THE INFLUENCE OF METHIONINE AND LIPOCAIC ON THE LIPID METABOLISM OF THE LIVER OF RATS IRRADIATED WITH X-RAYS

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Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 59-62, January, 1960

Original article submitted May 25, 1959

In the animal affected by radiation sickness, disturbance of the lipid metabolism takes place, and one manifestation of this is the development of fatty infiltration of the liver [8, 10, 12]. This is partly due to deprivation of the liver tissue of glycogen. Fatty infiltration of the liver may also be due to toxic products formed during irradiation.

There are contradictory reports in the literature concerning the content of phospholipids in the liver in radiation sickness: In some investigations [4, 9, 15] no changes were found, whereas in others [11] a slight decrease was particularly obvious before death of the animal.

Particular attention must be paid to the role of the lipotropic substances methionine and choline, which are necessary for the synthesis of phospholipids and prevention of fatty infiltration of the liver, especially because the content of methionine and choline in the liver of irradiated rats is decreased [2, 16].

In the present research an attempt was made to normalize the lipid metabolism in the liver tissue of irradiated rats by administration of methionine and lipocaic, which have been used clinically with success in the treatment of liver disease. Interest in the action of lipocaic in this case is accounted for by the fact that, as shown by the work of S. M. Leites and his co-workers, lipocaic possesses an inhibitory action on the development of toxic fatty infiltration of the liver [5, 6]. The indices selected for study were content of total lipids and phospholipids in the liver of rats.

METHOD

Experiments were carried out on male white rats weighing from 150 to 230 g. The animals were given a single exposure to total irradiation with x-rays (dose 650 r, current 15 ma, voltage 180 kv, dose rates 9.16 and 12.13 r/min, filters 0.5 mm Cu and 1 mm Al).

To ensure better utilization of the methionine and increase in its lipotropic action, the methionine was administered in combination with aminopeptide (a solution of the products of enzymic hydrolysis of the proteins of ox blood, consisting of aminoacids and peptides of low molecular weight; nitrogen content – 300 mg%). The compounds were injected subcutaneously, immediately after irradiation and then every 48 hours, in doses of 90 mg methionine and 25 ml aminopeptide per kg body weight.

Lipocaic was given at the same time by mouth, as an aqueous solution, in a dose of 1.3 units/kg body weight.

The lipids were extracted by a mixture of chloroform and methanol [13]. These were estimated in condensed form by oxidation with a chromate mixture the excess of which was titrated iodometrically [1]. The lipoid phosphorus was treated by mineralization and then estimated by the Fiske-Subba Row method, and the phospholipid content was then calculated (as lecithin) by multiplying this figure by 25.

RESULTS

The lipid content of the liver of healthy rats varied in 11 experiments from 5.3 to 7.73%, the mean value being 6.44% ($\pm \sigma = 0.8$; $\pm m = 0.24$).

In irradiated animals (Table 1), accumulation of lipids in the liver in the course of development of radiation sickness could be detected after 3-6 days, and it was very pronounced on the 9th day (mean 9.2%) and statistically significant. On the 12th day of the disease the total lipid content in individual animals reached 10-11%. The liver of these rats was anemic, yellowish in color and very friable. Meanwhile, individual animals were seen in which the content of liver lipids did not exceed normal even on the 9th-12th day of the disease. The phospholipid content in the liver of healthy rats varied in 11 experiments from 3.07 to 4.75%, the

TABLE 1 Total Lipid Content (Per Cent) in the Liver or Irradiated Rats under Different Experimental Conditions

Experimental conditions	Elements of the variational series	Days after irradiation				
		1	3—4	6—7	8—9	11—12
No preparations given	N	10	10	10	9	11
	V	5.39—8.0	6.38—9.12	4.66—9.45	7.3—11.0	6.0—11.6
	M	6.5	7.1	7.5	9.2	8.87
	±σ	0.93	0.86	1.53	1.3	1.57
	±m	0.29	0.27	0.48	0.43	0.47
Methionine with aminopeptide	N	10	10	8	10	11
	V	5.6—9.5	7.1—8.6	5.1—8.7	5.3—9.3	6.2—11.6
	M	6.9	7.7	7.1	7.7	8.1
	±σ	1.2	0.43	1.05	1.4	1.65
	±m	0.38	0.13	0.36	0.44	0.5
Lipocaic	N	10	10	10	11	8
	V	5.3—8.1	3.88—7.3	4.36—7.35	4.22—9.0	4.8—10.0
	M	6.7	5.9	6.1	6.85	6.94
	±σ	0.85	1.2	1.0	1.88	1.5
	±m	0.26	0.38	0.32	0.56	0.5

mean value being 3.55% (± σ = 0.43, ± m = 0.13). In the initial period of development of radiation sickness (on the first to third day) it fell slightly (Table 2). At subsequent periods the fall in the phospholipid content became marked: On the 6th day the phospholipid content averaged 2.31%, and on the 9th day their content reached its lowest mean value (2.13%). In some animals, however, even those which died, no such fall was observed.

The administration of methionine with aminopeptide slightly reduced the deposition of total lipids in the liver on the 8th-12th day after irradiation, and by comparison with controls, the phospholipid content was increased in the irradiated animals.

The therapeutic effect of administration of methionine which is a methyl group donor in the formation of choline, very probably is to improve the conditions for the formation of new choline-containing phospholipids, and primarily of lecithin.. There are findings which show that good results are also obtained from giving proteins rich in methionine, and especially casein [17]. It was impossible, however, to completely normalize the lipid metabolism by administration of methionine and aminopeptide. This was evidently because of the lowering of the activity of the enzyme system responsible for the processes of transmethylation [3].

