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MECHANISM OF "BIOCHEMICAL SHOCK"—III

COMPARATIVE STUDY OF BIOCHEMICAL EFFECTS INDUCED BY A RADIOPROTECTIVE AND A NON-RADIOPROTECTIVE COMPOUND OF ISOMER STRUCTURE

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Abstract—Intraperitoneal injection of DL-isocysteine into mice at doses of 300, 600 and 1000 mg/kg 10–15 min before γ-irradiation with 600 R had no effect on the survival of animals. Ineffectiveness of the drug was not connected with the peculiarities in its distribution in organs. A comparative study of isocysteine and cysteine effects at doses of 400 and 1000 mg/kg on thymidine kinase activity in rat thymus and spleen has demonstrated relatively weak inhibitory effect of the former (15 min after the administration). At the same time the effect of the radioprotective compound (cysteine leads to more pronounced inhibition in enzyme activity (12–15 and 30–50 per cent at a dose of 1000 mg/kg respectively). It was shown that the inhibitory action of cysteine on thymidine kinase was connected mainly with mixed disulfide formation between the radioprotector and the enzyme. The same patterns occurred for the partially purified thymidine–cytosine deoxyribosyl transferase from rat thymus. At the same time the ineffective isomer (isocysteine) did not form mixed disulfides to an appreciable extent. This data indicates that mixed disulfides play an important role in the production in mammals of a "biochemical shock" which is linked to a temporarily enhanced resistance to ionizing radiation.

RECENT observations have shown that some aminothiols and cysteine cause a temporal inhibition in the activity of some enzymes participating in the biosynthesis of DNA precursors in radiosensitive tissues.[1,2] The maximal inhibition of thymidine kinase and deoxyribosyl transferase activities was observed 10–15 min after intraperitoneal administration of the aminothiols and therefore coincided with their highest radioprotective effectiveness.

Previous studies have indicated that the inhibition observed was connected partly with mixed disulfide formation between the appropriate enzymes and radioprotector moiety.[3] Of all the types of bonds between β-mercaptoethylamine (MEA) and proteins in various organs and microstructures tested, only the formation and breakdown of mixed disulfides correlated with optimal radioprotective effectiveness of the drug (10–30 min after i.p. administration).[4,5] These data enable us to suggest that temporal inhibition of some biochemical processes in radiosensitive tissues, observed after the administration of aminothiol radioprotectors, reflects their characteristic feature, and may contribute partly to their radioprotective effect. The final answer probably depends on "whether or not this inhibition will take place only in the case of radioprotective aminothiols."

At present a rather wide number of compounds, structurally similar to radioprotectors, but differing in radiobiological properties are known. The pair of isomers–cysteine and isocysteine served as an example:

Cysteine (α-amino-β-mercaptopropionic acid)

Isocysteine (α-mercapto-β-aminopropionic acid)

The change in the position of the SH and NH_2 groups in cysteine leads to the formation of isocysteine which proved to exert no radioprotective effect.

Therefore these isomers were a convenient model for the comparative study of changes in the activity of one of the enzymes participating in DNA synthesis (thymidine kinase) in radiosensitive organs of rats after their intraperitoneal administration. Comparison of the character and mechanism of the changes observed would be valuable in determining the specificity of biochemical effects of radioprotective aminothiols which in turn would promote further studies on the mechanism of their action.

MATERIALS AND METHODS

Animals. Male albino rats (100–150 g) and mice (20 g) were used throughout.

Reagents. The following chemicals were used: l-cysteine HCl (Reanal, Hungary), m.p. 175–176°, 93% pure; DL-isocysteine HCl, m.p. 122–124° 90% pure was synthesized according to Schöberl and Braun,[6] and dithiothreitol (DTT) was prepared according to Evans *et al.*[7,8] by the Cleland modification. Thymidine was purchased from CalBiochem. We wish to acknowledge a generous gift from Dr. A. V. Titov of *N*-(4-hydroxy-1-naphthyl)-maleimide (NMI) and diazotized 5-nitro-*o*-anisidine (Diazol pink O).

For the determination of thymidine kinase (ATP:thymidine-5-phosphotransferase, EC 2.7.1.21) activity, [2-^{14}C]thymidine (sp. act. 7·7 mCi/mmole) was used. Activity of rat thymus thymidine:cytosine deoxyribosyl transferase (EC 2.4.2.6) was assayed with the use of [2-^{14}C]cytosine (sp. act. 2·3 mCi/mmole).

