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THE MECHANISM OF "BIOCHEMICAL SHOCK"—I

THE CORRELATION BETWEEN THE ACCUMULATION OF SOME THIOL RADIOPROTECTORS IN RAT TISSUES AND BIOCHEMICAL CHANGES INDUCED BY THEM

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Abstract—The various chemical forms of mercaptoethylamine (MEA) and l-cysteine and their content in rat thymus, spleen and liver have been determined during the first 3 hr after intraperitoneal (i.p.) drug administration at radioprotective doses. The determinations were made by quantitative spectrophotometric methods. The protectors studied accumulate in the organs mainly as free thiol. The amount of disulfides found was negligible and the mixed disulfides of MEA and 1-cysteine with cellular proteins were not determined because the methods used were not sufficiently sensitive. The maximum concentration of the protectors in tissues is reached 15–30 min after their i.p. administration. Comparison with previously published results on the effect of sulfhydryl compounds on nucleic acid metabolism indicates that the maximum radioprotective and biochemical effectiveness of the thiols coincides with their maximum concentration in tissues after i.p. administration. The possible biochemical mechanisms of radioprotective action of aminothiols are discussed.

ONE OF the most urgent problems of modern radiobiology is the study of radioprotective action of sulfur-containing compounds, especially those belonging to the aminothiols. Several hypotheses explaining the possible biochemical action of aminothiols have been proposed.[1–3] From our point of view one of the most fruitful is that of "biochemical shock" advanced by several authors,[2, 4–6] but the biochemical background of this hypothesis and its role in the realization of radioprotective action of sulfhydryl compounds is still uncertain. This may be due to lack of data on the aminothiol effect on the course of biochemical processes in mammals.

It is known that to exert maximum radioprotection aminothiols must be present in tissues at the moment of irradiation. Injected aminothiols are quickly distributed among various organs and tissues. The maximal level of radioactivity in various tissues is reached 2·5–30 min after the administration of ^{14}C- or ^{35}S-labelled aminothiols and disulfides,[7–14] and 15–30 min after cysteine-^{35}S injection.[15] The period of maximum accumulation of these drugs in tissues coincides in time with their maximal radioprotective action and with biochemical changes induced by them. As was pointed out by Smoliar[16] the first peak of radioprotective action of cystamine is reached 10 min after i.p. administration of the drug. At the same time La Salle and Billen[17] showed that a pronounced decrease in thymidine-^{14}C incorporation into DNA of mice bone-marrow cells develops 15 min after β-aminoethylisothiouronium (AET) administration. Four hr after injection of this drug its inhibitory action had disappeared but

the nature of the biochemically-active form of injected compounds is unknown, when distribution of exogenous aminothiols was studied with labelled compounds.

The injection of MEA, AET or cysteine into rats sharply inhibits uracil-2^{14}C incorporation into RNA of rat thymus nuclei.[20] MEA or AET administration 10–15 min before decapitation of animals leads to a pronounced decrease in the rate of thymidine-2^{14}C phosphorylation in rat thymus and spleen extracts. Three hr after i.p. injection of drugs this process is practically normalized.[21]

It was of interest therefore to know whether there is a correlation between the biochemical effects observed and the character of MEA and cystamine distribution in rat thymus and spleen. The form in which the administered aminothiols are accumulated in animal tissues and the time-course of this accumulation of thiol and disulfide forms of radioprotectors was studied.

MATERIALS AND METHODS

Animals

Male albino rats (120–200 g) were used in all experiments and maintained on rat cake and water *ad lib.*

Reagents

MEA-bitartrate (94·3%) and l-cysteine hydrochloride (Chemapol, Czechoslovakia) 91·7% pure were used. Sodium fluoropyruvate was synthesized as described earlier.[22] Dithiothreitol (DDT, Cleland's reagent) was obtained according to Evans *et al.* in Cleland modification.[23,24] We wish to acknowledge a generous gift from Dr. W. W. Cleland, University of Wisconsin, of the sample of tetraacetate of dithiothreitol, and the detailed method of DDT preparation.

Experimental procedures

l-cysteine hydrochloride and MEA-bitartrate was administered i.p. at doses of 1000 and 150 mg/kg respectively (as a free base). The animals were decapitated at various time-intervals after the injection of drugs. Thymus, spleen and liver were quickly removed and rinsed twice by water or 0·25 M sucrose.

