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QUARTERLY

Protection by pantothenol and β-carotene against liver damage produced by low-dose γ radiation*

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Rats were exposed to a total dose of 0.75 Gy of γ radiation from a ^{60}Co source, receiving three doses of 0.25 Gy at weekly intervals. During two days before each irradiation, the animals received daily intragastric doses of 26 mg pantothenol or 15 mg β-carotene per kg body mass. The animals were killed after the third irradiation session, and their blood and livers were analyzed. As found previously (Slyshenkov, V.S., Omelyanchik, S.N., Moiseenok, A.G., Trebukhina, R.V. & Wojtczak, L. (1998) *Free Radical Biol. Med.* 24, 894–899), in livers of animals not supplied with either pantothenol or β-carotene and killed one hour after the irradiation, a large accumulation of lipid peroxidation products, as conjugated dienes, ketotrienes and thiobarbituric acid-reactive substances, could be observed. The contents of CoA, pantothenic acid, total phospholipids, total glutathione and GSH/GSSG ratio were considerably decreased, whereas the NAD/NADH ratio was increased. All these effects were alleviated in animals supplied with β-carotene and were completely abolished in animals supplied with pantothenol. In the present paper, we extended our observations of irradiation effects over a period of up to 7 days after the last irradiation session. We found that most of these changes, with the exception of GSH/GSSG ratio, disappeared spontaneously, whereas supplementation with β-carotene shortened the time required for the normalization of biochemical parameters. In addition, we found that the activities of glutathione reductase, glutathione peroxidase, catalase and NADP-dependent malate (decarboxylating) dehydrogenase ('malic enzyme') in liver were also significantly decreased one hour after irradiation but returned to the normal

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level within 7 days. Little or no decrease in these activities, already 1 h after the irradiation, could be seen in animals supplemented with either β-carotene or pantothenol. It is concluded that pantothenol is an excellent radioprotective agent against low-dose γ radiation.

Protection by pantothenic acid and its derivatives against tissue damage produced by ionizing radiation and reactive oxygen species has been known since many years (Artom, 1954; Dombradi *et al.*, 1964; Szórády *et al.*, 1965; 1966; Perepelkin *et al.*, 1976; Kimura & Takahashi, 1981; Yoshikawa *et al.*, 1982; Nagiel-Ostaszewski & Lau-Cam, 1990; Utno, 1991, Kumerova *et al.*, 1991; 1992; Craciun *et al.*, 1992) and has been applied in medical practice, e.g. to ameliorate acute symptoms of radiotherapy (see e.g. Lokkevik *et al.*, 1996; and Mose *et al.*, 1997), and in cosmetics industry, though its mechanism remains unresolved. In our studies on Ehrlich ascites tumour cells (Slyshenkov *et al.*, 1995), we have demonstrated that this protective effect is not due to scavenging of free radicals but, rather, to promotion of repair mechanisms involving CoA of which pantothenic acid is the precursor. Furthermore, we have shown a considerable increase of glutathione content in cells preincubated in the presence of pantothenic acid or its derivatives (Slyshenkov *et al.*, 1996a). Since reduced glutathione is involved in enzymatic removal of hydrogen peroxide, phospholipid hydroperoxides and dioxygen anion radical ($O_2^{-\bullet}$), this findings pointed to an additional protective mechanism. Increased biosynthesis of phospholipids and cholesterol in pantothenic acid-supplemented cells could also contribute to the increased resistance of such cells against permeabilizing effect of digitonin (Slyshenkov *et al.*, 1996b).

Recently, we have described a protective effect of pantothenol, the immediate precursor of pantothenic acid, against liver damage produced by whole body exposure to low dose γ radiation in rat (Slyshenkov *et al.*, 1998). Since the deleterious effects of ionizing radiation are, to a great part, due to free radical generation, the mechanism of the observed effects might be similar to that found previously in Ehrlich ascites cells (Slyshenkov *et al.*, 1995; 1996a).

The protocol of these studies on whole body irradiation (Slyshenkov *et al.*, 1998) included three brief exposures of rats to γ radiation from a ^{60}Co source in weekly intervals. The animals were killed and their livers were removed for biochemical analyses exactly one hour after the last exposure. It was found that intragastric administration of pantothenol for two days preceding each exposure fully protected against lipid peroxidation, depletion of liver glutathione and a shift of its redox state as well as of the redox state of liver nicotinamide nucleotides. It also prevented a decrease of NADP-dependent malate dehydrogenase (decarboxylating), one of the enzymes involved in reducing cytosolic NADP. Administration of β-carotene exerted a partial protective effect.

