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Interaction of Low Doses of Ionizing Radiation, Potassium Dichromate and Cadmium Chloride in *Artemia franciscana* Biotest

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Abstract

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The influence of cadmium chloride (at concentrations of 100 and 200 mg·l^{-1}) and potassium dichromate (at a concentration of 50 mg·l^{-1}) along with the effect of gamma radiation ^{60}Co (at a dose of 10 and 50 Gy) on lethality to *Artemia franciscana* was investigated.

Four different interactions were studied, namely, those of potassium dichromate and gamma radiation, cadmium chloride and gamma radiation, and combinations of potassium dichromate and cadmium chloride in interaction with gamma radiation. A significant ($\alpha = 0.05$) decrease was observed in lethality due to exposure to radiation (10 Gy) in comparison with action of only potassium dichromate and cadmium chloride or their combination without exposure to gamma rays. These results support the theory of hormesis.

Artemia salina, hatching, nauplii, gamma radiation, hormesis, radiobiology, lethality

The present trend is to decrease the number of experiments using animals to a minimum. According to the EC Directive (1986), animals are all live vertebrates except for humans, including the free-living ones and their larvae capable of reproduction, but not the foetuses or embryos. Therefore we carried out this study on *Artemia franciscana* using an alternative biotest of second generation (Dvořák and Beňová 2002). With regard to taxonomic revisions of the nomenclature of the genus *Artemia*, this species is in the vast majority of older studies presented as *Artemia salina.* Invertebrates including the brine shrimp *Artemia salina* (Leach, 1814), are used as test organisms within second-generation bio-tests. Among them also *Artemia salina* (Leach, 1814) a brine crustacean.

A number of authors described the effect of higher doses of gamma radiation on higher vertebrates (Sesztáková 1996; Beňová et al. 2003; Falis et al. 2004) and *Artemia salina* (Dvořák and Beňová 2002).

On the other hand, low doses of ionizing radiation can have also positive effects (the so-called radiation hormesis). This effect is characterised as a stimulation of many processes, such as germination of seeds and growth of many plant species, increase in activity of individual enzymes, stimulation of bacterial division and isolated mammalian cells, prolongation of life of water plankton, drosophila, mice and rats after chronic irradiation with extremely low doses, increased radio-resistance to repeated irradiation with large doses (the so-called adaptation response), decreased mortality of humans due to oncological diseases (Hrnčíř 1999).

With the growing environmental pollution, the interest in the impact of xenobiotics, including hazardous chemical elements, on live systems increases (Kovalkovičová et al. 2000).

At present, the current research focuses on essential elements, such as Cu, Mo, Se and Zn,

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that are extremely important to the organism for many catalytic, enzymatic, activation and regulation processes (Amodio-Cocchieri et al. 2003; Davidson et al. 2004; De la Sienra et al. 2003).

On the other hand, cadmium is an element highly toxic to organisms living in water environment (Drastichová et al. 2004; Koréneková et al. 2002).

In our experiment we investigated the influence of cadmium chloride (at concentrations of 100 and 200 mg·l^{-1}) and potassium dichromate (at concentration 50 mg·l^{-1}) along with the effect of gamma radiation ^{60}Co (at a dose of 10 and 50 Gy) on lethality to *Artemia franciscana*.

Materials and Methods

The experiment was carried out on *Artemia franciscana* hatched in sea-water (Dvořák et al. 2005). Ten freshly hatched nauplii stages were placed into sea-water in polystyrene Petri dishes, 60 mm in diameter on average (the total volume of liquid and sample was 10 ml). Cadmium chloride ($CdCl_2.2H_2O$) solutions of concentrations 10, 100 and 200 mg·l^{-1} and potassium dichromate ($K_2Cr_2O_7$) solutions of concentrations of 5 and 50 mg·l^{-1}, prepared in sea-water, were used.

Nauplii stages were irradiated with gamma rays at the doses of 10 and 50 Gy (^{60}Co-source, CHISOSTAT) with a dose input of 11.36 Gy min^{-1}.

Fifteen experimental groups were formed (Table 1).

Each group consisted of 50 homogeneous individuals divided to 5 separate groups (dishes), 10 in each group. Altogether 800 *Artemia franciscana* were used in the experiment. Petri dishes were placed to a thermostat set to 20 ± 1 °C.

