Original Article

Influence of Local Peripheral Temporary Ischaemia on Biochemical and Histological Effects in Small Intestine and Serum of Rats Following Abdominal Irradiation

(ischaemic preconditioning / radiation / superoxide dismutase / jejunum crypts / lipid peroxidation)

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Abstract. The local temporary ischaemia effect on radiation-induced lipid peroxidation, superoxide dismutase isoenzyme activities, and intestinal crypt number was estimated in male WAG-strain rats *in vivo*. The animals were irradiated in the abdomen area with doses of 2 Gy for ten consecutive days using a Philips ^{60}Co source. The calculated dose rate was 0.595 Gy/min. Local temporary ischaemia was induced by clamping the tail base before each irradiation. The parameters evaluated were: TBA-RS level and enzymatic activities of CuZnSOD, MnSOD in serum and jejunum. The number of jejunum crypts was assigned as a histopathologic parameter. The results showed a clear protection by ischaemic preconditioning for crypt survival. The difference in the number of crypts in irradiated animals with and without local temporary ischaemia was statistically significant (Student's *t*-test $P < 0.05$). Also, significant enhancement of TBA-RS was observed in the serum of irradiated animals. Local temporary ischaemia application diminished the concentration of radiation-induced TBA-RS. The differences in the levels of TBA-RS in the serum were statistically significant (ANOVA $P < 0.002$). In contrast, there was no evident effect on the level of TBA-RS in tissue homogenates in any investigated groups. Some fluctuation of CuZnSOD isoenzyme activity in intestinal tissue was noted; however, the differences were not significant. Local temporary ischaemia had no influence on MnSOD activity in serum, and in both irradiated groups the behaviour of this isoenzyme was similar. Also, there were no differences in MnSOD activity measured in tissue homogenates. These findings support results of our previous *in vivo* studies, suggesting that local temporary ischaemia can prevent oxidative effects of fractionated radiotherapy

Introduction

The relationship between effects of irradiation and status of circulation are bilateral. Cardiomyopathy with symptoms of circulatory insufficiency (Stewart and Fajardo, 1971) and arteriosclerosis may develop after irradiation (Fajardo, 1977; Rutqvist et al., 1992). On the other hand, it has been found that radiotherapy is more effective for cells close to arteries (Thomlinson and Gray, 1955). Radiation induces free radical chain reactions which cause cell damage, and the concentration of free radicals depends on the oxygen in irradiated tissues (McMillan and Steel, 1997; Van der Kogel, 1997). It is well established that the level of oxygen in the irradiated area changes the effectiveness of the radiation (Elkind et al., 1965). On the other hand, exposure to brief ischaemia has been shown to induce free radical processes (Moncada and Higgs, 1991; Parratt, 1994), indicating free radical-nitric oxide involvement in vascular intima response to ischaemic preconditioning. Marked elevation of lipid peroxides in response to severe ischaemia was also observed in rat brain exposed to ischaemia-reperfusion (Islekel et al., 1999a). One of the many elements of antioxidant defence against the destructive action of reactive oxygen species is the activity of superoxide dismutase - SOD (EC 1.15.1.1) and its mito-

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Abbreviations: MDA – malondialdehyde, SOD – superoxide dismutase, TBA-RS – thiobarbituric acid-reactive substances.

chondrial (MnSOD), cytoplasmic (CuZnSOD) and extracellular (EC-SOD) isoenzymes (Mates et al., 1999; Fattman et al., 2003). The activities of the above-mentioned types of SOD modulate the effect of ischaemia (Deshmukh et al., 1997; Nakajima et al., 2001; Hoshida et al., 2002; Salvemini and Cuzzocrea, 2002; Jones et al., 2003) and also the effects of irradiation (Lee et al., 2001; Motoori et al., 2001; Ueta et al., 2001; Guo et al., 2003). Previously published results support the hypothesis that temporary ischaemia changes the effects of irradiation (Walichiewicz et al., 2002, 2003). The present study has been undertaken to extend insight into the biochemical events and histo-pathological consequences underlying the interaction between local temporary ischaemia and radiation.