EXPERIMENTAL PROCEDURES

Studies on radioprotective effect of isocysteine. 79 mice were used in the experiments. The animals were totally irradiated with 600 R of γ-rays ^{60}Co. Dosimetry was performed by means of an ionization method ("Phillips" Universal Dosimeter) or with ferrous sulfate dosimeter. Isocysteine was administered i.p. at doses of 300, 600 and 1000 mg/kg (as a free base) 10–15 min before the irradiation. After irradiation the animals were observed for a period of 30 days.

Quantitative spectrophotometric determination of isocysteine in tissues. For the determination of isocysteine in tissue extracts the method proposed by Titov[9] was used with slight modifications. This method was specially developed by the author for the determination of MEA in the blood and tissues. SH-groups of the thiol in tissue extracts are blocked with NMI and after paper chromatography of the extracts the

complexes thiol–NMI are stained by azo dye and the absorbancy of the appropriate spot eluates was determined.

Rats were given an i.p. injection of isocysteine at doses of 400 and 1000 mg/kg, and the animals were decapitated at various time-intervals. Thymus, spleen and liver were removed and thoroughly extracted in 2·5% HPO_3 in 50% ethanol, proteins were discarded and 0·2–0·3 ml of supernatant was treated with $NaBH_4$ for the reduction of disulfides. One vol. of freshly prepared 1% solution of NMI in ethanol was added to 3 vol. of the supernatant. A few seconds later the dark cherry-coloured solution turned straw-coloured. 0·025–0·05 ml of mixture were spotted on Whatman No. 1 paper strips of *n*-propanol–5% acetic acid (4:1) at 20° for 18 hr and chromatographed in dry paper sprayed with freshly prepared 5-nitro-*o*-anisidine solution (20 mg of the dye in 25 ml of 0·05 M $NaHCO_3$). NMI and its complexes with thiols were developed as spots of intensive pink colour. After the extraction of the spots by ethanol–5% acetic acid (5:1) the absorbancy of eluates at 524 nm was measured. Absorption spectrum of the product of interaction of diazotized 5-nitro-*o*-anisidine with isocysteine–NMI complex is shown in Fig. 1.

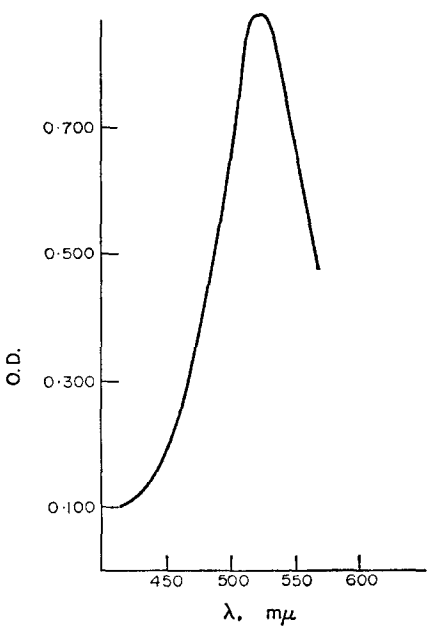

FIG. 1. Absorption spectrum of the product of interaction of isocysteine–NMI complex with diazotized 5-nitro-*o*-anisidine.

The isocysteine content of tissue extracts was calculated by standard curves obtained for the solutions of different isocysteine concentrations prepared on the extracts of each organ separately. Values were expressed as micromoles of isocysteine per gram of fresh tissue.

Evaluation of thymidine kinase activity in extracts of spleen and thymus. Rats were injected with cysteine or isocysteine at doses of 400 and 1000 mg/kg i.p. and 10–15 min later the animals were decapitated. Thymus and spleen were quickly removed and homogenized in 0·1 M Tris–HCl buffer pH 7·4. The supernatant after centrifugation at 105,000 *g* for 1 hr was used as the enzyme source.

The reaction mixture (in a total volume of 0·2 ml) contained: 0·14 ml (0·9–2·0 mg of protein) of thymus or spleen extracts; 14 μmole of Tris–HCl pH 7·4; 30 mμmole of [2-^{14}C]thymidine (0·23 μCi); 5·0 μmole of ATP, and 2·5 μmole of $MgCl_2$. In control tubes ATP was omitted. The mixtures were incubated for 60 min at 37°, and thymidine kinase was assayed as previously described.[10]

Assay of thymidine:cytosine deoxyribosyl transferase. The partially purified enzyme from rat thymus was prepared as described previously.[11] "Fraction V" (after the elution from DEAE–cellulose) was used in the experiments as the enzyme source. The assay of thymidine:cytosine deoxyribosyl transferase was carried out with [2-^{14}C]cytosine according to Filippovich *et al.*[3] The activity of enzyme after cysteine and isocysteine addition and DTT treatment was expressed as per cent of the appropriate control (no thiol added).

