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Serum Proteins in Rats Exposed to Ionizing Radiation and Thermal Injury

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Summary

Changes of the serum protein concentration were examined in the following groups of rats: a) those continuously irradiated at a dose rate of 1 Gy/day up to a total dose of 7 Gy [IR], b) burned with dry heat of 110 °C for 20 s on a 1590 mm^2 area of body surface [B], c) subjected to both (irradiation and thermal injury) treatments [MIX], d) an intact control group [C]. It has been ascertained that the dynamics of change in serum prealbumin, A_1-macroglobulin, haptoglobin and haemopexin in all experimental groups were in principle similar from the 7th day after the onset of irradiation. However, compared to the control group, a significant difference was observed particularly after thermal damage and after the effect of both treatments. Alterations in serum protein concentrations after irradiation alone had only a temporary effect.

Key words

Serum proteins – Continuous gamma irradiation – Thermal injury

Introduction

The understanding of the mode of serum protein response to various physical noxa is of importance mainly in clinical practice. In case of physical damage, the most important is ionizing radiation. Radiation-induced protein changes as well as their modification by numerous radioprotective agents have been studied experimentally in considerable detail. It can be stated that the protein response in irradiated organisms correlates well with the development of radiation disease (Balish *et al.* 1970, Markoe *et al.* 1976).

Similarly, there is a vast amount of experimental data on the effect of burns on serum proteins. Electrophoretic analyses clearly show a similar pattern of changes after irradiation, the values being dependent on the extent of the burnt body surface (Baar 1970, Waxman *et al.* 1987). However, little is known about the combined effect of both radiation and subsequent burning on serum proteins. Therefore, we have tried to assess such a combined injury, the emphasis being placed on the contribution of either treatments on the induced changes in serum protein concentration.

Methods

Male Wistar SPF rats weighing 180–200 g were used throughout. They were fed pelleted Larsen diet and water *ad libitum*. Four groups of rats were used: a) rats continuously irradiated at a dose rate of 1 Gy per day (^{60}Co source) up to a total dose of 7 Gy [IR], b) rats exposed to dry heat of an electric source (110 °C for 20 seconds) without making contact with 1590 mm^2 surface of the clean-shaven animal's back [B], c) rats exposed to both treatments, 7 Gy gamma dose before surface burning [MIX] and d) healthy control rats [C]. All the animals were kept in a naturally lighted regimen, the burnt ones being maintained under sterile conditions (bedding, food). Experimental burning has been done under the total anaesthesia of animals. Before thermal injury experimental animals were anaesthetized with Pentobarbital (Spofa) in a dose of 35 mg/kg i.p. Thermal injury was performed about 15 min after the start of the anaesthesia. The degree of anaesthesia was tested by a stretch of the hindleg. Blood was collected after rapid decapitation at intervals 1, 7, 14, 30 and 60 days after cessation irradiation or thermal damage had been discontinued. There were 7 animals in each group.

Serum protein concentrations were determined by quantitative two-dimensional immunoelectrophoresis as described previously (Chlebovská *et al.* 1981).

Individual precipitation lines correspoding to the serum proteins followed were evaluated using software FOREZA on a computer. The results were evaluated statistically by Student's t-test with a 95 % confidence interval for the means. Statistical significance was expressed for $P<0.05$.

Table 1

The mean serum prealbumin, albumin and A_1-globulin concentration (%) after irradiation (IR), thermal injury (B) and irradiation with thermal injury (MIX) in rats compared to the the control group (C)

Intervals	Groups	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.
Days		Prealbumin	Albumin	A_1-globulin
1	MIX	86.96±36.24	116.66±17.17	93.49±13.23
	B	125.36±38.63+ + +	111.62±12.99	105.75±16.25
	IR	39.13±6.15**	94.30±17.98	73.12±19.49
	C	100.00±20.71	100.00±18.08	100.00±10.79
7	MIX	60.02±21.21	80.67±6.35* +	70.37±10.36*
	B	86.52±24.86	95.71±8.90	96.03±10.40
	IR	63.93±19.93	96.39±6.44	88.36±12.80
	C	100.00±24.96	100.00±4.02	100.00±12.87
14	MIX	80.78±30.62	96.66±6.36	75.79±7.14*
	B	89.64±23.00	97.96±7.29	105.95±28.14
	IR	100.45±32.39	102.96±1.74	120.95±20.05
	C	100.00±29.99	100.00±4.13	100.00±10.11
28	MIX	167.14±48.71*	99.11±7.28	97.43±16.32
	B	139.87±51.12	96.93±4.79	104.93±17.34
	IR	135.82±22.10	85.96±9.06*	101.90±12.63
	C	100.00±33.66	100.00±5.74	100.00±11.48
60	MIX	78.44±11.17*	84.57±8.11*	76.55±9.55**
	B	83.64±23.41	85.77±6.00*	105.20±9,05
	IR	81.76±20.05	99.28±8.05	92.18±21.27
	C	100.00±16.82	100.00±6.08	100.00±6.80

*The significance of values (P<0.05, P<0.01, P<0.001): *, **, *** vs control; +, + +, + + + vs irradiation*

Results

The following proteins were evaluated by quantitative two-dimensional immunoelectrophoresis: prealbumin, albumin, A_1-globulin, A_1-macroglobulin, haptoglobin and haemopexin.