Material and Methods

Animals

Male WAG-strain rats weighing 230–250 g (5–6 months old) were used in the experiments. The animals were housed four per cage, under controlled temperature (20–22 °C), humidity (60–70 %) and lighting (12-h light/dark cycle) and were provided with food and water *ad libitum*.

Experimental protocol

The Ethical Committee of Warsaw Medical University reviewed and accepted all the procedures. All rats were anaesthetized using 0.05 mg/g Brietal (Lilly, Indianapolis, IN) s.c. 15 min before treatment. Irradiation was performed with a Philips Co-60 source (Philips, Eindhoven, The Nederlands). Animals were divided into four groups. In the first group (N = 42), animals were irradiated on the abdomen only with doses of 2 Gy per day for ten consecutive days. Rats were irradiated individually in well-ventilated plexiglass immobilization chambers. The calculated dosage rate was 0.595 Gy/min. In the second group (N = 42), animals were irradiated as in the first group, but before each irradiation a local temporary ischaemia was induced in each animal by clamping the tail base (three times for 3 min, with 1-min pause in between). During this procedure, performed immediately before each irradiation, rats were immobilized in the individual chambers. Rats in the third group (N = 42) were not irradiated, but a local temporary ischaemia was applied to them for ten consecutive days. The changes in cutaneous microcirculation due to the clamping-induced tail ischaemia were monitored in a non-invasive way, using laser Doppler flowmetry (Laser Flowmeter type BRL-100, Hugo Sachs Elektronik, March-Hugstetten, Germany) (Walichiewicz et al., 2002, 2003). Control group (N = 12) rats were sham-exposed to local temporary ischaemia and/or irradiation.

Assays

The controlled biochemical parameters were: the thiobarbituric acid-reactive substances (TBA-RS) in serum and jejunum homogenates, activity of mitochondrial and cytoplasmic isoenzymes of SOD in serum and jejunum homogenates. The TBA-RS levels were estimated as previously described (Walichiewicz et al., 2005). SOD isoenzyme activity was estimated by the method of Oyanagui (1984). Activity of the isoenzymes was expressed in nitrate units (NU/ml of serum) and in jejunum homogenates in nitrate units per milligram of protein as proposed by Spitz and Oberley (1989). The number of crypts in jejunum was assigned as the morphologic parameter. The small intestines were preserved in formalin and then cut into 5-μm thin slices. The cutting plane was perpendicular to the long axis of the jejunum. The specimens were stained with haematoxylin&eosin. The morphometric analysis was performed with a KS400 photo-analyser (Carl Zeiss Jena, Germany) connected to an Axioplan 2 MTT microscope and a Sony 3CCD camera. The numbers of crypts were calculated in each of the areas of mucosa (Walichiewicz et al., 2003). Samples were collected on 1st, 3rd, 5th, 8th, 11th and 17th day following the first irradiation or sham-irradiation.

Presentation of data

The data are given as means of four determinations with $P < 0.05$ used as the level of significance. The analysis of variance (ANOVA) and Student's *t*-test for independent probes were used as the statistical methods. Further on, more detailed post-hoc analysis was performed. Values of means together with standard errors are presented in Tables (1–5).

Table 1. Concentration of thiobarbituric acid-reactive substances in serum (expressed as nM of MDA). Values of means and standard errors

Day of experiment	control group	ischaemic preconditioning	irradiation	irradiation + ischaemic preconditioning
day 1	216.50 ± 29.13	253.50 ± 15.84	367.52 ± 97.34	395.50 ± 100.59
day 3		21.45 ± 52.56	471.37 ± 46.58	274.70 ± 87.40
day 5		207.40 ± 27.92	593.02 ± 101.57	353.725 ± 74.56
day 8	143.30 ± 20.93	109.15 ± 32.59	403.80 ± 97.70	192.60 ± 42.02
day 11		127.00 ± 29.84	256.85 ± 58.13	105.75 ± 58.74
day 14		242.90 ± 15.84	92.60 ±13.83	66.23 ± 24.12
day 17	129.10 ± 26.59	135.79 ± 17.32	149.97 ± 80.13	67.42 ± 18.59

