ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 2, 26 SECTIO DDD 2010

Bukovinian State Medical University, Department of Medical Chemistry, Department of Pharmacy

IGOR GERUSH, OLEG GERUSH

The state of oxidant and antioxidant systems in the liver at exposure to low-dose ionizing radiation and influence of Echinacea purpurea tincture

Stan układu oksydacyjnego i antyoksydacyjnego wątroby eksponowanej na niskie dawki promieniowania jonizującego i wpływ nalewki z *Echinacea purpurea*

INTRODUCTION

Ionizing radiation has multiple and various effects on the living organisms. They can stimulate or inhibit the physiological and biochemical processes, which leads to a deficiency which can affect the normal cell growth and their normal evolution. Ionizing irradiation was reported to induce oxidative stress with overproduction of reactive oxygen species (ROS), which react rapidly with almost all structural and functional organic molecules, including proteins, lipids and nucleic acids causing disturbance of cellular metabolism. To avoid oxidative damage, animal have evolved various protective mechanisms to counteract the effects of reactive oxygen species in cellular compartments [1].

Exposure to low-dose gamma radiation is common in certain occupations but the biological and health effects of such exposure remain to be determined. Everyone agrees that cellular responses to low values of absorbed doses of ionizing radiation are not readily predictable by extrapolation of responses observed at high doses.

The species which are pharmaceutically important belonging to *Echinacea purpurea* are well-known for their therapeutic qualities being some of the fewest herbs with immunostimulative and antiviral effects. They possess a stimulating influence upon the CNS, they are used in the treatment of infection diseases and have antioxidant properties [2, 3, 5].

The aim of this study was to investigate radioprotective and antioxidant properties of *Echinacea purpurea* tincture (E.P.t) in the liver of rats under conditions of experimental irradiation in low doses.

MATERIAL AND METHODS

The study involved 35 white mongrel rats weighing 140–160 g. The animals were exposed hemibody (below rib cage) to single total X-ray irradiation in the dose of 0.3 Gy. Experimental animals were divided into three groups: I – irradiated animals and not treated; II – irradiated animals

receiving E.P.t. 7 days after irradiation; III – irradiated animals receiving E.P.t. 7 days before and 7 days after irradiation. The tincture made of a dried root of *Echinacea purpurea* was introduced intragastrically every day in the dose of 0.25 ml/kg.

The liver of animals of the I–III groups was examined on the 7th days after the irradiation and compared with the results of the intact group of animals. The liver was rapidly removed, weighed and homogenized in appropriate buffers for determination of glutathione concentration and antioxidant system enzyme activities.

The reduced form of glutathione (GSH) was measured by the Meshchishen method, glutathione-S-transferase (G-S-T), glutathione reductase (GR), glutathione peroxidase (GPx) and glucose-6-phosphate dehydrogenase (G6PD) activities, and the total protein concentration were determined as described previously [5]. Superperoxid dismutase (SOD) and catalase (CAT) activities were determined according to Nashikimi [9] and Koroluk [7], respectively.

Values are presented as mean±SEM and were compared by a Student test. A P-value <0.05 was considered statistically significant.

RESULTS

Irradiation of the animals in the dose of 0.3 Gy caused an increase of superperoxid dismutase (on 7.8%), catalase (on 15.5%) activity (Table 1). The research showed that ionizing irradiation caused considerable changes in the liver glutathione system. Thus, there was found increased activity of G6PD (on 5.5%), GR (on 15.8%) and the content of GSH (on 10.6%) in the liver of rats after irradiation. These changes are a protective, compensatory body reaction to a substantial increase of lipid peroxide oxidation (LPO) ionizing irradiation. It should be noted that the activity of GPx and G-S-T in the liver decreases by 19.1% and 19.2 % in comparison with the indices of intact animals.

Oral administration of E.P.t. to the irradiated rats during 7 days after irradiation contributed normalization of SOD and catalase activity as well as glutathione content. Administration of the medication prevented disorders in the activity of the key enzymes of the glutathione system GPx and G-S-T substantially, and it even resulted in an increase of their activity by 26.9% and 43.0% in comparison with the indices of the irradiated group of animals, it was reliably higher than in the intact group of animals. Althought G6PD activity had a tendency to increase, and GR activity was reliably higher than that of the control, increase of GSH in the liver was not found, which might be connected with its intensive use to render the products of LPO harmless by GPx and G-S-T enzymes.