However, these results left several unanswered questions: (1) whether pantothenol really prevented the deleterious symptoms in livers of the irradiated animals or, rather, only delayed the onset of these defects; (2) whether β-carotene was a weaker protective agent than pantothenol or its protective effect could be observed on a longer time scale; and (3) were the metabolic effects of low doses of γ radiation persistent or could they be reversed without any treatment. To answer these question, we now extended the previous work (Slyshenkov *et al.*, 1998) by examining livers of irradiated animals over a longer period following the last exposure, namely after 24 h and 7 days. In addition, we extended our study over three more enzymes involved in protection against oxidative stress: glutathione reductase, glutathione peroxidase and catalase.

MATERIALS AND METHODS

Animals. Female albino rats (140–180 g body mass) were kept on standard laboratory diet. The experimental protocol was the same as described previously (Slyshenkov *et al.*, 1998). The animals were divided into four groups, fifteen rats in each. Animals of the first group were the controls and were kept without further treatment. Animals of groups II, III and IV were subjected to whole body γ irradiation from a cobalt bomb (model AGAT-C, U.S.S.R.) three times at weakly intervals, receiving a total dose of 0.75 Gy. Two days before each irradiation session, i.e. on Monday and Tuesday, the animals of group III were given daily, *via* a stomach catheter, β-carotene, 15 mg/kg body mass, and the animals of group IV, D-pantothenol, 26 mg/kg body mass. Every Wednesday, the rats were exposed to γ irradiation for 24 s, accumulating 0.25 Gy during each exposure. The animals were killed by decapitation one hour, 24 h and 7 days after the third exposure, five animals from each group each time. Blood (prevented from clotting by heparin) and fragments of liver were taken immediately and kept in liquid nitrogen for the determination of the NAD^+/NADH ratio and the contents of glutathione and CoA. The remaining liver was briefly perfused with cold 0.9% NaCl and, after removal of large blood vessels, cut into large pieces, rinsed with the same solution, blotted with filter paper and preserved in liquid nitrogen for all other analyses.

Analytical procedures. Conjugated dienes and ketone trienes were determined in heptane extracts at 235 and 275 nm, respectively (Recknagel & Ghoshal, 1966). Thiobarbituric acid-reactive substances were measured as described previously (Slyshenkov *et al.*, 1995) and expressed as malondialdehyde. Cytoplasmic NAD^+/NADH ratio was calculated from the pyruvate/lactate ratio (Williamson *et al.*, 1967) determined enzymatically (Williamson & Corkey, 1964). Reduced and oxidized glutathione were measured with methylglyoxal plus glyoxalase I and with NADPH plus glutathione reductase, respectively (Akerboom & Sies, 1981). Total CoA was determined with phosphotransacetylase (McDougal & Dargar, 1979) after previous alkaline hydrolysis of CoA esters. Pantothenic acid in blood was determined by gas chromatography as the total amount of pantolactone-forming compounds (Moiseenok *et al.*, 1984; Slyshenkov *et al.*, 1998).

Total lipids were extracted from liver by the procedure of Folch *et al.* (1957) and determined gravimetrically. Phospholipids were determined by the amount of lipid phosphorus after combustion in concentrated perchloric acid at 180°C. Cholesterol was measured by the Liebermann-Burchard colour reaction with acetic anhydride (Abell *et al.*, 1952).

Determination of enzyme activities. Fragments of livers were homogenized in 200 mM KCl containing 5 mM Tris/HCl (pH 7.4) and 0.1% Triton X-100 and centrifuged for 30 min at 14000 × ***g*** at 4°C. Enzyme activities were measured in the supernatant fraction. NADP-dependent malate (decarboxylating) dehydrogenase ('malic enzyme', EC 1.1.1.40) was measured according to the method of Brdiczka & Pette (1971) as described previously (Slyshenkov *et al.*, 1998). Catalase (EC 1.11.1.6) was determined by following the decomposition of H_2O_2 at 240 nm (Aebi, 1984). Glutathione reductase (EC 1.6.4.2) and glutathione peroxidase (EC 1.11.1.9) were assayed by following the rate of NADPH oxidation in appropriate systems (Flohé & Günzler, 1984; Carlberg & Mannervik, 1995). All measurements were performed at room temperature (about 20°C).

Chemicals. D-Pantothenol was from Hoffmann-La Roche (Basel, Switzerland) and β-carotene from N.P.O. Vitaminy (Moscow, Russia).

Statistical methods. All values are expressed as means for 5 animals ± SEM. Statistical significance was evaluated using the Student's *t*-test.

RESULTS

Changes in blood

Irradiation resulted in a large increase of conjugated dienes which are an indication of lipid peroxidation, whereas the levels of GSH and pantothenic acid and/or its derivatives (estimated as pantolactone-producing compounds) were decreased. These changes diminished with time so that they disappeared or were much less pronounced 7 days after irradiation (Fig. 1). Administration of β-carotene or pantothenol to rats before irradiation considerably decreased the accumulation of conjugated dienes and completely prevented the decrease in GSH. β-Carotene exhibited, however, no significant effect on the decrease in blood pantothenic acid after irradiation. As expected, administration of pantothenol greatly increased its concentration in blood which returned to the normal level after 7 days.