Effect of cysteine and isocysteine on thymidine kinase in thymus extracts in vitro. The incubation mixtures contained in a total vol. of 0·45 ml: 0·4 ml (3–6 mg of protein) of thymus extracts, 40 μmole of Tris–HCl pH 7·4; 30 mμmole of thymidine* and cysteine or isocysteine in the quantities given in the legends of Fig. 5. The mixtures were incubated at 37° for 10 min, chilled to zero and dialyzed at 4° against 0·1 M Tris–HCl buffer pH 7·4 for 30 min. After dialysis, DTT solution was added (to a final concentration of 7×10^{-3} M), and tubes were incubated at room temperature for 15 min. In the control experiments water instead of DTT was added. DTT alone had only slight stimulatory action on the enzyme activity. During the incubation of protein with DTT cleavage of mixed disulfides occurred; thiols and DTT are removed in the course of dialysis. Aliquots (0·24 ml) served as the enzyme source in the incubation mixture for the assay of thymidine kinase.

Effect of cysteine and isocysteine on partially purified preparations of thymidine: cytosine deoxyribosyl transferase in vitro. 0·2 ml of "fraction V" (20 μg of protein), 12 μmole of Tris–HCl buffer pH 7·4 and cysteine or isocysteine added at various concentrations (see Fig. 5) were incubated for 15 min at 37°. Following dialysis against 0·1 M Tris–HCl buffer pH 7·4 the mixtures were treated with DTT as described above for thymidine kinase. Aliquots were used as the enzyme source for the assay of transferase.

RESULTS

From our results on toxicity studies LD_{50} for DL-isocysteine equals 1650 mg/kg i.p. In the experiments with irradiation the drug was administered at doses of 300, 600 and 1000 mg/kg (as a free base).

It can be seen from Table 1, that i.p. injection of isocysteine into mice 10–15 min before irradiation did not result in either protection or sensitization of animals. On the basis of χ^2 value the difference in survival between the control and experimental groups of

* Preincubation without the substrate leads to inactivation of the enzyme. Therefore thymidine was included into the incubation mixtures.