Table 1. Experimental *Artemia* were divided to 15 groups as follows

Identification of groups	Concentration $K_2Cr_2O_7$ (mg·l^{-1})	Concentration $CdCl_2$ $2H_2O$ (mg·l^{-1})	Dose of ionising radiation (Gy)
0 Gy	0	0	0
10 Gy	0	0	10
50 Gy	0	0	50
100 Cd 0 Gy	0	100	0
50 Cr 0 Gy	50	0	0
50 Cr 10 Gy	50	0	10
50 Cr 50 Gy	50	0	50
200 Cd 0 Gy	0	200	0
200 Cd 10 Gy	0	200	10
200 Cd 50 Gy	0	200	50
100 Cd 50 Cr 0 Gy	50	100	0
100 Cd 50 Cr 10 Gy	50	100	10
100 Cd 50 Cr 50 Gy	50	100	50
200 Cd 50 Cr 0 Gy	50	200	0
200 Cd 50 Cr 10 Gy	50	200	10
200 Cd 50 Cr 50 Gy	50	200	50

At the time intervals 24, 48, 72, 96 and 120 h we counted all live *Artemia*. The results were compared with the control group and evaluated statistically. In order to eliminate the remote results for individual dishes, we used the Dean-Dixon test (Dvořák and Šucman 1995). Significance of differences between means of individual groups was tested according to Wayland and Hayes (1991).

Results

Fig. 1 and Table 2 show differences between the groups at 48 or 72 h after beginning of the experiment.

With all four observed interactions, i.e. potassium dichromate and gamma radiation, cadmium chloride and gamma radiation and combinations of potassium dichromate and

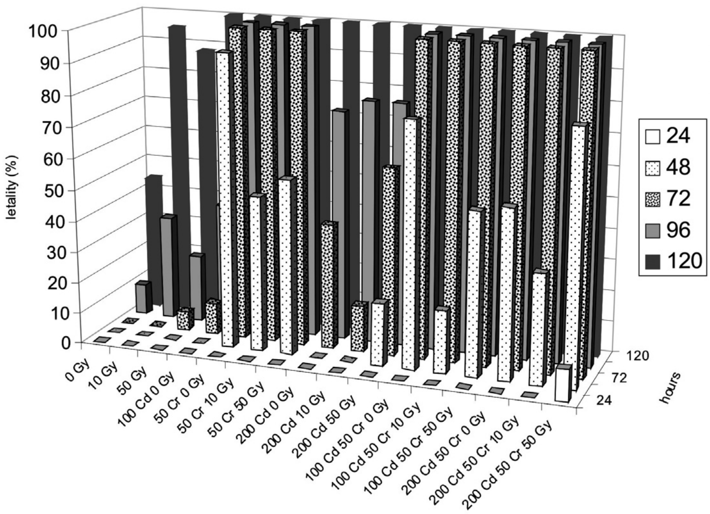

Fig. 1. Lethality in *Artemia franciscana* caused by interaction of cadmium chloride ($CdCl_2.2H_2O$), potassium dichromate ($K_2Cr_2O_7$) and gamma radiation

cadmium chloride with gamma radiation, we observed a significant decrease in lethality after irradiation with gamma rays at a dose of 10 Gy in comparison with the action of potassium dichromate and cadmium chloride or their combinations without the influence of gamma radiation.

The effect of the higher dose of ionizing radiation (50 Gy) together with potassium dichromate was only slightly higher compared to the dose of 10 Gy, however, the 56% lethality was significantly lower than 94% lethality caused by potassium dichromate at a concentration of 50 $mg{\cdot}l^{-1}$.

When applying cadmium chloride at a concentration of 200 $mg{\cdot}l^{-1}$, observation after 72 h interval showed that the 50 Gy dose caused significantly higher lethality in comparison with the toxicity of cadmium chloride alone. Evaluation at the 72 h interval was related to lower toxicity of 200 $mg{\cdot}l^{-1}$ cadmium chloride in comparison with the toxicity of 50 $mg{\cdot}l^{-1}$ potassium dichromate. Moreover, cadmium chloride at concentration of 200 $mg{\cdot}l^{-1}$ exhibited about 4-fold higher lethality in comparison with 100 $mg{\cdot}l^{-1}$ (40% or 10% at delay of 72 h).