Changes in liver

Low molecular mass compounds

The amounts of conjugated di- and trienes and of thiobarbituric acid-reactive substances (expressed as malondialdehyde) in liver almost doubled 1 h after irradiation but returned to the normal level within 7 days or less (Fig. 2, panels A and B). Irradiation resulted in a decrease of total liver CoA (panel C) and total glutathione (panel D) by 20% and 30%, respectively. These changes were, however, more persistent as they were only partly alleviated after 7 days.

Gamma irradiation not only decreased the total amount of glutathione but drastically shifted the proportion between its reduced and oxidized forms. As shown in Fig. 2 (panel E), GSH/GSSG ratio decreased 1 h after irradiation to 16% of its value in livers of control animals and still amounted to 60% seven days after the last irradiation. Exposure to γ radia-

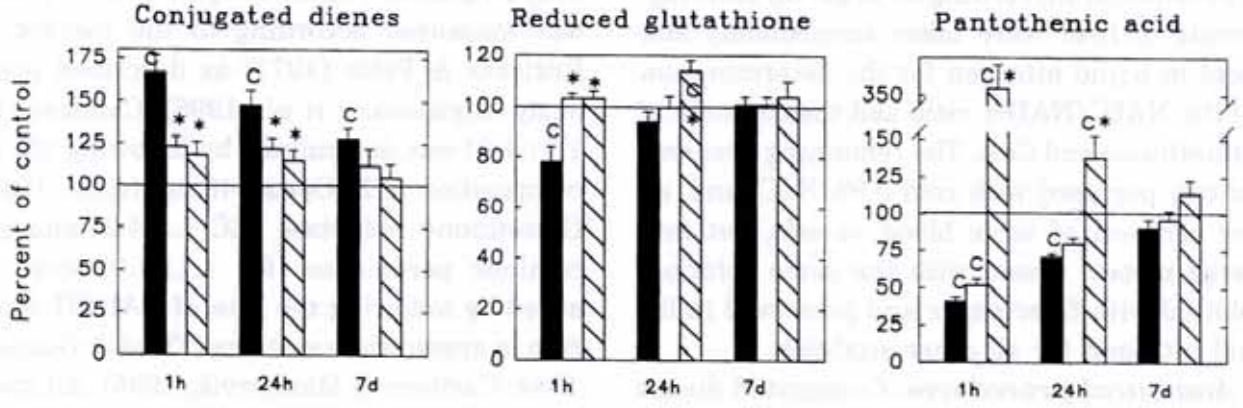

Figure 1. Changes in the levels of conjugated dienes, reduced glutathione and pantothenic acid and its derivatives (determined as pantolactone-forming compounds) in the blood of γ-irradiated rats, and the effects of β-carotene and pantothenol.

All values are expressed in percentage of corresponding values in the blood of non-irradiated animals which amounted to 0.62 ± 0.05 absorbancy unit/ml for conjugated dienes, to 1.25 ± 0.03 μmol/ml for GSH and to 10.2 ± 0.8 nmol/ml for pantolactone-forming substances (here designated as pantothenic acid). Black columns, irradiated animals without further treatment; white columns, irradiated animals supplemented with β-carotene; dashed columns, irradiated animals supplemented with pantothenol. Statistically significant differences ($P < 0.05$) with respect to non-irradiated controls are indicated by 'c' and with respect to irradiated but otherwise untreated rats, examined at the same time after the last irradiation, are marked by '*'.

tion also changed the proportion between oxidized and reduced forms of liver cytosolic NAD which, however, returned to its control level within a few days (Fig. 2, panel F).

Administration of β-carotene to rats before each radiation exposure resulted in a lower level of conjugated di- and trienes and strongly diminished the formation of thiobarbituric acid-reactive compounds (Fig. 2, panels A and B). It had no effect on the decrease of CoA (panel C), but partly protected against diminution of total glutathione and shifts of