Table 2. Concentration of thiobarbituric acid-reactive substances in intestine homogenate (expressed as nM of MDA). Values of means and standard errors

Day of experiment	control group	ischaemic preconditioning	irradiation	irradiation + ischaemic preconditioning
day 1	4.727 ± 1.17	4.222 ± 0.89	3.576 ± 1.20	4.616 ± 0.89
day 3		1.872 ± 0.21	4.035 ± 1.52	3.263 ± 1.74
day 5		1.535 ± 0.49	2.444 ± 1.64	2.232 ± 0.95
day 8	2.986 ± 0.38	3.489 ± 0.59	2.163 ± 0.64	1.249 ± 0.69
day 11		0.769 ± 0.03	4.093 ± 2.99	3.838 ± 1.79
day 14		5.907 ± 0.16	2.157 ± 0.65	3.573 ± 1.93
day 17	3.276 ± 1.81	4.004 ± 0.34	4.778 ± 1.07	2.331 ± 1.04

Table 3. CuZn-superoxide dismutase (cytoplasmic isoenzyme) activity in serum (expressed as NU/ml of serum). Values of means and standard errors

Day of experiment	control group	ischaemic preconditioning	irradiation	irradiation + ischaemic preconditioning
day 1	25.25 ± 0.07	17.70 ± 0.05	18.32 ± 0.57	19.17 ± 0.12
day 3		18.237 ± 0.75	20.56 ± 0.15	19.12 ± 0.55
day 5		17.63 ± 2.25	21.84 ± 1.12	19.66 ± 1.97
day 8	24.65 ± 0.11	19.28 ± 1.07	23.90 ± 0.73	18.60 ± 2.61
day 11		21.47 ± 0.06	23.30 ± 1.45	18.71 ± 1.16
day 14		21.00 ± 0.07	20.10 ± 3.04	21.16 ± 2.41
day 17	24.96 ± 2.20	19.85 ± 2.67	19.59 ± 2.17	21.31 ± 0.69

Table 4. Mn-superoxide dismutase (mitochondrial isoenzyme) activity in serum (expressed as NU/ml of serum). Values of means and standard errors

Day of experiment	control group	ischaemic preconditioning	irradiation	irradiation + ischaemic preconditioning
day 1	11.9697 ± 1.06	11.513 ± 0.65	10.792 ± 0.45	9.554 ± 0.61
day 3		9.855 ± 0.65	7.275 ± 1.39	7.652 ± 0.56
day 5		9.797 ± 1.22	7.333 ± 0.57	7.304 ± 0.80
day 8	11.362 ± 1.22	10.377 ± 0.33	9.855 ± 1.25	11.021 ± 1.80
day 11		9.101 ± 0.05	8.985 ± 0.99	8.657 ± 1.08
day 14		11.00 ± 0.05	9.849 ± 2.57	11.00 ± 0.05
day 17	11.784 ± 1.11	12.503 ± 2.13	12.732 ± 1.54	11.523 ± 0.52

Table 5. Numbers of crypts in intestine slices (expressed as percentage of number of crypts on mucosa area). Values of means and standard errors

Day of experiment	control group	ischaemic preconditioning	irradiation	irradiation + ischaemic preconditioning
day 1	43.00 ± 8.18	44.90 ± 17.33	46.55 ± 11.45	50.11 ± 11.63
day 3		47.00 ± 12.33	56.51 ± 10.22	58.59 ± 11.15
day 5		44.01 ± 9.56	46.95 ± 2.66	54.35 ± 5.61
day 8	38.19 ± 7.95	43.86 ± 11.07	43.19 ± 4.28	54.21 ± 11.54
day 11		41.62 ± 5.48	48.19 ± 7.55	52.43 ± 5.36
day 14		40.39 ± 6.72	38.83 ± 4.28	37.88 ± 4.38
day 17	40.57 ± 1.67	42.09 ± 10.82	47.14 ± 7.95	47.86 ± 9.03