Prealbumin

Marked changes in serum prealbumin in response to irradiation could be seen as early as 24 h after its cessation (Table 1). At that time, the concentrations lower than control were found in both IR and MIX groups, significantly in the former one. By 7 days the decrease became more profound in the MIX group only. On the contrary, an increase in prealbumin, though non-significant, was observed 24 h after burning. Between 7–14 days, there was a transient recovery to norm in all three groups, followed by an increase on day 28, significant again in the MIX group only. During the period of 60 days the prealbumin concentration recovered in the B and IR groups, while it remained significantly changed in the MIX rats.

Table 2
The mean serum haptoglobin, haemopexin and A_1-macroglobulin concentration (%) after irradiation (IR), thermal injury (B) and irradiation with thermal injury (MIX) in rats compared to the control group (C)

Intervals	Groups	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.
Days		Haptoglobin	Haemopexin	A_1-macroglobulin
1	MIX	151.96±11.55***	109.45±9.97	122.97±24.23
	B	161.10±8.62*** +	102.64±13.14	129.48±30.54
	IR	106.27±27.74	92.68±9.72	93.84±14.79
	C	100.00±14.13	100.00±9.85	100.00±23.52
7	MIX	85.90±44.93	74.44±11.08	57.89±10.52**
	B	103.84±8.30	60.00±10.62*	63.15±16.64*
	IR	91.02±30.25	85.27±17.20	66.66±11.84*
	C	100.00±33.97	100.00±29.00	100.00±13.72
14	MIX	112.71±47.99	96.23±27.34	87.50±5.32
	B	128.39±36.04	104.11±19.11	101.39±13.13
	IR	102.12±48.68	98.87±23.53	102.78±13.23
	C	100.00±36.76	100.00±21.94	100.00±32.33
28	MIX	95.93±16.91	110.54±12.91	105.21±8.20
	B	109.46±16.72	113.79±11.52	102.55±11.42
	IR	107.74±15.11	118.82±18.58	114.36±24.41
	C	100.00±13.08	100.00±5.80	100.00±3.60
60	MIX	76.38±23.99*	72.98±10.49**	77.85±11.79
	B	67.38±13.26**	76.33±6.95**	89.06±1.98
	IR	92.40±9.29	91.39±7.91	99.97±14.29
	C	100.00±7.85	100.00±7.15	100.00±17.46

*The significance of values ($P<0.05$, $P<0.01$, $P<0.001$): *, **, *** vs control; +, + +, + + + vs irradiation*

Albumin

As follows from Table 1, a significant decrease occurred in the MIX rats at 7 and 60 days and in IR rats at 30 days. At other intervals the albumin concentrations corresponded to the values of unirradiated controls.

A_1-globulin

Serum A_1-globulin (Table 1) was affected primarily by the combined treatment which was followed by a statistically significant fall at 7, 14 and 60 days. In the other groups, no such marked decrease was observed. The increase at 14 days after irradiation appeared to be only transient.

A_1-macroglobulin

The values of A_1-macroglobulin (Table 2) were slightly elevated in the MIX and B groups after the first 24 h before decreasing in all experimental groups at 7 days. However, from day 14 the concentration returned to control levels.

Haptoglobin

The greatest changes were detected at the first estimation time when the haptoglobin concentration (Table 2) reached 152 % and 161 % of the control levels in the MIX and B rats, respectively. A lower increase could still be seen after 2 weeks. This was subsequently followed by a steady decrease with the lowest values being attained by 60 days. This was significant in all the groups as compared to the controls.

Haemopexin

Haemopexin, like haptoglobin, exhibited the greatest changes which persisted till the end of the observation period (60 days) in B and MIX rats. The significant decrease in the haemopexin concentration 7 days after burning alone (Table 2) appears to be of special interest.

Discussion

The present experiments were modelled as a hypothetical nuclear incident where ionization radiation was combined with a thermal noxa.

When evaluating the results, it can be stated that no permanent results in the concentration of the studied proteins were caused by the continuous irradiation dose employed. Certain decrease of the serum prealbumin, albumin, A_1-globulin and A_1-macroglobulin were observed mainly up to 30 days after irradiation. They were temporary in character and were mitigated by subsequent autoregulation. A long-term drop of serum concentration in rats irradiated with continuous exposure to 1 Gy/day was found in our previous experimentsat the terminal period of their life, i.e. by the 44th day when the damage of organs synthetizing proteins had probably occurred (Chlebovská *et al.* 1978).

