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The effect of silymarin and gamma radiation on nucleic acids in rat organs

H. HAKOVÁ, E. MIŠÚROVÁ, *Department of Cellular and Molecular Biology, P. J. Šafárik University, Košice, Slovakia*

Abstract—The influence of silymarin on radiation-induced changes in concentrations of RNA and DNA was followed in male Wistar rats. The liver, spleen and bone marrow were examined at 30 h, 7, 14 and 21 days after 6 Gy whole-body gamma irradiation or 30 h and 7 days after 3 Gy whole-body gamma irradiation. Following silymarin treatment, a mild increase in the concentration and total content of RNA and DNA in liver and bone marrow at days 7 and 14 after administration was found; in spleen, total content of DNA significantly increased at 30 h. Silymarin administered 1 h before irradiation, moderated radiation-induced changes in nucleic acids in its target organ, liver, and in spleen and bone marrow. We suggest that beneficial effects of silymarin on radiation injury to the membranes of liver cells resulted primarily from its antioxidative ability and its ability to act as a radical scavenger, thereby preventing membrane permeability changes.

Silymarin, frequently used in the treatment of liver diseases, is a flavonoid complex consisting of silybin, silydianin and silychristin. It is capable of protecting liver cells directly by stabilizing the membrane structures, including endoplasmic reticulum, by an effective decrease of membrane permeability and a change of lipid content in the membrane (Fassati & Fassati 1973; Seeger 1971). Silymarin can also influence intracellular metabolism including RNA synthesis (Sonnenbichler et al 1975). This drug was found to protect mice from flyagaric toxins (Vogel & Temme 1969). Silymarin application alleviates the injury to mitochondria, endoplasmic reticulum and other structures in rat liver induced by tetrachloromethane.

Several experimental studies have been done to assess whether silymarin can influence the course of radiation illness. Flemming (1971) found that oral doses of silymarin to mice prevented the radiation-induced loss of body weight, increased the percentage of animal survival and accelerated the recovery of surviving mice.

In regenerating liver (after partial hepatectomy) of irradiated rats, silymarin partly alleviates the changes in nucleic acids, histones and some cytological indicators of the damage (Haková & Mišúrová 1992; Kropáčová & Mišúrová 1992; Kožurková et al 1992).

The aim of our study was to assess the effect of silymarin on radiation-induced nucleic acid changes in its target organ, the liver, and in morphologically and functionally different tissues, bone marrow and spleen. As in other combined treatments, the results may be influenced by the sequence of treatments applied. In the present work we have assessed the radioprotective effect of silymarin applied before irradiation.

Materials and methods

Adult male Wistar rats, 240–280 g at the beginning of the experiments, were kept under standard conditions and were allowed free access to laboratory food and water.

Silymarin (Flavobion, Spofa, CSFR) was administered orally at a dose of 70 mg kg^{-1} by tube, 1 h before total body irradiation, as recommended by the manufacturer.

Rats were irradiated with a single total body dose of gamma radiation (3 or 6 Gy) from a ^{60}Co source (Chisostat, CSFR) at a dose rate of 276 mGy min^{-1}.

Rats were examined 30 h, 7, 14 and 21 days after irradiation or silymarin treatment. Animals which received 3 Gy were examined only at the critical intervals of 30 h and 7 days following irradiation.

Nucleic acid concentrations in the liver, spleen and bone marrow were determined spectrophotometrically (Tsanev & Markov 1960) in hydrolysates of purified samples.

Statistical significance was evaluated by the Peritz' F-test (Harper 1984). In control and experimental groups there were from 6 to 10 animals. However, since the experiments were performed repeatedly, the individual data are based on examination of 12–18 animals.

Results and discussion

Liver. In liver of non-irradiated rats, silymarin alone did not cause a statistically significant increase in the concentration and total content of RNA between 30 h and 14 days after administration (Table 1).

In 6 Gy-irradiated animals protected by silymarin, both the concentration and total content of RNA were higher than in non-protected irradiated animals during the entire period that followed. Statistically significant differences were noted 7 and 14 days after irradiation. In 3 Gy-irradiated animals, the differences between silymarin-protected and non-protected responses were found to be more marked.

The effect of silymarin alone on the DNA and RNA concentration and total content was similar; an increase of 10–15% as compared with intact controls was seen between 30 h and 14 days after administration (Table 1).