The simultaneous 48-h action of cadmium chloride at a concentration of 100 $mg{\cdot}l^{-1}$ and potassium dichromate at a concentration of 50 $mg{\cdot}l^{-1}$ together with irradiation by a dose of 50 Gy resulted in a significantly higher mortality (52%) in comparison with the action of 10 Gy (20%). However, also in this case the joint action of chemicals and gamma rays at a dose of 50 Gy was significantly lower than the action of both potassium dichromate and cadmium chloride without exposure to ionizing radiation (52% and 78%, respectively).

Contrary to that, 48 h after beginning of the experiment, the interaction of 200 $mg{\cdot}l^{-1}$ cadmium chloride (i.e. double concentration than that used in the previous case) and 50 $mg{\cdot}l^{-1}$ potassium dichromate with 50 Gy dose of gamma radiation caused significantly higher lethality in comparison with the action of both chemicals without exposure to gamma rays.

This result is similar to the above mentioned results of interactions observed after 72 h when exposing *Artemia* to 200 $mg{\cdot}l^{-1}$ cadmium chloride and gamma radiation. However, the lethality was higher by approximately 14 to 20%.

Surprising results were obtained after 48 h-long toxic action of potassium dichromate and cadmium chloride without gamma rays. In this case, 48 h after the beginning of the experiment, the highest lethality (94%) was caused by the singular action of potassium dichromate at a concentration of 50 mg·l^{-1}. When cadmium chloride at a concentration of 100 mg·l^{-1} was added on top of potassium dichromate, a significant decrease in lethality to 78% was observed. Still more marked decrease in lethality (to 54%) was observed when the concentration of cadmium chloride was increased to 200 mg·l^{-1}.

Table 2. Lethality in *Artemia franciscana* (%) caused by interaction of cadmium chloride ($CdCl_2.2H_2O$), potassium dichromate ($K_2Cr_2O_7$) and gamma radiation

	24			48			72			96		
	x	n	SD	x	n	SD	x	n	SD	x	n	SD
0 Gy	0	5	0.0	0	5	0.0	0	4	0.0	10	5	8.6
10 Gy	0	5	0.0	0	5	0.0	0	5	0.0	34	5	21.5
50 Gy	0	5	0.0	0	5	0.0	6	5	8.6	22	5	12.9
100 Cd 0 Gy	0	5	0.0	0	4	0.0	10	4	0.0	40	5	30.1
50 Cr 0 Gy	0	5	0.0	94+	5	8.6	100	5	0.0	100	5	0.0
50 Cr 10 Gy	0	5	0.0	50*	5	8.6	100	4	0.0	100	5	0.0
50 Cr 50 Gy	0	5	0.0	56*	5	17.2	100	4	0.0	100	4	0.0
200 Cd 0 Gy	0	5	0.0	0+	5	0.0	40+	5	17.2	74	5	17.2
200 Cd 10 Gy	0	5	0.0	0+	5	0.0	15*	4	4.9	78	5	17.2
200 Cd 50 Gy	0	4	0.0	20*	5	12.9	60*	4	9.7	78	5	25.8
100 Cd 50 Cr 0 Gy	0	4	0.0	78*	5	25.8	100	5	0.0	100	5	0.0
100 Cd 50 Cr 10 Gy	0	5	0.0	20+	5	8.6	100	4	0.0	100	4	0.0
100 Cd 50 Cr 50 Gy	0	5	0.0	52*	4	8.6	100	5	0.0	100	5	0.0
200 Cd 50 Cr 0 Gy	0	5	0.0	54+	5	34.4	100	5	0.0	100	5	0.0
200 Cd 50 Cr 10 Gy	0	5	0.0	35*	5	4.9	100	4	0.0	100	5	0.0
200 Cd 50 Cr 50 Gy	10	4	0.0	80*	5	17.2	100	5	0.0	100	5	0.0

(+ *) Differences between the values were significant ($\alpha = 0.05$)

n = number of Petri dishes, each containing 10 *Artemia franciscana*, included in the experiment

x = arithmetic mean

SD = standard deviation

Discussion

In addition to other authors, Ochi and Ohsawa (1985) described toxicity of cadmium. The theory of its mechanism is based on blocking the SH groups in proteins. This effect is similar to that of ionizing radiation. In all tissues cadmium is bound to metallothionein. Cd, Zn or Cu ions are bound through sulphydryl groups and may constitute up to 11% of metallothionein molecular weight (Frazier 1982; Kottferová et al. 2002). When metallothionein synthesis is insufficient, toxicity of cadmium becomes manifest because the free Cd^{2+} ions are more dangerous for the intracellular enzyme systems (Jayawickreme and Chatt 1990).