More important alterations of protein composition appeared after the thermal injury and combined damage. On the basis of the changes in haptoglobin and A_1-macroglobulin it may be concluded that the thermal noxa is the main factor in inducing differences of the MIX group. It is known that both damage (i.e. heat and irradiation) lead to an inflammatory reaction characterized by an increase of the protein content in the acute phase and an alteration of their serum concentration related to the extent of damage caused by these traumas (Daniels *et al.* 1974, Henneberg *et al.* 1975). Because such an increase was not present in the irradiated group, the obvious increase of haptoglobin values up to 160 % was already found on the second day in the B and MIX groups. This is probably a reaction to inflammatory processes caused by the thermal damage. Similar findings have been reported by Šimša and Mráz (1974). These authors compared the effects of acute $LD_{25/30}$ irradiation and 30 % surface burning. The increase of haptoglobin concentration on day 14 after thermal injury is considered to be caused by the spread of skin infection which culminates exactly at this period.

The decisive contribution of thermal damage was not evident in relation to the alterations related to the prealbumin, albumin, A_1-globulin, A_1-macro-globulin and haemopexin serum concentration decrease in the MIX group. Notwithstanding that, the authors observing the results of burns on serum proteins stated that the significant decrease of albumin fractions at early time intervals after this noxa lasted untill 60 days after (Bonate 1990). We have observed this on day 7 after the combined damage only. It is of interest that on the 60th day of our experiments the albuminaemia, as a result of the thermal noxa, was decreased to the same extent in B and MIX groups. It can be stated that irradiation combined with thermal injury induced a more marked response of rats than irradiation alone, which was followed by slower and long-term reparation of all the parameters studied.

Attention should be drawn to the different character of prealbumin changes on the first day of examination among groups IR and B or MIX, respectively. These differences suggest that different mechanisms are involved in modifying the serum values in irradiated and burnt animals. The radiation-induced prealbumin decrease, in agreement with the data reported by Henneberg *et al.* (1975), is attributed to a reduced offer of tryptophan – its structural amino-acid – due to postradiation anorexia. In burnt children, Lytle *et al.* (1960) found a fraction functionally different from the prealbumin in healthy organisms before the albumin electrophoretic line. This fraction, denoted as pseudoprealbumin (Daniels *et al.* 1974), multiplies during catabolic processes after thermal injury.

The dynamics of serum protein concentration changes described here was studied in laboratory rats. As far as the species specificity is concerned, it is possible, with a certain tolerance, to apply these results to estimating the effect of similar noxa in man.

References

BAAR S.: Plasma protein changes in mild thermal trauma. *J. Trauma* **10:** 684–689, 1970.

BALISH E., PEARSON T.A., CHASKES S.: Irradiated humans: Microbial flora, immunoglobulins, complement [C3] transfferin, agglutinins, and bacteriocidins. *Radiat. Res.* **43:** 729–756, 1970.

BONATE P.L.: Pathophysiology and pharmacokinetics following burn injury. *Clin. Pharmacokin.* **18:** 118–130, 1990.

CHLEBOVSKÁ K. , CHLEBOVSKÝ O., PRASLIČKA M.: Effect of magnitude of daily continuous radiations dose on serum proteins and survival in rats. *Folia Biol.* **24:** 51–58, 1978.

CHLEBOVSKÁ K., PRASLIČKA M., CHLEBOVSKÝ O.: Character of changes in the serum proteins of continuously irradiated rats. *Physiol. Bohemoslov.* **30:** 557–567, 1981.

DANIELS J.C., LARSON D.L., ABSTON S., RITZMANN E.: Serum protein profiles in thermal burns. I. Serum electrophoretic patterns, immunoglobulins, and transport proteins. *J. Trauma* **14**: 137–152, 1974.

HENNEBERG R., MESSERSCHMIDT O., MAURER G.: Studies on combined injuries. XXII. Investigations on serum proteins of mice after whole-body irradiation combined with open skin wounds. *Strahlentherapie* **149**: 636–649, 1975.

LYTLE R.J., MILLER L.F., ROSENTHAL S.R.: A protein moiety inhibitory to Hela cell growth in sera of burned children. *Fed. Proc.* **19**: 73, 1960.

MARKOE A.M., ASBELL S.O., WEIDANZ W.P., SALUK P., ANTONIADES K., BRADYL. W.: Effect of acute and fractionated partial body X-irradiation on circulating immunoglobulin levels in mice. *Int. J. Radiat. Oncol. Biol. Phys.* **1**: 465–470, 1976.

ŠIMŠA J., MRÁZ J.: Changes in the contents of some rat serum proteins under pathologic conditions. *Radiobiol. Radiother.* **4**: 509–519, 1974.

WAXMAN K., REBELLO T., PINDERSKI L., O'NEAL K., KHAN N., TOURANGEAU S., HIMES E., CORDILL K.: Protein loss across burn wounds. *J. Trauma* **27**: 136–140, 1987.

Reprint Requests

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