Thymus nuclei were isolated according to Klouwen and Betel.[25] Cysteine, cystine and mixed disulfides of these amino acids with cellular proteins (after the treatment with DDT) were determined spectrophotometrically with reagent 2 by Gaitonde method,[26] pH-value in samples was adjusted by adding of 1 M Tris-HCl, pH 8·0. MEA, cystamine and mixed disulfides of MEA with proteins (after the treatment with DDT) were measured by method of Herrington *et al.*[27, 28] The content of cysteine, MEA and corresponding disulfides was determined in aliquots of tissue homogenates and expressed as micromoles per gram of fresh tissue. The extracts from the appropriate tissues of control rats (not injected with radioprotector) were used as the blank.

RESULTS

The time-course of changes in MEA, l-cysteine and corresponding disulfides content in thymus, spleen and liver is shown on Figs. 1 and 2. The maximal accumulation of free MEA in spleen and liver is reached 15 min after the administration of drug into animals and amounted to 2·73 $\pm$ 0·2 and 1·77 $\pm$ 0·05 μ-moles/g of tissue respectively. The maximal MEA content in thymus (Fig. 1a) was noted 30 min after

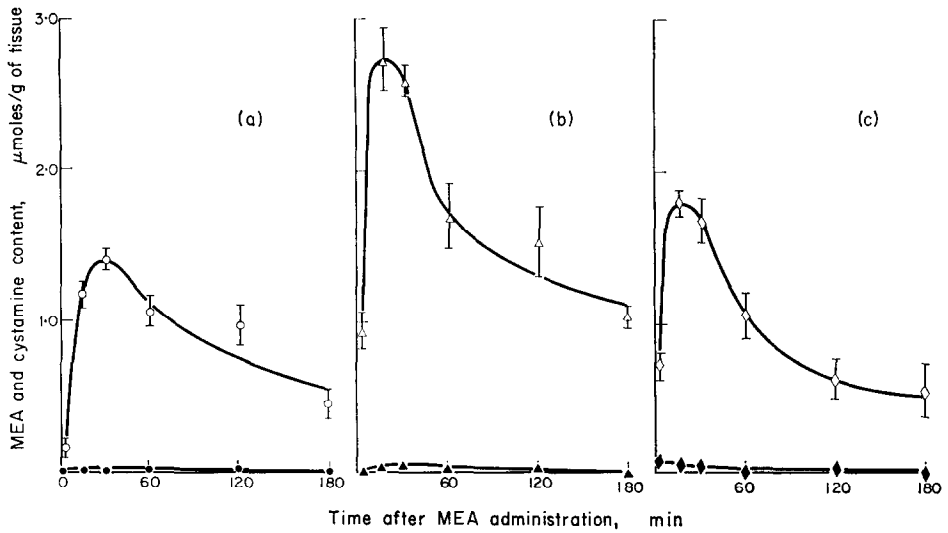

FIG. 1. MEA and cystamine content in various rat tissues at different time-intervals after i.p. administration of MEA. a-thymus; b-spleen; c-liver. Open symbols, MEA content; full symbols, cystamine content.

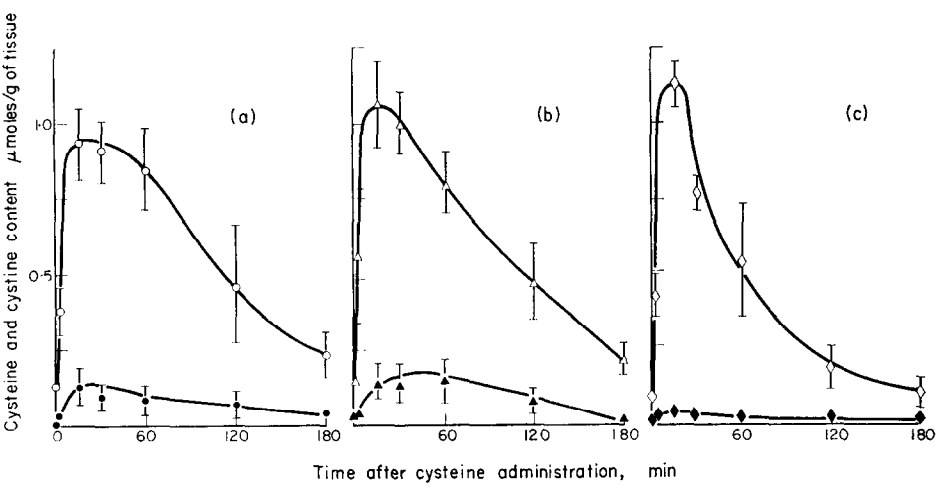

FIG. 2. Cysteine and cystine content in various rat tissues at different time-intervals after i.p. administration of cysteine. a-thymus; b-spleen; c-liver. Open symbols, cysteine content; full symbols, cystine content.

the drug administration and amounted to 1·39 ± 0·02 μ-moles/g. The content of SH-form of radioprotector is sharply decreased with time and by the third hour the level is reduced to one third. It is interesting to note that cystamine content in all tissues studied during the first 3 hr after i.p. MEA administration is negligible.