Results

The level of peroxidation products in serum, expressed as malondialdehyde (MDA) concentration, was almost doubled, compared to controls, in both irradiated groups one day post irradiation. A further increase was observed up to the 5th day in the irradiated group, whereas local temporary ischaemia prevented a further increase in lipid peroxidation formation. The normalization of MDA up to the control level was observed on the fourth day after the termination of irradiation (Fig. 1). There were no regular changes in the MDA level in tis-

sue homogenates in any group (Fig. 2). It was striking that any manipulation, i.e. irradiation only, irradiation with preconditioning and/or preconditioning ischaemia, initially diminished serum activity of the cytoplasmic isoenzyme (CuZnSOD) relative to its activity in control groups. A slow increase of this enzyme activity was observed over the next few days, the quickest being in animals irradiated for a few days and which almost reached the control level on the 8th day (Fig. 3). Some fluctuation of CuZnSOD isoenzyme activity in intestinal tissue was observed in both irradiated groups with a tendency to increase compared to controls, although differences were not significant (Fig. 4). Figure 5 shows that preconditioning ischaemia had no influence on MnSOD mitochondrial isoenzyme activity and in both irradiated groups the behaviour of this enzyme was similar, e.g. it decreased during the first few days of fractionated irradiation. Also, there were no differences in MnSOD isoenzyme activity measured in tissue homogenates (Fig. 6). Over the course of the experiment in the control group and in rats with applied ischaemia, the number of crypts in mucosa of jejunum showed only small fluctuations. Irradiation caused increase of the crypt number in animals irradiated with or without preconditioned hypoxia on day 3, which was followed by subsequent decrease, more rapid in only irradiated rats. On day 14, the number of crypts was comparable in all experimental groups. The difference in the number of crypts in irradiated animals with and without local temporary ischaemia was statistically significant (Student's *t*-test $P < 0.05$) (Fig. 7). Post-hoc analysis revealed that in the case of MDA measured in serum, statistically important differences exist first between the group with ischaemia and the irradiated group and second, between the irradiated group and the group where ischaemia together with irradiation was applied. The same situation pertains to

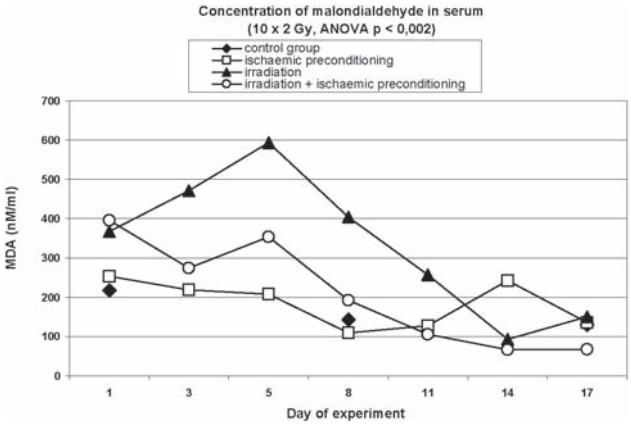

Fig. 1. Concentration of TBA-RS in serum of untreated control, ischaemia-preconditioned control, ischaemia-preconditioned and 10 x 2 Gy irradiated, and 10 x 2 Gy irradiated animals. Values and standard errors are presented in Table 1.

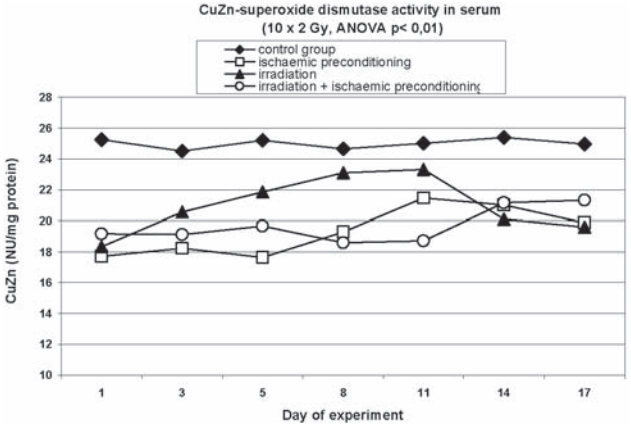

Fig. 3. CuZn-SOD (cytoplasmic isoenzyme) activity in serum of untreated control, ischaemia-preconditioned control, ischaemia-preconditioned and 10 x 2 Gy irradiated, and 10 x 2 Gy irradiated animals. Values and standard errors are presented in Table 2.