In the animals which received the preparation before and after irradiation, the studied indices were at the control level.

Table 1. *Echinacea purpurea* tincture influence upon the state of indices of the liver antioxidant system after single irradiation at the dose of 0.3 Gy (M±m, n=7 - 12)

Groups of animals	Examined indices (measurement units)						
	SOD (U/ min/mg protein)	CAT (μmol / min/mg protein)	GSH (μmol/g liver)	GPx (nmol/min/ mg protein)	GR (nmol/min/mg protein)	G-6-PD (nmol/ min/mg protein)	G-S-T (nmol/min/ mg protein)
Control	0.51 ± 0.01	186.5 ± 6.4	6.99 ± 0.05	182.4 ± 2.7	3.85 ± 0.08	8.75 ± 0.05	61.2 ± 1.4
I – irradiation	0.56 ± 0.01**	215.5 ± 9.1*	7.73 ± 0.09**	147.6 ± 4.4**	4.46 ± 0.08**	9.22 ± 0.09**	49.4 ± 2.9**
II – irradiation + E.P.t.	0.50 ± 0.01	188.2 ± 8.4	6.93 ± 0.13	187.4 ± 4.9	4.26 ± 0.06**	8.94 ± 0.09	70.8 ± 3.0*
III – E.P.t. + irradiation + E.P.t.	0.50 ± 0.02	184.0 ± 6.6	7.19 ± 0.07	180.5 ± 9.7	3.96 ± 0.07	8.74 ± 0.12	60.3 ± 4.2

* Possible changes in comparison with intact animals (P<0.05) ** Possible changes in comparison with intact animals (P<0.01)

DISCUSSION

Total single irradiation of rats was established to cause activation of lipid peroxidation in the liver. To avoid oxidative damage, animal have evolved various protective mechanisms to counteract the effects of reactive oxygen species in cellular compartments. One of the protective mechanisms was the enzymatic system, which operates with the sequential and simultaneous actions of a number of enzymes including SOD and CAT [4].

SOD activity, as well as that of catalase, is characterized by increasing under the influence of irradiation, which is indicative of their radioresistance. A stimulating effect of low-dose radiation upon SOD activity might be the result of an increased amount of the superoxide anion radical and induction of the enzyme synthesis [10].

The most substantial changes were observed in the liver glutathione system of irradiated animals, which was manifested by the increased content of GSH and activity of GR and G6PDH as compared with the control, which is indicative of the mobilization of the liver endogenic antioxidant resources. On the other hand, we can observe inhibition of the ways to use GSH by GPx and G-S-T enzymes, which activity is inhibited under ionizing radiation eventually resulting in the increase of GSH content in the liver of the irradiated animals. Another way should not be rejected, for example, induction of GSH synthesis in the liver and its increase in the liver of the irradiated animals.

It should be noted that reduction of GPx activity and an increase of SOD activity promote substantial accumulation of hydrogen peroxide. The latter recombining with superoxide-anione-radacal, promotes formation of hydroxyl radical and further peroxidation of bimembrane lipids. Reduction of antioxidant enzymes activity result from their dyscoordinated action and their gradual affliction by active LPO products, which create favorable conditions for greater free radical oxidation in the organs and tissues.

Administration of E.P.t. for 7 days after irradiation caused a reliable normalizing action upon all the examined indices. The use of the tincture before and after the exposure proved to be more effective than that only after irradiation.

Biologic effectiveness of the preparation in the destruction of peroxidation products and their toxic metabolites, stabilization of the organism's antioxidant system state has been caused by biologically active substances in its composition such as polyphenols, phlavonoids, alkaloids, caffeine acid, betain, vitamins, macro- and microelements [1, 8] that stimulate opportunities of the body antioxidant system and stimulate GSH synthesis. This is due to antioxidant and membrane-stabilizing properties of E.P.t. [5].