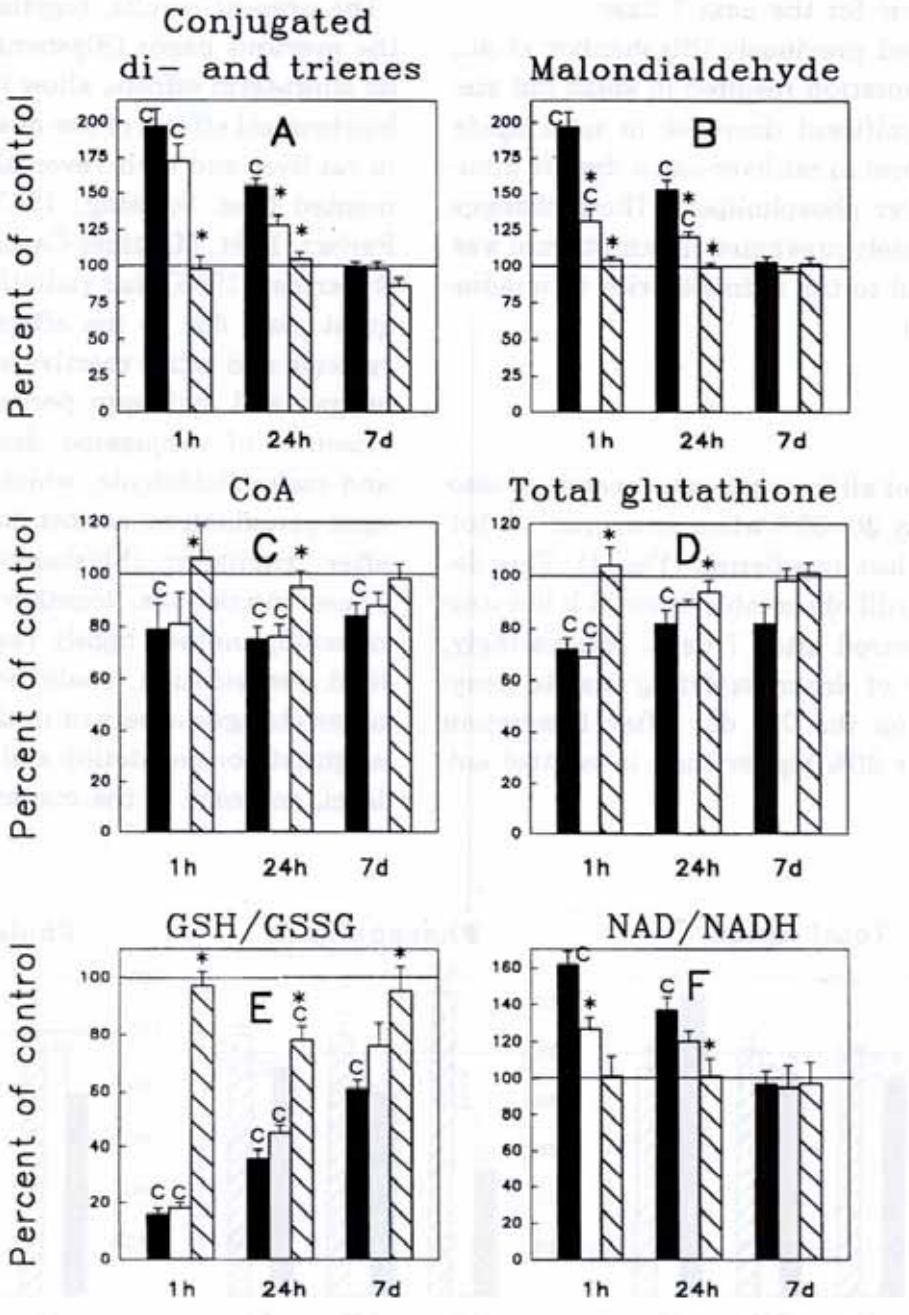

Figure 2. Changes of several biochemical parameters in the liver of γ-irradiated rats and the effects of β-carotene and pantothenol.

Values for non-irradiated rats (taken as 100%) were: the sum of conjugated dienes and ketotrienes, 1.06 ± 0.06 absorbancy units/mg total lipid; malondialdehyde, 34.3 ± 1.6 nmol/g wet mass; CoA, 308 ± 13 nmol/g wet mass; total glutathione, 5.7 ± 0.4 μmol/g wet mass; GSH/GSSG ratio, 37.2 ± 3.5; NAD/NADH ratio, 579 ± 47 (see also Slyshenkov *et al.*, 1998). Description of columns and statistical significance as in Fig. 1.

glutathione and cytosolic NAD towards their oxidized forms (panels D, E and F).

As shown previously (Slyshenkov *et al.*, 1998), administration of pantothenol fully protected against changes in all six parameters illustrated in Fig. 2 when measured 1 h after irradiation and these values remained at the same level for the next 7 days.

As described previously (Slyshenkov *et al.*, 1998), γ irradiation resulted in small but statistically significant decrease in total lipids and cholesterol in rat liver and a drastic diminution of liver phospholipids. These changes were completely prevented if pantothenol was administered to the animals prior to irradiation (Fig. 3).

Enzymes

Activities of all four enzymes examined also decreased by 20–30% when measured 1 h following the last irradiation (Fig. 4). This decrease was still observable after 24 h but usually disappeared after 7 days. Interestingly, the activity of decarboxylating malate dehydrogenase on the 7th day after irradiation was by over 30% higher than in control animals.

Both β-carotene and pantothenol prevented these changes in enzyme activities observed already 1 h after irradiation (Fig. 4).