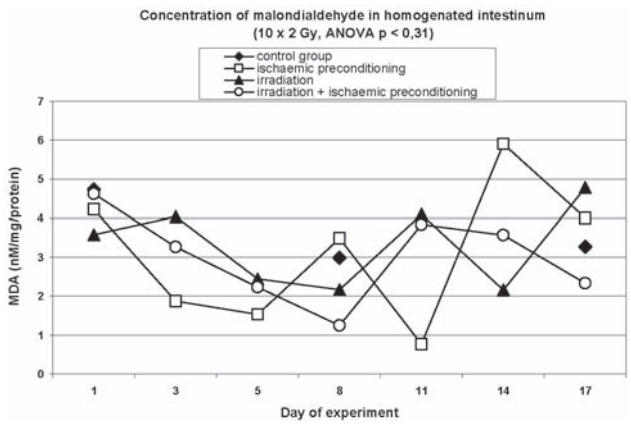

Fig. 2. Concentration of TBA-RS in intestinum homogenate of untreated control, ischaemia-preconditioned control, ischaemia-preconditioned and 10 x 2 Gy irradiated, and 10 x 2 Gy irradiated animals.

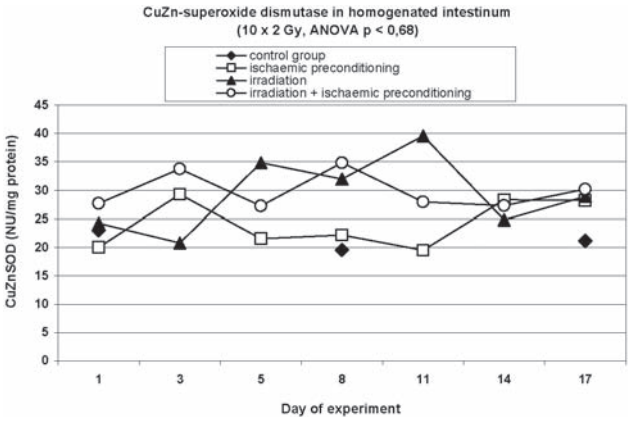

Fig. 4. CuZn-SOD (cytoplasmic isoenzyme) activity in intestinum homogenate of untreated control, ischaemia-preconditioned control, ischaemia-preconditioned and 10 x 2 Gy irradiated, and 10 x 2 Gy irradiated animals. Values and standard errors are presented in Table 3.

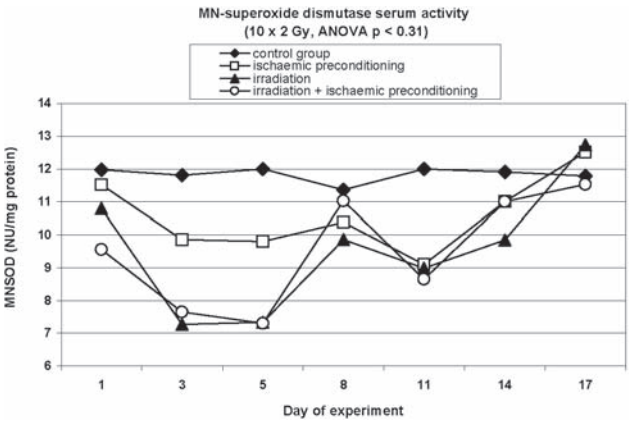

Fig. 5. Mn-SOD (mitochondrial isoenzyme) activity in serum of untreated control, ischaemia-preconditioned control, ischaemia-preconditioned and 10 x 2 Gy irradiated, and 10 x 2 Gy irradiated animals. Values and standard errors are presented in Table 4.