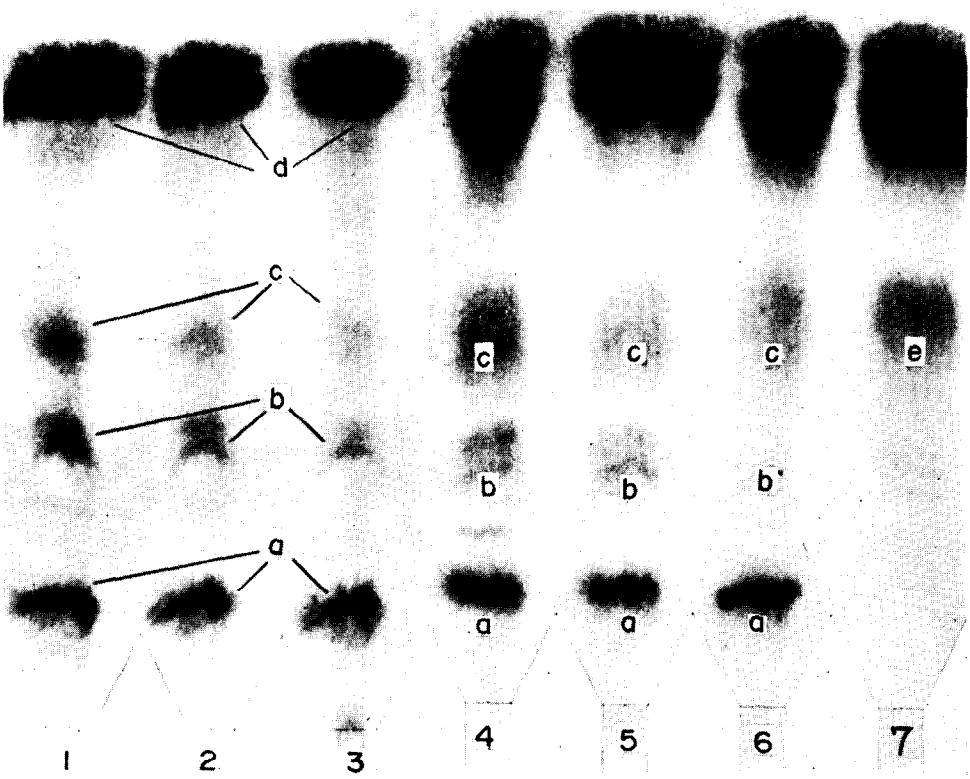

FIG. 2. (left) Chromatograms of the extracts of organs of intact rats after the treatment with $NaBH_4$ and NMI, (1) thymus, (2) spleen, (3) liver; (a) reduced glutathione–NMI; (b) complexes of NMI with non-identified endogenous thiols; (c) cysteine–NMI (d) NMI.

FIG. 3. (right) Chromatograms of the extracts of organs of rats 15 min after i.p. isocysteine administration at a dose of 1000 mg/kg (4–6) and the position of isocysteine–NMI complex (7). (4) thymus, (5) spleen, (6) liver; (e) isocysteine–NMI; (b,c,d) as in Fig. 2.

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TABLE 1. EFFECT OF ISOCYSTEINE ON THE SURVIVAL OF MICE AFTER γ-IRRADIATION. MALE MICE, WHOLE-BODY IRRADIATION WITH 600 RDL-ISOCYSTEINE HCl INJECTED INTRAPERITONEALLY 10–15 min BEFORE IRRADIATION

Group of animals	No. of animals	Survival (%)	Mean survival time (days) ± S.E.	χ^2
1. Control	20	20·0	17·8 ± 1·15	
2. Isocysteine, 300 mg/kg	20	20·0	21·3 ± 0·24	0
3. Isocysteine, 600 mg/kg	18	33·3	19·6 ± 1·12	1·0
4. Isocysteine, 1000 mg/kg	21	30·0	17·8 ± 2·10	1·0

$$\chi^2 = \frac{(k_1 n_2 - l_1 n_2)^2 (n_1 + n_2 - 1)}{(k_1 + l_1)(k_2 + l_2) n_1 n_2},$$

Where n_1-number of animals in the experimental group, k_1-number of animals which survived irradiation, k_2-number of dead animals, n_2-number of animals in the control group, l_1 and l_2-values in the control group corresponding to k_1 and k_2.

animals is statistically insignificant. Therefore isocysteine may be regarded as devoid of protective power.

The ineffectiveness of isocysteine may be connected with peculiarities in drug distribution in various organs of animals. We studied the distribution of this compound in thymus, spleen and liver of rats at various time intervals after i.p. administration of isocysteine at doses of 400 and 1000 mg/kg. The method of quantitative spectrophotometric assay for isocysteine permits the drug to be extracted from the majority of endogenous thiols.

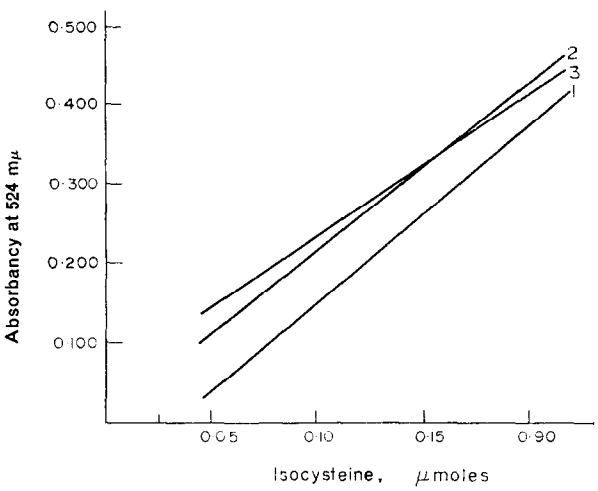

FIG. 4. Standard curves for isocysteine were determined in the extracts of tissues, (1) liver, (2) spleen, (3) thymus. Samples contained 0·2 ml of supernatant obtained after centrifugation of homogenate of corresponding organ in 2·5% HPO_3–50% ethanol (1:2, w/v), and varying amounts of isocysteine in 0·1 ml. The conditions of the subsequent treatment of samples with NMI, paper chromatography and elution were described in the text under Quantitative spectrophotometric determination of isocysteine in tissues. Aliquots of the samples spotted on chromatograms contained isocysteine at the amounts given on the abscissa. Each line was obtained from three to five separate determinations at given isocysteine concentrations.