DISCUSSION

The present results, together with those of the previous paper (Slyshenkov *et al.*, 1998) on short-term effects, allow to evaluate some biochemical effects of low doses of γ radiation in rat liver and their reversal. It is well documented (von Sonntag, 1987; Hagen, 1989; Farber, 1994; Martínez-Cayuela, 1995; Leyko & Bartosz, 1986) that radiation injury is, to a great part, due to the effect of oxygen free radicals and other reactive species as singlet oxygen and hydrogen peroxide. In fact, the amounts of conjugated dienes, ketotrienes and malondialdehyde, which are products of lipid peroxidation, almost doubled within 1 h after irradiation (Slyshenkov *et al.*, 1998). These compounds, together with 4-hydroxynonenal, another highly reactive product of lipid peroxidation, could be responsible for other changes observed in this investigation, as glutathione oxidation and diminution of its level, decrease of the content of CoA and of

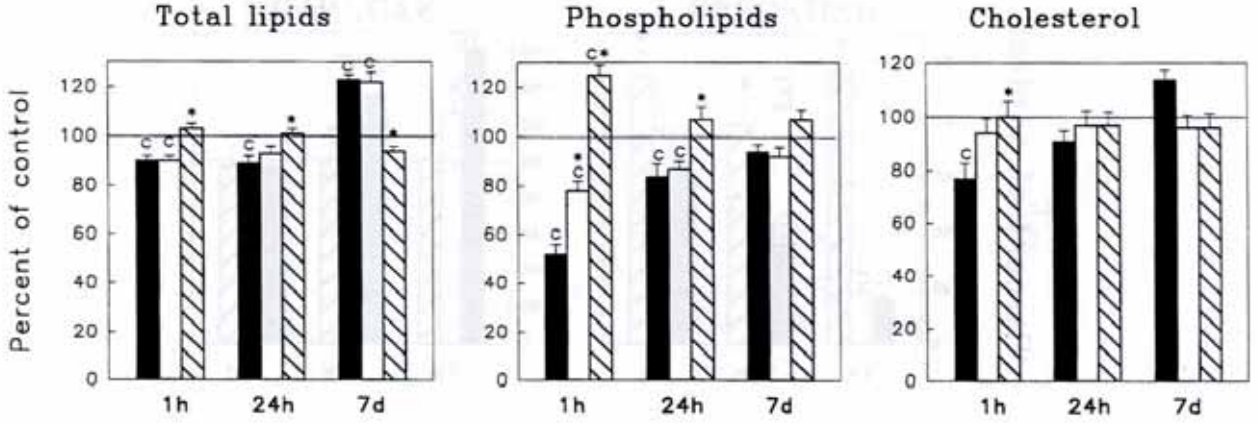

Figure 3. Changes in the content of lipidic substances in liver upon γ irradiation.

Values for non-irradiated animals were: total lipids, 54 ± 2 mg/g wet mass; phospholipids, 24 ± 1 mg/g wet mass; cholesterol, 3.5 ± 0.2 μmol/g wet mass (Slyshenkov *et al.*, 1998). Description of columns and statistical significance as in Fig. 1.

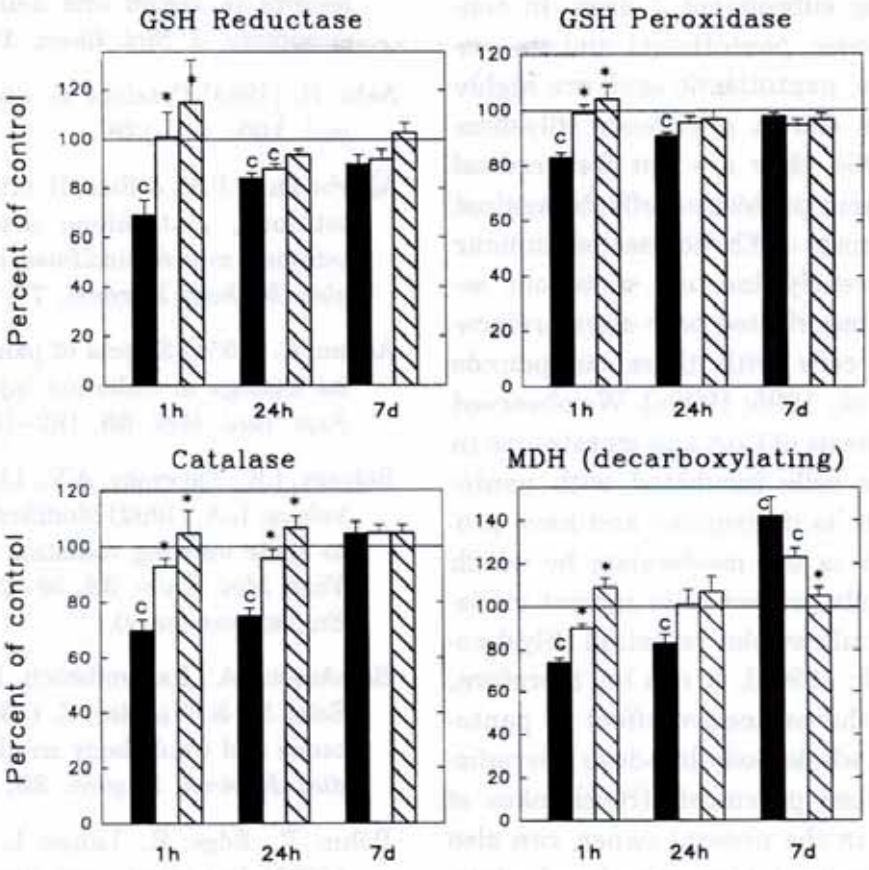

Figure 4. Changes in the activities of glutathione reductase, glutathione peroxidase, catalase and NADP-dependent malate (decarboxylating) dehydrogenase in livers of γ-irradiated rats.