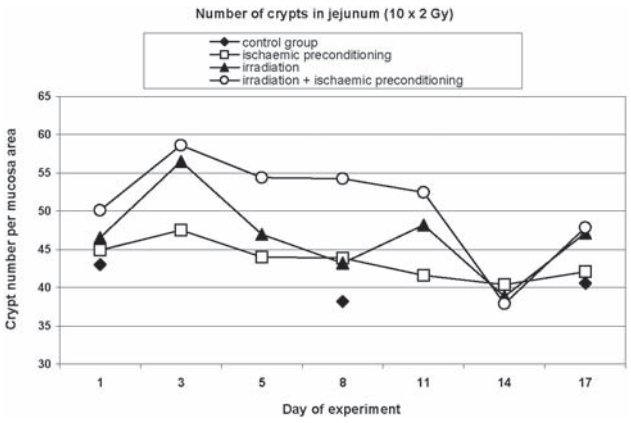

Fig. 7. Numbers of crypts in intestinum slices of untreated control, ischaemia-preconditioned control, ischaemia-preconditioned and 10 x 2 Gy irradiated, and 10 x 2 Gy irradiated animals. Values and standard errors are presented in Table 5.

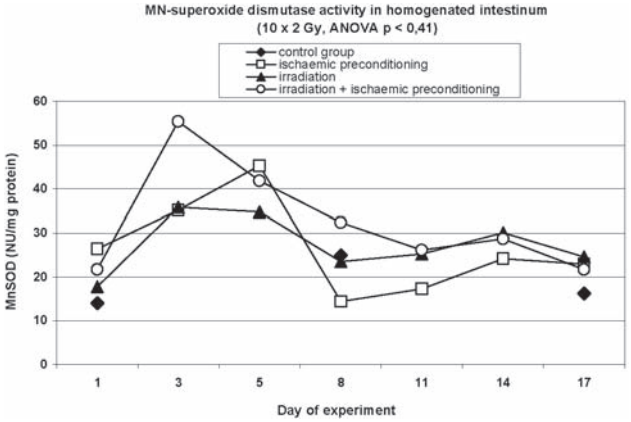

Fig. 6. Mn-SOD (mitochondrial isoenzyme) activity in intestinum homogenate of untreated control, ischaemia-preconditioned control, ischaemia-preconditioned and 10 x 2 Gy irradiated, and 10 x 2 Gy irradiated animals.

the case of CuZnSOD isoenzyme activity in serum. It means that there are no statistical differences between groups where irradiation together with ischaemia was applied and where ischaemia was applied alone. In case of MDA measured in homogenates, statistically important differences are those seen between measurement points but not the differences among groups. One can see the differences between days 1 and 5, 1 and 8, 5 and 14, 5 and 17, 8 and 17, and also between 11 and 17.

Discussion

The exposure to low doses (2–3 Gy) of ionizing radiation with brief local ischaemia was recently reported to modify the effect of total body irradiation (Walichiewicz et al., 2002, 2003). Related data obtained from experimental models *in vitro* and *in vivo* provide information that organisms can be protected against a potentially lethal stress by first exposing them to a temporary treatment of the same or a different inducer. This preconditioning mode of action has been investigated in many models of protection against oxidative stress including gamma-ray exposure (Walichiewicz et al., 2005). Stress-inducing stimuli, such as ionizing radiation, cause a broad spectrum of lesions in cell macromolecules, among them lipid peroxidation (Valko et al., 2007). Our data show that the concentration of lipid peroxides gradually increased up to the 5th day of the experiment and then slowly decreased to the control level. Local temporary ischaemia before each irradiation induced significant amelioration of this effect, but only in serum (Fig. 1). On the contrary, the values of TBA-RS were not significantly changed in the jejunum by applying brief local ischaemia (Fig. 2). The insignificance of these concentrations might probably be the result of their simultaneous formation and removal to blood, where they establish the total level of TBA-RS observed in serum. On the other hand, a significant attenuation of the lipid level by hypoxic preconditioning treatment was observed. After exposure to one run of hypoxia, the content of phospholipids and free fatty acids increased significantly, contrary to a profound decrease observed after exposure to four runs of hypoxia. It seems to be a kind of adaptive response of animals to hypoxia (Duan et al., 1999). A question remains why no statistical difference was observed, concerning TBA-RS, between the control group and the group with ischaemic preconditioning or between irradiated and non-irradiated animals when ischaemia was applied. We suppose that the part of animal body where ischaemia was applied in our experiments was too small to generate remarkable changes in the serum level of TBA-RS. On the other hand, local low-molecular-weight antioxidant involved in binding of transition metal ions such as copper and (mainly) iron plays a substantial role in preventing free radical lipid peroxidation. Another, probably also important, reason for the lack of statistical significance may be chemical reactivity of alkanals, which are formed