Trivalent chromium is a biogenic element playing an important role in metabolism of saccharides or metabolism of insulin as the so-called “glucose tolerance factor” (Mertz et al. 1979). It is also an important factor in various enzymatic reactions, e.g. activation of thromboplastin, β-glucuronidase and bacterial urease (Langard and Norseth 1979). Trivalent chromium does not pass across the cellular membrane contrary to hexavalent chromium that is transported through this membrane by ion transmitters. Then it is metabolised to trivalent chromium (O’Flaherty 1993; Singh et al. 1998). On the cellular level, chromium can lead to cessation of the cellular cycle, apoptosis or neoplastic transformation.

Examination of groups exposed to Cd and Cr, either separately or in combination, showed better viability in irradiated groups. With higher doses of radiation a protective effect of the mentioned metals and their compounds is assumed (Luckey et al. 1975; Luckey and Venugopal 1977). Skalická et al. (2005) described attenuation of the negative effects of cadmium and its decrease in an organism with subsequent positive effect on health in Japanese quails when their diet was supplemented with organic chromium. The effect of essential elements, including chromium, on reduction in toxicity of heavy metals was described also by Chowdhury and Chandra (1987).

On the other hand, this may involve the so-called hormesis, i.e. a protective effect of low doses of ionizing radiation and simultaneous action of other toxic factors. Low doses of ionizing radiation stimulate DNA synthesis and in this way improve the adaptive response of the respective species (Hrnčíř 1999). Low doses stimulate certain phenomena whereas high doses may inhibit them. This means that low doses have favourable effects but the high ones have a harmful influence on biological systems. Considerable number of expert epidemiological studies support the view that low doses of ionizing radiation are, in fact, beneficial also to human health (Luckey 1991; Cohen 1995; Pollycove 1997).

Similar to radiobiology, hormesis is encountered also in toxicology where it can also explain different effects of low and high concentrations (Hrnčíř 1999).

Summation of the effects of cadmium and radiation was studied using higher doses of ionizing radiation (Dvořák and Beňová 2002; Klimová and Mišúrová 2004). These results support the theory of hormesis.

Interakcie nízkych dávok ionizujúceho žiarenia, dichromanu draselného a chloridu kademnatého pomocou biotestu s *Artemia franciscana*

V rámci európskej Konvencie o ochrane stavovcov používaných pre pokusné a iné vedecké účely je jedna z hlavných požiadaviek obmedziť počet pokusov na stavovcoch na minimum. Súčasným trendom je znižovanie počtu pokusných zvierat. To sú dôvody na využívanie alternatívnych biotestov na bezstavovcoch medzi ktoré patrí aj *Artemia franciscana.*

V našom experimente sme sledovali vplyv chloridu kademnatého (v koncentrácii 100 a 200 $mg \cdot l^{-1}$) a dichromanu draselného (v koncentrácii 50 $mg \cdot l^{-1}$) súčasne s účinkom gamma žiarenia ^{60}Co (dávkou 10 a 50 Gy) na letalitu *Artemia franciscana*.

Vo všetkých 4 sledovaných interakciách, dichroman draselný a žiarenie gama, chlorid kademnatý a žiarenie gama, rovnako aj u kombinácií dichromanu draselného a chloridu kademnatého v interakcií so žiarením gama sme pozorovali signifikantné ($\alpha = 0.05$) zníženie letality pri expozícii dávkou žiarenia 10 Gy v porovnaní s pôsobením dichromanu draselného a chloridu kademnatého, alebo ich kombinácií bez vplyvu žiarenia gama. Výsledky tejto práce podporujú teóriu hormezy.

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