Protective effects of silymarin on 6 Gy-induced changes in DNA can be seen during the entire period that followed, most markedly on day 7 after irradiation. At that time, irradiation alone decreased the concentration and content of DNA temporarily by 24% compared with controls; in silymarin-protected irradiated animals no post-irradiation loss in DNA was observed, and DNA values were higher by 40–45% compared with non-protected animals.

The lower radiation dose (3 Gy) caused a 10–15% decrease in both the DNA concentration and content in liver. In this group, silymarin also prevented a radiation-induced loss in DNA and the concentration of DNA was statistically higher compared with non-protected rats.

Spleen. In spleen, silymarin alone produced a transient increase in total content of nucleic acids, primarily of the DNA at 30 h; the slight increase in the content of nucleic acids found on the 21st day after administration was without statistical significance (Table 2).

The 6 Gy dose alone resulted in a considerable reduction in nucleic acid concentration; total content of both RNA and DNA per organ was reduced by day 7, to 28 and 40% of control values, respectively. A rapid recovery took place with an overshooting of control values as seen 21 days after irradiation.

The 3 Gy dose caused much less reduction in nucleic acid concentration and total RNA and DNA content in spleen; the loss in RNA and DNA was 35–40% lower than after 6 Gy.

Correspondence: H. Haková, Department of Cellular and Molecular Biology, P. J. Šafárik University, Moyzesova 11, 041 67 Košice, Slovakia.

Table 1. Concentration and total content of RNA and DNA (means ± s.e.m.) in the liver of rats after application of silymarin and gamma irradiation.

		RNA		DNA	
Time	Treatment	Concn (mg g^{-1})	Content (mg)	Concn (mg g^{-1})	Content (mg)
	Control	7·36 ± 0·71	74·7 ± 0·08	1·11 ± 0·09	11·3 ± 1·2
30 h	Silymarin (70 mg kg^{-1})	8·04 ± 0·68	84·1 ± 0·8	1·20 ± 0·13	12·9 ± 0·9
	Irradiation (6 Gy)	7·55 ± 0·42	68·9 ± 0·3	1·22 ± 0·13	11·1 ± 0·8
	Silymarin (70 mg kg^{-1}) + irradiation (6 Gy)	7·94 ± 0·53	75·2 ± 2·4	1·21 ± 0·61	11·7 ± 0·6
	Irradiation (3 Gy)	7·45 ± 0·29	71·9 ± 0·9	1·14 ± 0·76	10·9 ± 1·4
	Silymarin (70 mg kg^{-1}) + irradiation (3 Gy)	8·05 ± 0·54	81·9 ± 1·3	1·20 ± 0·71	11·9 ± 0·5
7 days	Silymarin (70 mg kg^{-1})	8·47 ± 0·49	87·9 ± 4·1	1·26 ± 0·04	12·9 ± 1·1
	Irradiation (6 Gy)	7·64 ± 0·33	76·9 ± 1·5	0·86 ± 0·05*	8·7 ± 0·8
	Silymarin (70 mg kg^{-1}) + irradiation (6 Gy)	8·59 ± 0·52*°	81·0 ± 0·8	1·37 ± 0·18*°°	13·0 ± 0·2
	Irradiation (3 Gy)	7·25 ± 0·65	78·7 ± 1·7	1·03 ± 0·12	10·2 ± 1·4
	Silymarin (70 mg kg^{-1}) + irradiation (3 Gy)	8·75 ± 0·59*°	85·2 ± 0·9	1·33 ± 0·19*°°	11·4 ± 1·1
14 days	Silymarin (70 mg kg^{-1})	8·32 ± 0·48	82·6 ± 1·1°	1·30 ± 0·10	12·7 ± 0·9
	Irradiation (6 Gy)	6·98 ± 0·67	58·6 ± 3·8*	1·22 ± 0·12	10·0 ± 0·9
	Silymarin (70 mg kg^{-1}) + irradiation (6 Gy)	7·99 ± 0·69	70·1 ± 1·1°	1·42 ± 0·18*°	12·1 ± 0·6
21 days	Silymarin (70 mg kg^{-1})	7·58 ± 0·81	76·9 ± 2·9	1·17 ± 0·08	12·4 ± 0·9
	Irradiation (6 Gy)	6·59 ± 0·75	59·8 ± 1·2	1·32 ± 0·14	11·5 ± 0·9
	Silymarin (70 mg kg^{-1}) + irradiation (6 Gy)	7·42 ± 0·38	71·3 ± 1·4	1·43 ± 0·13	12·7 ± 0·7

*$P < 0·05$ compared with control. °$P < 0·05$, °°$P < 0·01$ compared with unprotected animals.