together with MDA and can react each other (Ohya, 1993). Such situation may result in the loss of MDA concentration induced by the used procedures and non-significant levels of TBA-RS. Since ionizing radiation is known to induce reactive oxygen species, among them the superoxide radical anions, we investigated local temporary ischaemia and the involvement of SOD isoenzymes in the protection against radiation. We observed high variability of cytoplasmic SOD isoenzyme (CuZnSOD) activity in serum and also in jejunum homogenates. It should be noted that the diminished serum activity of cytoplasmic SOD isoenzyme resulted from all treatments constituting the experimental procedure. Considering the study of Maczewski et al. (2004), lack of influence of ischaemia preconditioning upon SOD activities might be the result of endothelial protection. Namely, endothelial cells might activate an SOD-like anti-$^{\bullet-}O_2$-mechanism which attenuates the $^{\bullet-}O_2$ burst whilst at the same time causing an increased $^{\bullet}OH$ burst. Thus, specific SOD activity might not have been detectable because it was limited to a small, although functionally important enzyme fraction, like that bound to the endothelial glycocalyx. The superoxide radical anion formed can dismutate into oxygen and hydrogen peroxide. Superoxide and hydrogen peroxide can then react with transition metals' ions to create the strongly oxidative hydroxyl radical (Valko et. al., 2006). It has been reported that, contrary to a significant decrease of SOD activities induced by ischaemia, a significant increase in catalase activities was observed (Islekel, 1999b). Taken together, it is therefore not surprising that rising levels of $^{\bullet}OH$ burst stimulate catalase activities. Data reported in an *in vivo* study reveal a significant decrease not only in SOD, but also in glutathione peroxidase activities, contrary to the high increment of catalase activities (Islekel et al. 1999b). Glutathione peroxidase shares hydrogen peroxide with catalase, but it can also react with lipid hydroperoxides. Although CuZnSOD isoenzyme activities from ischaemia-preconditioned rats, in response to the radiation treatment, were lower than those from radiation-treated animals, the differences were not always significant. The variability of CuZnSOD from serum and jejunum homogenates might be explained by serum presence of extracellular and intracellular fractions of CuZnSOD from the irradiated area. The level of CuZnSOD increased gradually in irradiated rats. Local temporary ischaemia protected against radiation and, because of this, the CuZnSOD level activity in serum was low and rose on the 14th day (on the 4th day after the last local temporary ischaemia) when the protection ceased to act. In non-irradiated animals, during the first eight days the level of CuZnSOD in serum was low, which may be the sign that local temporary ischaemia diminished the transport of CuZnSOD isoenzyme through cell membranes, causing its build-up in the cells until the level of this enzyme, by the 11th day, was so high that it began to be secreted into the blood. MnSOD isoenzyme activity in tissue homogenate (Fig. 6) gradually increased during the first few days, then slowly recovered, reaching after 17 days a value close to that of the control. Radiation alone also induced an increase of MnSOD activity in jejunum during the first days of treatment, although much lower than the combined treatment of local temporary ischaemia followed by radiation. Levels of MnSOD isoenzyme activity depended on local temporary ischaemia but because this is low, the differences in activity of mitochondrial isoenzyme might be insignificant statistically. Simultaneous variability of MnSOD levels in blood and in jejunum homogenates suggests that cellular resistance against radiation produced by local temporary ischaemia depends on MnSOD expression and activity. It should be underlined that the superoxide radical reacts rapidly with nitric oxide, forming peroxynitrite, and sustaining this radical anion-driven effects is nitration and inactivation of MnSOD isoenzyme (Yamakura et al., 1998), which is closely linked with ischaemia and reperfusion (McMillan-Crow and Cruthirds, 2001).