Along with antioxidant and membrane-stabilizing effects in the mechanism of antiirradiation and antistress influence on E.P.t. in people, a certain role is played by its property to restore and stimulate physical and mental ability to work, to alleviate accompanying psychoemotional stresses [6].

CONCLUSIONS

Administration of *Echinacea purpurea* preparation significantly reduces the incidence of antioxidant system. Its powerful radioprotective capacities are achieved through free radical

scavenging performed by biologically active substances in its composition. *Echinacea purpurea* posseses remarkable protective properties and its biological activity could be important in many other diseases where lipid peroxidation products have been reported.

In conclusion, biological effects observed after administration of *Echinacea purpurea* preparation suggest that it may be beneficial for the prevention of adverse health effects in workers exposed to ionizing radiation.

REFERENCES

1. Barrett B.: Medicinal properties of Echinacea: a critical review. Phytomedicine, 10, 66, 2003.
2. Bauer R., Wagner H.: Echinacea Handbuch für Ärzte, Apotheker und andere Naturwissenschaftler. WBA, Stuttgart 1990.
3. Benderitter M., Vincent-Genod L. et al.: The Cell Membrane as a Biosensor of Oxidative Stress Induced by Radiation Exposure: A Multiparameter Investigation. Radiat. Res., 159, 471, 2003.
4. Dalle-Donne I., Rossi R., Colombo R. at al.: Biomarkers of Oxidative Damage in Human Disease. Clin. Chem., 52, 601, 2006.
5. Gerush I., Meshchyshen I., Gerush O.: Glutathione system in the liver of animals with acute toxic hepatitis receiving *Echinacea purpurea* tincture. Annales UMSC, Sect. DDD, 19, N1, 44, 211, 2006.
6. Joksić G., Petrović S. et al.: Biological effects of *Echinacea purpurea* on human blood cells. Arh. Hig. Rada Toksikol., 60, 165, 2009.
7. Koroluk M., Ivanova L., Maiorova I.: Metod opredelenia aktivnosti katalazy. Lab. Delo, 1, 16, 1988.
8. Moskaug J., Carlsen H. et al.: Polyphenols and glutathione synthesis regulation. Am. J. Clin. Nutr., 81, 277, 2005.
9. Nashikimi N., Appajik R., Jagi K.: The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. and Biophys. Res. Communs., 46, 2, 849, 1972.
10. Otsuka K., Koana T. et al.: Activation of antioxidative enzymes induced by low-dose-rate whole-body gamma irradiation: adaptive response in terms of initial DNA damage. Radiat Res., 166, 474, 2006.

SUMMARY

It was established that total single irradiation of rats in the dose of 0.3 Gy caused activation of lipid peroxidation in the liver and considerable changes in the liver antioxidant system. Administration of *Echinacea* tincture to the irradiated rats contributed to normalization of SOD and catalase activity as well as glutathione system enzymes. Biologic effectiveness of the preparation in the destruction of peroxidation products and their toxic metabolites, stabilization of the organism's antioxidant system state has been caused by biologically active substances in its composition. The use of the tincture before and after the exposure proved to be more effective than that only after irradiation.

Key words: *Echinacea purpurea*, glutathione system, low-dose ionizing radiation, liver, antioxidant-provident status

STRESZCZENIE

Stwierdzono, że jednorazowe napromienienie szczurów dawką całkowitą o wielkości 3 Gy powodowało aktywację peroksydacji lipidów w wątrobie i zmiany w systemie antyoksydacyjnym wątroby. Podanie napromienionym szczurom nalewki z jeżówki prowadziło do normalizacji aktywności SOD i katalazy, jak również enzymów systemu glutationowego. Biologiczna efektywność preparatu w niszczeniu produktów peroksydacji i ich toksycznych metabolitów, stabilizacja stanu systemu antyoksydacyjnego organizmu była związana z substancjami biologicznie czynnymi i ich składem. Zastosowanie nalewki przed i po ekspozycji na promieniowanie jonizujące było bardziej skuteczne niż podanie jedynie po napromienieniu.

Słowa kluczowe: *Echinacea purpurea*, system glutationowy, niskie dawki promieniowania jonizującego, wątroba, status antyoksydacyjny