The protective effect of silymarin in 6 Gy-irradiated rats was manifested as a more rapid increase in RNA concentration than in unprotected rats, within an initial post-irradiation period. A statistically significant difference was seen on the 7th day after exposure, when a concentration 35% higher than in unprotected irradiated rats was found. Moreover, silymarin moderated the overshoot of both RNA concentration and content on day 21 after irradiation.

The favourable effect of silymarin on nucleic acid changes was more marked after a dose of 3 Gy than after a 6 Gy-dose at both intervals that followed. Differences were seen in DNA content per organ: in protected animals it reached the level of the silymarin group 7 days after irradiation, i.e. it was 30% higher than in non-protected rats.

Bone marrow. Silymarin alone did not cause a statistically significant increase in either the concentration or total content of RNA or DNA in bone marrow (Table 3).

In irradiated rats, silymarin application moderated the post-irradiation loss in both nucleic acids. On the other hand, there was a more marked overshoot of values compared with that in non-protected irradiated animals during the recovery period. In bone marrow, as in liver and spleen, a more favourable effect of silymarin could be seen after a dose of 3 Gy than 6 Gy.

Considering the pattern of changes in the three tissues, it can be seen that the modifying effect of silymarin predominated in liver, whereas the effect of irradiation, primarily with the higher dose (6 Gy) predominated in spleen and bone marrow. The moderation of the initial response of cells to irradiation, as a

Table 2. Concentration and total content of RNA and DNA (means ± s.e.m.) in the spleen of rats after application of silymarin and gamma irradiation.

		RNA		DNA	
Time	Treatment	Concn (mg g^{-1})	Content (mg)	Concn (mg g^{-1})	Content (mg)
	Control	7·4 ± 0·2	5·2 ± 0·1	4·6 ± 0·2	3·2 ± 0·1
30 h	Silymarin (70 mg kg^{-1})	8·4 ± 0·4	6·7 ± 0·7	5·0 ± 0·3	4·8 ± 0·8**
	Irradiation (6 Gy)	4·2 ± 0·1**	1·3 ± 0·1**	3·4 ± 0·2	1·3 ± 0·2**
	Silymarin (70 mg kg^{-1}) + irradiation (6 Gy)	4·1 ± 0·2**	1·6 ± 0·1**	3·7 ± 0·2	1·5 ± 0·1**
	Irradiation (3 Gy)	5·1 ± 0·4*	3·4 ± 0·4*	3·6 ± 0·3	2·4 ± 0·2
	Silymarin (70 mg kg^{-1}) + irradiation (3 Gy)	5·9 ± 0·5	4·6 ± 0·3	4·5 ± 0·5	3·0 ± 0·2
7 days	Silymarin (70 mg kg^{-1})	7·7 ± 0·5	5·9 ± 0·5	4·8 ± 0·6	3·6 ± 0·4
	Irradiation (6 Gy)	4·2 ± 0·9**	1·3 ± 0·1**	4·6 ± 0·4	1·4 ± 0·1**
	Silymarin (70 mg kg^{-1}) + irradiation (6 Gy)	6·5 ± 0·7	1·9 ± 0·3**	5·3 ± 0·6	1·5 ± 0·1**
	Irradiation (3 Gy)	5·3 ± 0·4*	3·8 ± 0·2	4·1 ± 0·5	2·5 ± 0·4
	Silymarin (70 mg kg^{-1}) + irradiation (3 Gy)	6·9 ± 0·4	4·6 ± 0·5	5·4 ± 0·4°	3·6 ± 0·6°
14 days	Silymarin (70 mg kg^{-1})	7·9 ± 0·5	5·4 ± 0·4	4·6 ± 0·4	3·6 ± 0·3
	Irradiation (6 Gy)	9·7 ± 0·7*	4·4 ± 0·1	6·8 ± 0·5**	3·0 ± 0·3
	Silymarin (70 mg kg^{-1}) + irradiation (6 Gy)	9·4 ± 0·5*	4·4 ± 0·1	6·9 ± 0·7**	3·1 ± 0·3
21 days	Silymarin (70 mg kg^{-1})	8·9 ± 0·4	6·5 ± 0·1	4·6 ± 0·6	3·9 ± 0·5
	Irradiation (6 Gy)	12·6 ± 0·5**	10·3 ± 0·5**	5·6 ± 0·3	4·6 ± 0·3**
	Silymarin (70 mg kg^{-1}) + irradiation (6 Gy)	10·7 ± 0·6**°	8·1 ± 0·7**°	6·8 ± 0·4**°	5·1 ± 0·3**

*$P < 0·05$, **$P < 0·01$ compared with control. °$P < 0·05$ compared with unprotected animals.