As presented in Fig. 7, the changes in the number of crypts are substantial. Up to the 3rd day of irradiation the number of crypts had increased but then subsequently decreased. By the 11th day of the experiment (after 10 days of irradiation) the number of crypts increased again in irradiated animals that had not received local temporary ischaemia. Such an effect may indicate that some of the cells undergoing apoptosis, induced by low radiation doses, release agents that initiate proliferation of surviving clonogenic cells. This is most probably a typical reaction of mucosa in response to radiotherapy and it may imply that the population of the clonogenic cells is heterogenous in terms of radiosensitivity (Hendry et al., 1992; Roberts et al., 2003). On the other hand, as suggested by Thames et al. (1981), the population of intestinal crypt cells has a significant ability for the repair of sublethal damage. Small doses of irradiation stimulate proliferation in fast-responding tissue. Higher doses destroy proliferating cells. Between 7th and 11th day of radiotherapy with a daily dose of 2 Gy, mucosa (and also jejunum crypts) regenerate (Trott and Kummermer, 1993; Dorr et al., 1994). Local temporary ischaemia applied immediately before each irradiation showed protective action against the destructive effects of radiation, even though stimulation to proliferation was the same in irradiated animals with or without ischaemia. The number of crypts in rats that had received local temporary ischaemia did not decrease during subsequent days of radiotherapy. By the 14th day of the experiment the number of crypts decreased in both irradiated groups. The protective effects of local temporary ischaemia are present for up to a maximum of four days after the last ischaemia (Walichiewicz et al. 2002, 2003) and it is probable that the decrease in the number of crypts was the result of termination of the protective effects from local temporary ischaemia.

Ionizing radiation treatment can induce many radiotoxic effects *in vivo*. However, at least some of these effects can be slowed down by inducing local temporary ischaemia. Local temporary ischaemia seems to result

from the formation of oxygen radicals, which react directly with unsaturated lipids, and this leads to cell membrane peroxidation. Antioxidant enzymes SOD, catalase and radical scavengers are able to detoxify oxygen radicals, thus attenuating the post-ischaemic lesions of the mucosa, and prevent reperfusion damage of the intestine. This suggests the role for preventive and therapeutic strategies focusing on counteracting oxidative damage in situations involving oxidative stress, i.e. use of antioxidant. The putative underlying mechanism may involve direct molecular recombination of free radicals induced both by brief ischaemia and by ionizing radiation. It seems that ischaemia may prevent ionizing radiation damage of the intestine when the irradiation starts during the reperfusion period. In addition, we have previously shown that the protective effect of ischaemic preconditioning disappeared with the use of higher doses of radiation (Walichiewicz et al., 2005). Thus, it may be concluded that the nature of ischaemic preconditioning limits its capacity to buffer massive radicals' bursts created by high doses of ionizing radiation. Since the conditions of radiation treatment employed by us, i.e. relatively low dose and/or short exposure, were the same as in usual clinical practice, the observation that some radiation toxicity symptoms in non-ischaemic tissues can be ameliorated by local temporary ischaemia is obviously valuable. The use of ischaemic preconditioning as a potential protective treatment against radiation toxicity *in vivo* should be carefully evaluated under various conditions of administration. Possible alterations in antioxidant defence may be useful indicators for monitoring radiotoxicity and the effectiveness of protective attempts such as local ischaemic preconditioning.

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