The values for non-irradiated rats were: GSH reductase, 48 ± 3 nmol NADPH/min per mg protein; GSH peroxidase, 775 ± 17 nmol NADPH/min per mg protein; catalase, 300 ± 21 μmol H_2O_2/min per mg protein; malate dehydrogenase, 46 ± 2 nmol NADPH/min per mg protein. Description of columns and statistical significance was as in Fig. 1.

the activities of the enzymes that are important in prevention of the oxidative stress.

All these effects of low-dose γ irradiation were, however, transient and most of them completely disappeared within 7 days after the last irradiation. β-Carotene administered to the animals during two days before each irradiation session considerably shortened the time needed for the various biochemical parameters studied to return to their normal levels. This is compatible with the well documented antioxidant (Rousseau *et al.*, 1992; Chen *et al.*, 1993; Ozhogina & Kasaikina, 1995; Postaire *et al.*, 1995; Sies & Stahl, 1995; Tsuchihashi *et al.*, 1995) and radioprotective (Belyaev *et al.*, 1992; Postaire *et al.*, 1995; Salvadori *et al.*, 1996; Umegaki *et al.*, 1997; Różanowska *et al.*, 1998; Konopacka *et al.*, 1998; Böhm *et al.*, 1998; Fuchs, 1998) actions of β-carotene in biological systems. Ben-Amotz *et al.* (1996) observed protection by β-carotene feeding against growth disturbance of rats subjected to whole body irradiation. In the experiments presented here, β-carotene was radioprotective against liver damage. Being highly lipophilic, β-carotene may preferentially protect cell membranes, acting as free radical quencher, singlet oxygen scavenger and lipid antioxidant.

The protective effect of pantothenol was even more pronounced. With all biochemical parameters studied, a complete prevention of the damaging effects of γ radiation was obvious already 1 h after the last irradiation (Slyshenkov *et al.*, 1998) and no deterioration of the biochemical status of the liver could be

observed during subsequent 7 days. In contrast to β-carotene, pantothenol and its oxidized derivative, pantothenic acid, are highly hydrophilic. As shown previously (Slyshenkov *et al.*, 1995), they are not free radical scavengers. Their protective effects against free radical damage of Ehrlich ascites tumour cells was apparently due to a metabolic action, as it was manifested only after preincubation of the cells with these compounds (Slyshenkov *et al.*, 1995; 1996a). We observed enhanced synthesis of CoA and glutathione in Ehrlich ascites cells incubated with pantothenic acid and its derivatives and have proposed that this is the mechanism by which these compounds protect cells against oxidative stress and ultraviolet radiation (Slyshenkov *et al.*, 1995; 1996a). It can be, therefore, assumed that the protective effect of pantothenol against whole body low-dose γ irradiation, as described previously (Slyshenkov *et al.*, 1998) and in the present paper, can also be due to enhanced biosynthesis of glutathione and CoA.

In conclusion, pantothenol, pantothenic acid and their derivatives are excellent protective agents against liver damage by low-dose γ radiation. Their mechanism of action is different than that of β-carotene and, in the experimental model employed in the present study, appeared more effective. Both pantothenic acid (and its derivatives) and β-carotene may be of practical importance in abolition or alleviation of health hazards of inhabitants of areas of elevated background radiation, as those in some parts of Ukraine and Belarus after the Chernobyl disaster, and for protective measures for individuals professionally exposed to ionizing radiation.

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REFERENCES

Abell, L.L., Brodie, B.B. & Kendall, F.E. (1952) A simple method for the estimation of total cholesterol in serum and determination of its specificity. *J. Biol. Chem.* **195**, 357–386.

Aebi, H. (1984) Catalase in vitro. *Methods Enzymol.* **105**, 121–126.

Akerboom, T.P.M. & Sies, H. (1981) Assay for glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. *Methods Enzymol.* **77**, 373–382.

Artom, C. (1954) Effects of pantothenic acid and its analogs in radiation injury by ^{32}P. *Proc. Expt. Biol. Med.* **86**, 162–165.

Belyaev, I.K., Zaraysky, A.V., Limberg, V.K. & Vakulova, L.A. (1992) Modification of resistance to acute ionizing radiation using β-carotene. *Vopr. Med. Khim.* **38**, 39–42 (in Russian with English summary).

