zmeny svaloviny *Poecilia reticulata*

Táto práca popisuje útok žiarenia gama na mikrobiálnu kontamináciu kože a orgánov a na histologické zmeny kostrovej svaloviny ryby. Akvárijské ryby *Poecilia reticulata* boli vystavené útokom žiarenia ^{60}Co v dávkach 20 a 30 Gy (11,36 Gy/min). Intravitálne boli od 8. dňa a po ožiarení pozorované rozsiahle krvácaniny. Celkové vyblednutie tela sa prejavilo dlhodobo 10. - 30. dňom experimentu. Na 7., 14., 21. a 28. dňoch bolo urobené mikrobiologické a histologické vyšetrenie. Makroskopicky bola pozorovaná vodnatejšia svalovina. Došlo k intenzívnejšej väzbe vody v riedkomych vláknach, čím boli svalové vlákna od seba viac oddialené. Mikrobiologickým vyšetrením steru z povrchu tela a akváriovej vody bola preukázaná prítomnosť *E. coli* a *Aeromonas hydrophila*. V prípade rýb oboch ožiarených skupín (20 a 30 Gy) došlo k miernemu zvýšeniu počtu *E. coli* a *A. hydrophila* v závislosti na dobe od ožiarenia (od $1 \cdot 10^1$ po $6 \cdot 10^3$ CFU·g⁻¹). Srdce, pečeň a svalovina však boli sterilné. V tomto pokuse nebola preukázaná mikrobiálna kontaminácia svaloviny intravitálnou dávkou ionizujúceho žiarenia 30 Gy ani u rýb usmrtených 28 dní po ožiarení.

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