Table 3. Concentration and total content of RNA and DNA (means ± s.e.m.) in the bone marrow of rats after application of silymarin and gamma irradiation.

		RNA		DNA	
Time	Treatment	Concn (mg g^{-1})	Content (mg)	Concn (mg g^{-1})	Content (mg)
	Control	3·7 ± 0·6	0·12 ± 0·00	2·7 ± 0·2	0·08 ± 0·00
30 h	Silymarin (70 mg kg^{-1})	3·6 ± 0·9	0·13 ± 0·00	2·7 ± 0·2	0·10 ± 0·00
	Irradiation (6 Gy)	1·7 ± 0·2**	0·06 ± 0·00**	1·3 ± 0·1*	0·05 ± 0·00*
	Silymarin (70 mg kg^{-1}) + irradiation (6 Gy)	2·1 ± 0·2*	0·09 ± 0·00	1·4 ± 0·2*	0·06 ± 0·00*
	Irradiation (3 Gy)	1·8 ± 0·3**	0·06 ± 0·00**	1·7 ± 0·4*	0·05 ± 0·00*
	Silymarin (70 mg kg^{-1}) + irradiation (3 Gy)	2·9 ± 0·4°	0·10 ± 0·00	2·7 ± 0·2	0·07 ± 0·00
7 days	Silymarin (70 mg kg^{-1})	3·9 ± 0·6	0·13 ± 0·00	3·0 ± 0·3	0·09 ± 0·00
	Irradiation (6 Gy)	4·0 ± 0·6	0·11 ± 0·00	3·2 ± 0·6	0·09 ± 0·00
	Silymarin (70 mg kg^{-1}) + irradiation (6 Gy)	4·3 ± 0·9	0·14 ± 0·00	4·0 ± 0·3*	0·11 ± 0·00*
	Irradiation (3 Gy)	3·0 ± 0·6	0·10 ± 0·00	3·3 ± 0·7	0·07 ± 0·00
	Silymarin (70 mg kg^{-1}) + irradiation (3 Gy)	4·2 ± 0·5°	0·13 ± 0·00	4·1 ± 0·4*	0·10 ± 0·00°
14 days	Silymarin (70 mg kg^{-1})	4·7 ± 1·2	0·16 ± 0·00	3·0 ± 0·1	0·9 ± 0·00
	Irradiation (6 Gy)	4·4 ± 0·7	0·21 ± 0·00**	2·1 ± 0·4	0·10 ± 0·00
	Silymarin (70 mg kg^{-1}) + irradiation (6 Gy)	5·2 ± 1·1	0·24 ± 0·00**	2·6 ± 0·4	0·09 ± 0·00
21 days	Silymarin (70 mg kg^{-1})	4·0 ± 0·9	0·15 ± 0·00	3·0 ± 0·2	0·09 ± 0·00
	Irradiation (6 Gy)	5·8 ± 0·5*	0·18 ± 0·00	2·1 ± 0·8	0·09 ± 0·00
	Silymarin (70 mg kg^{-1}) + irradiation (6 Gy)	4·4 ± 0·2	0·19 ± 0·00*	2·3 ± 0·2	0·09 ± 0·00

*$P < 0.05$, **$P < 0.01$ compared with control. °$P < 0.05$ compared with unprotected animals.

monitor of radioprotective effectiveness, was more marked in radiosensitive tissues (spleen, bone marrow) after 3 Gy irradiation. This finding may relate to the fact that, in contrast to slowly proliferating and more radioresistant tissues, the dominating injury in highly proliferating tissues, is the chromatin breakdown (Skalka et al 1965; Matyášová et al 1984). Therefore, silymarin (enhancing mainly the cell membrane stability) could not prevent the cell loss induced by chromatin breakdown in these tissues.

Free radicals arising due to irradiation can cause a cumulation of destroyed or atypic molecules which can consequently influence the membrane permeability (for example, lysosome membranes) and metabolism as a whole. Serious metabolic dysfunction can be caused by biological mediators released due to the action of radicals produced by the radiolysis of water (Donlon & Walden 1988). Mediators, such as prostaglandins and histamine, interact with specific receptors, thereby controlling physiological responses at the local, organ and whole animal level.

We suggest that alleviation of the radiation consequences in tissues with different proliferating activities is a result of silymarin's ability to act as a radical scavenger, thereby preventing the membrane permeability changes.

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