Ben-Amotz, A., Rachmilevich, B., Greenberg, S., Sela, M. & Weshler, Z. (1996) Natural β-carotene and whole body irradiation in rats. *Radiat. Environ. Biophys.* **35**, 285–288.

Böhm, F., Edge, R., Lange, L. & Truscott, T.G. (1998) Enhanced protection of human cells against ultraviolet light by antioxidant combinations involving dietary carotenoids. *J. Photochem. Photobiol. B.* **44**, 211–215.

Brdiczka, D. & Pette, D. (1971) Intra- and extramitochondrial isozymes of (NADP) malate dehydrogenase. *Eur. J. Biochem.* **19**, 546–551.

Carlberg, I. & Mannervik, B. (1985) Glutathione reductase. *Methods Enzymol.* **113**, 484–490.

Chen, H., Pellett, L.J., Andersen, H.J. & Tappel, A.L. (1993) Protection by vitamin E, selenium, and β-carotene against oxidative damage in rat liver slices and homogenate. *Free Radic. Biol. Med.* **14**, 473–482.

Craciun, C., Ghircoiasiu, M. & Craciun, V. (1992) Electron-microscopic studies on the effect of calcium pantothenate upon rat liver and locally irradiated epidermis. *Acta Morphol. Hung.* **40**, 231–248.

Dombradi, G.A., Szepesvári, E. & Szórády, I. (1964) The possible mode of action of pantothenic acid in radioprotection. *Acta Physiol. Lat. Am.* **14**, 327–329.

Farber, J.L. (1994) Mechanisms of cell injury by activated oxygen species. *Envir. Health Persp.* **102** (Suppl. 10), 17–24.

Flohé, L. & Günzler, W.A. (1984) Assays of glutathione peroxidase. *Methods Enzymol.* **105**, 114–121.

Folch, J., Lees, M. & Sloane Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497–502.

Fuchs, J. (1998) Potentials and limitations of the natural antioxidants RRR-α-tocopherol, L-ascorbic acid and β-carotene in cutaneous photoprotection. *Free Radic. Biol. Med.* **25**, 848–873.

Hagen, U. (1989) Biochemical aspects of radiation biology. *Experientia* **45**, 7–12.

Kimura, S. & Takahashi, Y. (1981) Preventive effects of L-ascorbic acid and calcium pantothenate against photosensitive actions induced by pheophorbide and hematoporphyrin. *J. Nutr. Sci. Vitaminol.* (Tokyo) **27**, 521–527.

Konopacka, M., Widel, M. & Rzeszowska-Wolny, J. (1998) Modifying effect of vitamins C, E and β-carotene against γ-ray-induced DNA damage in mouse cells. *Mutat. Res.* **417**, 85–94.

Kumerova, A.O., Silova, A.A. & Utno, L.Y. (1991) Effect of pantethine on post-heparin lipolytic activity and lipid peroxidation in the myocardium. *Biull. Eksp. Biol. Med.* **111**, 33–35 (in Russian with English summary).

Kumerova, A.O., Utno, L.Y., Lipsberga, Z.E. & Shekestere, I.Y. (1992) Myocardial protection by derivatives of pantothenic acid in heart model with experimental ischemia and reperfusion. *Biull. Eksp. Biol. Med.* **113**, 373–375 (in Russian with English summary).

Leyko, W. & Bartosz, G. (1986) Membrane effects of ionizing radiation and hyperthermia. *Int. J. Radiat. Biol.* **49**, 743–770.

Lokkevik, E., Skovlund, E., Reitan, J.B., Hannisdal, E. & Tanum, G. (1996) Skin treatment with bepanthen cream *versus* no cream during radiotherapy – a randomized controlled trial. *Acta Oncol.* **35**, 1021–1026.

Martínez-Cayuela, M. (1995) Oxygen free radicals and human disease. *Biochimie* **77**, 147–161.

McDougal, D.B., Jr. & Dargar, R.V. (1979) A spectrophotometric cycling assay for reduced coenzyme A and its ester in small amounts of tissue. *Analyt. Biochem.* **97**, 103–115.

Moiseenok, A.G., Slyshenkov, V.S., Sunozova, E.V. & Gurinovich, V.A. (1984) Quantitative chromatographic estimation of pantolactone. *Vopr. Med. Khim.* **30**, 126–128 (in Russian with English summary).

Mose, S., Adamietz, I.A., Saran, F., Thilmann, C., Heyd, R., Knecht, R. & Bottcher, H.D. (1997) Can prophylactic application of immunoglobin decrease radiotherapy-induced oral mucositis? *Am. J. Clin. Oncol.* **20**, 407–411.

Nagiel-Ostaszewski, I. & Lau-Cam, C.A. (1990) Protection by pantethine, pantothenic acid and cystamine against carbon tetrachloride-induced hepatotoxicity in the rat. *Res. Commun. Chem. Pathol. Pharmacol.* **67**, 289–292.