A better action was obtained from administration of lipocaic. In this case, on the 9th-12th day after irradiation, the mean figures of the lipid content in the liver of the rats (see Table 1) were considerably below those in the series of experiments without treatment. Lipocaic also had a favorable action, to a lesser degree than methionine with aminopeptide, on the normalization of the content of phospholipids in the liver (see Table 2). As has recently been shown [7], the mechanism of the lipotropic action of lipocaic consists of activation of the processes of oxidation of fatty acids, the conversion of lipids into glycogen and the increase in their removal from the liver. Provided that the intake of methionine or choline into the body is adequate, lipocaic also activates the phospholipid metabolism. This may, possibly, explain why lipocaic, when used in this case without other lipotropic agents, was more successful in preventing the development of fatty infiltration, whereas methionine was more effective in relation to phospholipid metabolism.

In a separate series of experiments on 75 animals, observations were made for a period of one month on the rate of survival of the irradiated rats.

Administration of methionine with aminopeptide had a beneficial effect on the rate of survival: This reached 40% by comparison with 8% in control experiments. It is possible that aminopeptide, being a protein preparation, played the role of a nutrient substance, sustaining the animals through the crisis of the radiation sickness, when their digestive powers were rather diminished.

No increase was observed in the rate of survival of the irradiated animals treated with lipocaic (12% survived). This, however, is not an argument in favor of its omission from combined treatment. Fatty infiltration of the liver seriously interferes with the normal function of this organ, and attempts to overcome it are completely justified.

TABLE 2 Phospholipid Content (Per Cent) in the Liver of Irradiated Rats

Experimental conditions	Elements of the variational series	Days after irradiation				
		1	3—4	6—7	8—9	11—12
No preparations given	N	10	10	10	10	10
	V	2,82—3,79	2,45—3,5	1,69—2,9	1,27—3,07	1,7—3,5
	M	3,21	2,87	2,31	2,13	2,54
	±σ	0,4	0,85	0,14	0,57	0,45
	±m	0,12	0,27	0,04	0,18	0,14
Methionine with aminopeptide	N	10	10	8	11	11
	V	1,9—3,6	2,57—3,5	2,42—3,55	2,3—3,6	1,55—3,69
	M	2,71	2,93	2,74	2,92	2,71
	±σ	0,5	1,65	0,3	0,43	0,57
	±m	0,16	0,52	0,1	0,13	0,17
Lipocaic	N	10	10	9	11	8
	V	2,92—3,55	2,7—3,85	2,25—3,08	1,92—3,2	2,15—3,37
	M	3,15	3,16	2,61	2,68	2,87
	±σ	0,23	0,89	0,09	0,4	0,4
	±m	0,07	0,28	0,03	0,12	0,14

SUMMARY

Following X-ray irradiation (650 r), the content of fat in the liver gradually rises, while that of phospholipids decreases. Administration of lipocaic greatly reduces the developmnet of fatty infiltration in the irradiated rats. Employment of methionine with aminopeptide has asimiliar, although less prounced effect.

The fall in the phospholipid content of the liver of irradiated rats, to which these substances were given, was less than that in the control irradiated animals.

Administration of methionine with aminopeptide also increased the percentage of surivival of the irradiated

LITERATURE CITED

[1] S. D. Balakhovskii and I. S. Balakhovskii, Methods of Chemical Analysis of the Blood [in Russian] (Moscow, 1953).

[2] M. P. Eleazarova, Vestnik rentgenol. i radiol., 2, 22 (1956).

[3] M. P. Eleazarova and V. S. Stepanova, Proceedings of Sections of the All-Union Conference on Medical Radiology, Section of Experimental Radiology [in Russian] (Moscow, 1956) p. 48.

[4] I. I. Ivanov, V. S. Balabukha, E. F. Romantsev, et al., Metabolisin in Radiation Sickness [in Russian] (Moscow, 1956).

[5] S. M. Leites and T. S. Yakusheva, Arkh. patol. 4, 44 (1949).

[6] S. M. Leites, Uspekhi sovremennoi biol. 18, 2, 214 (1944).

[7] S. M. Leites, Problemy endokrinol. i gormonoterap. 1, 71 (1955).

[8] M. Ariell, Radiology, 1951, v. 57, p. 561.

[9] J. G. Coniglio, W. J. Darby, J. A. Efner and others, Am. J. Physiol., 1956, v. 184, p. 113.

[10] W. E. Comatzer, J. P. Davidson and O. D. Engelstad and others, Radiation Res., 1954, v. 1, p. 546.

[11] J. P. Davidson, C. Simonson, and W.E. Cornatzer, Fed. Proc., 1954, v. 13, p. 197.

[12] F. Ellinger, Radiology, 1945, v. 44, p. 241.

[13] J. Folch and others, J. Biol. Chem. 1951, v. 191, p. 833.

[14] E. Geiger, Science, 1950, v. 111, p. 594, Fed. Proc., 1951, v. 10, p. 670.

[15] E. J. King, Biochem. J., 1932, v. 26, p. 292.

[16] U. S. Kumta, S. U. Gurnani, and M. B. Sahasrabudhe, Current Sc., 1955, v. 24, p. 362.

[17] C. C. Lucas and J. H. Ridout, Canad. J. Biochem. a. Physiol., 1955, v. 33, p. 25.