The accumulation pattern is similar for l-cysteine (Fig. 2). The maximum cysteine content is reached 15 min after i.p. administration and then gradually decreases. At

the end of the third hour the cysteine content in liver tissue is normal, and in thymus and spleen is 1·5–2 times greater than the endogenous level. Cystine content in the liver (Fig. 2c) after cysteine administration is less than in two other tissues (Fig. 2a and 2b) at all the time-intervals studied. The content of cysteine and cystine in control animals is 0·14 ± 0·02 and 0·023 ± 0·012 μ-moles/g in the spleen; 0·13 ± 0·01 and 0·012 ± 0·01 μ-moles/g in the thymus; 0·11 ± 0·01 and 0·011 ± 0·01 μ-moles/g of tissues in the liver.

The protectors distribution among the nucleus and cytoplasm of thymocytes at various time-intervals after i.p. MEA administration are shown in Fig. 3. It can be

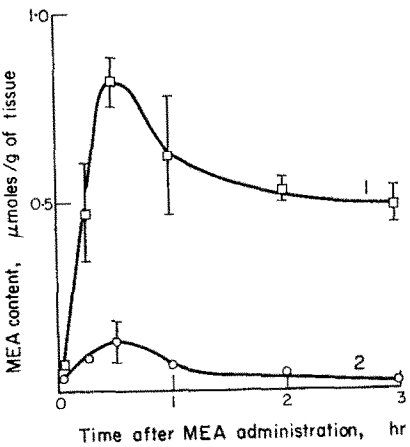

FIG. 3. MEA distribution at different times between nuclei and cytoplasm after i.p. MEA administration. On the abscissa—time after MEA administration, hours. On the ordinate—MEA content, micromoles/g of tissue. 1—cytoplasm; 2—nuclei.

seen that penetrating into the cell the aminothiol is localized mainly in the cytoplasm. The MEA content in nuclei is negligible but the changes in its content with time coincide well with those in the cytoplasm and in whole homogenate. In the present experiment the attempt to determine cystamine concentration in nuclei and cytoplasm gave no results. It must be stressed that we could not obtain reproducible results in the course of studies of radioprotectors binding to cellular proteins by means of mixed disulfide bonds in whole tissue homogenates as well as in cytoplasm and nuclei of thymocytes. The negative results obtained do not mean that this binding does not take place (e.g. see 29), but depends on the method used and will be discussed in the Discussion section.

DISCUSSION

On the base of MEA and l-cysteine content the tissues studied during the first 30 min after radioprotectors administration may be arranged in the following order: spleen > liver > thymus. Analogous character of radioactivity distribution among these organs is observed 15 min after cysteine-^{35}S and cystamine administration.[14, 30, 31] A further trace of radioprotector distribution among the organs and tissues of rats takes place after i.v. administration of MEA hydrochloride.[28]

In our experiments the maximal content of MEA was determined 15–30 min after aminothiol administration. At the same time according to Betz *et al.*[32] the amount of protector in thymus and spleen is maximal 2 min after cystamine administration and determined mainly in protein-bound form. Thirty min after the administration the amounts of free and bound radioprotector in the same organs are nearly of the same order.

The fact that we could not detect the mixed disulfides of radioprotectors with proteins testifies, probably, to the relatively low susceptibility of spectrophotometric methods employed.

So the maximal accumulation of protectors studied in rat tissues is noted 15–30 min after i.p. administration, and the injected drugs are determined mainly as thiols. At the same time it is known that *in vivo* these drugs exert the notable inhibitory effect on nucleic acid metabolism.[33, 36] Therefore it was of interest to compare the data obtained in the distribution of the protector among the rat tissues with changes in some processes of nucleic acid metabolism in time after i.p. MEA or cysteine administration.

At the period of maximal protective effectiveness (10–15 min after MEA administration) the significant decrease in the rate of thymidine phosphorylation is noted in thymus and spleen. By the third hour when the amount of protector in tissues decreases approximately three times, the rate of thymidine phosphorylation is practically normalized (Table 1).