Ozhogina, O.A. & Kasaikina, O.T. (1995) β-Carotene as an interceptor of free radicals. *Free Radic. Biol. Med.* **19**, 575–581.

Perepelkin, S.R., Egorova, N.D., Kovalenko, V.A. & Demin, V.I. (1976) Protective effect of pantothenic acid (vitamin B3) in radiation sickness. *Radiobiologiya* **16**, 722–726.

Postaire, E., Regnault, C., Simonet, L., Rousset, G. & Bejot, M. (1995) Increase of singlet oxygen protection of erythrocytes by vitamin E, vitamin C, and β-carotene intakes. *Biochem. Mol. Biol. Int.* **35**, 371–374.

Recknagel, R.O. & Ghoshal, A.K. (1966) Lipoperoxidation of rat liver microsomal lipids by carbon tetrachloride. *Nature* **210**, 1162–1163.

Rousseau, E.J., Davison, A.J. & Dunn, B. (1992) Protection by β-carotene and related compounds against oxygen-mediated cytotoxicity and genotoxicity: Implications for carcinogenesis and anticarcinogenesis. *Free Radic. Biol. Med.* **13**, 407–433.

Różanowska, M., Wessels, J., Boulton, M., Burke, J.M., Rodgers, M.A., Truscott, T.G. & Sarna, T. (1998) Blue light-induced singlet oxygen generation by retinal lipofuscin in non-polar media. *Free Radic. Biol. Med.* **24**, 1107–1112.

Salvadori, D.M., Ribeiro, L.R., Xiao, Y., Boei, J.J. & Natarajan, A.T. (1996) Radioprotection of β-carotene evaluated on mouse somatic and germ cells. *Mutat. Res.* **356**, 163–170.

Sies, H. & Stahl, W. (1995) Vitamins E and C, β-carotene, and other carotenoids as antioxidants. *Am. J. Clin. Nutr.* **62**, 1315S–1321S.

Slyshenkov, V.S., Rakowska, M., Moiseenok, A.G. & Wojtczak, L. (1995) Pantothenic acid and its derivatives protect Ehrlich ascites tumor cells against lipid peroxidation. *Free Radic. Biol. Med.* **19**, 767–772.

Slyshenkov, V.S., Moiseenok, A.G. & Wojtczak, L. (1996a) Noxious effects of oxygen reactive species on energy coupling processes in Ehrlich ascites tumor mitochondria and the protection by pantothenic acid. *Free Radic. Biol. Med.* **20**, 793–800.

Slyshenkov, V.S., Rakowska, M. & Wojtczak, L. (1996b) Protective effect of pantothenic acid and related compounds against permeabilization of Ehrlich ascites tumor cells by digitonin. *Acta Biochim. Polon.* **43**, 407–410.

Slyshenkov, V.S., Omelyanchik, S.N., Moiseenok, A.G., Trebukhina, R.V. & Wojtczak, L. (1998) Pantothenol protects rats against some deleterious effects of γ radiation. *Free Radic. Biol. Med.* **24**, 894–899.

Szórády, I., Gazdag, I. & Kocsor, S. (1966) Weitere Beobachtungen über die Strahlenschutzwirkung der Pantothensäure. *Naturwiss.* **53**, 527.

Szórády, I., Tóth, Gy. & Gazdag, I. (1965) X-Ray protection by means of pantothenic acid. *Progr. Biochem. Pharmacol.* **1**, 533–536.

Tsuchihashi, H., Kigoshi, M., Iwatsuki, M. & Niki, E. (1995) Action of β-carotene as an antioxidant against lipid peroxidation. *Arch. Biochem. Biophys.* **323**, 137–147.

Umegaki, K., Uramoto, H., Suzuki, J. & Esashi, T. (1997) Feeding mice palm carotene prevents DNA damage in bone marrow and reduction of peripheral leukocyte counts, and enhances survival following X-ray irradiation. *Carcinogenesis* **18**, 1943–1947.

Utno, L.Y. (1991) Effects of pantethine on metabolism in myocardial mitochondria under the conditions of deep hypothermia. *Biull. Eksp. Biol. Med.* **111**, 577–578 (in Russian with English summary).

von Sonntag, C. (1987) *The Chemical Basis of Radiation Biology.* Taylor & Francis, London.

Williamson, J.R. & Corkey, B.E. (1964) Assays of intermediates of the citric acid cycle and related compounds by fluorometric enzyme methods. *Methods Enzymol.* **13**, 434–512.

Williamson, D.H., Lund, P. & Krebs, H.A. (1967) The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. *Biochem. J.* **103**, 514–527.

Yoshikawa, T., Furukawa, Y., Tamai, M., Murakami, M. & Kondo, M. (1982) The increase of lipid peroxidation in experimental hepatitis in rats induced by carbon tetrachloride or D-galactosamine and its inhibition by pantethine. *J. Appl. Biochem.* **4**, 228–233.