TABLE 1. EFFECT OF I.P. MEA-BITARTRATE ADMINISTRATION (300mg/kg) ON THYMIDINE-2^{14}C PHOSPHORYLATION IN RAT THYMUS AND SPLEEN EXTRACTS[21]

Experimental conditions	Rate of thymidine-2^{14}C phosphorylation, per cent to the control	
	Thymus	Spleen
Control	100 (6)	100 (6)
10–15 min	69±8 (6)	45±10 (10)
3 hr	100±20 (6)	121±11 (4)

The number of the experiments is indicated in parentheses.

The results on the effect of i.p. MEA administration on uracil-2^{14}C incorporation into RNA of isolated rat thymus nuclei are represented on Fig. 4. In the period of maximal aminothiol accumulation in thymus tissue the pronounced inhibition of nuclear RNA synthesis takes place. It should be stressed however that regardless of low MEA content in isolated nuclei (Fig. 3) the rate of label incorporation is sharply decreased already 4 min after radioprotector administration, kept at this low level during the 2-hr period, and then gradually normalized reaching the control 3·5–4 hr after administration of the drug

The same character of the dynamics of cysteine and cystine accumulation in thymus tissue and uracil-2^{14}C incorporation into nuclear RNA took place after i.p. cysteine administration. The data obtained are represented in Fig. 5. From Fig. 5 the strike

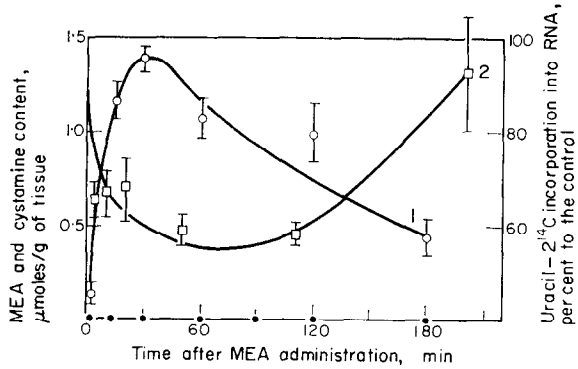

FIG. 4. Relationship between MEA content in the tissue and uracil-2^{14}C incorporation into RNA of isolated rat thymus nuclei.[20] On the abscissa—time after MEA administration, min. On left ordinate MEA and cystamine content, micromoles/g of tissue. On right—uracil-2^{14}C incorporation into RNA, per cent to the control. 1—MEA content; 2—uracil-2^{14}C incorporation.

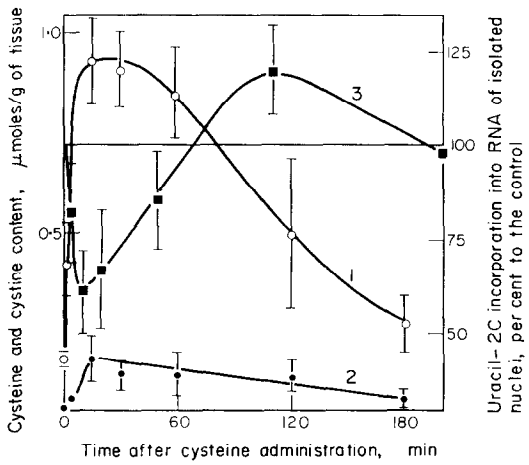

FIG. 5. Relationship of cysteine and cystine content in the tissue and uracil-2^{14}C incorporation into RNA of isolated rat thymus nuclei.[20] On the abscissa—time after cysteine administration, min. On left ordinate—cysteine and cystine content, micromomoles/g of tissue. On right—uracil-2^{14}C incorporation into RNA of isolated rat thymus nuclei. 1—cysteine content; 2—cystine content; 3—uracil-2^{14}C incorporation into RNA, per cent to the control.

correlation between cysteine and cystine content in rat thymus tissue and biochemical effect can be seen.

Therefore the experiments described point to the interesting correlation between biochemical effect, changes in radioprotectors content in the tissue and their radioprotective action. According to modern concepts the biochemical mechanism of radioprotective action of aminothiols and disulfides is complex and carried out along several directions simultaneously.[2-6, 37-39] The administration of these compounds leads to temporal inhibition of metabolic processes ("biochemical shock") conditioned

by their direct interaction with subcellular structures, components of DNP, mitotic proteins and sulfur-containing enzymes participating in the metabolism of nucleic acids, proteins and other metabolites essential for life.

The administration of aminothiols and disulfides induced the significant and reversible inhibition of radiosensitive processes coinciding in time with their maximal protective effectiveness and accumulation in tissues. The "biochemical shock" may interfere with the destructive processes by strengthening the rate of reparation.

As a result of biological detoxication the amount of exogenous sulfur-containing compounds in organs and tissues is diminshed, and this can be followed by the normalization of metabolic processes.

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