Chromatography of tissue extracts from animals treated with $NaBH_4$ and NMI resolved compounds corresponding to complexes of NMI with glutathione, $R_f = 0{\cdot}19$ (a); non-identified thiols $R_f = 0{\cdot}44$ (b); and cysteine $R_f = 0{\cdot}54$ (c) (Fig. 2 (1–3)). The upper spot (d) was identified as unchanged NMI. Figure 3 (4–6) shows chromatogram of extracts of the same organs from rats which received isocysteine at a dose of 1000 mg/kg i.p. 10–15 min before decapitation. Unfortunately the R_f-value of isocysteine–NMI complex (7e) coincides with that for cysteine. This strongly reduces the sensitivity of the method. Therefore the standard curves for isocysteine were obtained with the use of standard isocysteine solution prepared on extracts of appropriate organs (Fig. 4). These lines were plotted on the basis of an average value of OD_{524} (from 3 to 5 determinations) for each isocysteine concentration.

Recovery of isocysteine added to the tissue extracts (0·15 μmole) shows that the method is reproducible although it is not very sensitive (Table 2).

TABLE 2. RECOVERY OF ISOCYSTEINE ADDED TO TISSUE EXTRACTS

Extract	Amount of isocysteine added (μmoles)	Amount of isocysteine determined	
		μmole	Recovery %
Thymus	0·150	0·172	114
		0·150	100
		0·165	110
Liver	0·150	0·151	102
		0·161	107
Spleen	0·150	0·153	102
		0·158	105
		0·160	106
		0·162	108

Intraperitoneal administration of DL-isocysteine into rats at doses of 400 and 1000 mg/kg, and the determination of its content in several tissues gave the following results (Table 3). At 15 and 30 min after i.p. administration at a dose of 400 mg/kg, isocysteine was found only in the liver. This may be explained probably by the relatively low sensitivity of the method. Indeed, 15 min after the administration of isocysteine at a higher dose (1000 mg/kg), the drug was found in all organs studied (Table 3).

Data on comparative effect of cysteine and isocysteine on the activity of thymidine kinase in thymus and spleen of rats are given in Table 4. This shows that the inhibition of thymidine kinase after isocysteine administration (1000 mg/kg) is proportional to drug concentration in the tissues (Table 3). The inhibitory action of cysteine injected at a dose of 1000 mg/kg was more pronounced compared to isocysteine, and the inhibitory effect of the latter was not dose-dependent.

TABLE 3. DL-ISOCYSTEINE CONTENT IN THE ORGANS OF RATS AT VARIOUS TIME INTERVALS AFTER i.p. ADMINISTRATION

Time after isocysteine administration (min)	Dose (mg/kg)	Isocysteine content in organs studied (μmole/g of tissue)		
		Thymus	Spleen	Liver
15	400	N.D.	N.D.	5·0 ± 0·2(3)
30	400	N.D.	N.D.	3·0 ± 0·1(2)
120	400	N.D.	N.D.	N.D.
15	1000	2·6 ± 0·5(2)	3·1 ± 0·5(2)	5·6 ± 0·3(2)

Values are expressed as mean ± S. E M.
The number of the experiments are indicated in parentheses.
N.D., not detectable.

So, the difference in radiobiological effectiveness of cysteine and its isomer coincides well with absolute values of their inhibitory effect on thymidine kinase activity. The non-radioprotective compound, isocysteine, administered at a dose of 1000 mg/kg leads to a less pronounced inhibition in enzymatic activity compared to the radioprotector, cysteine, administered at the same dose.

TABLE 4. EFFECT OF i.p. CYSTEINE AND ISOCYSTEINE ADMINISTRATIONS ON THYMIDINE KINASE ACTIVITY IN THYMUS AND SPLEEN OF THE RATS (15 min AFTER THE DRUG INJECTION)

Drugs	Dose (mg/kg)	Inhibition in thymidine kinase activity (control %)	
		Thymus	Spleen
Cysteine	400	5·0 ± 0·5 (4)	10·0 ± 2·0 (4)
	1000	29·0 ± 7·0 (4)	49·0 ± 9·0 (4)
Isocysteine	400	8·0 ± 1·0 (3)	15·0 ± 3·0 (4)
	1000	12·0 ± 2·0 (3)	15·0 ± 2·0 (4)

Values are expressed as mean ± S. E. M.
The number of the experiments are indicated in parentheses.

A study of the mechanism of inhibition of thymidine kinase activity in the thymus gave results which are summarized in Fig 5.

Inhibition of thymidine kinase activity which was more pronounced after addition of cysteine (Fig. 5a) than after addition of equimolecular amounts of isocysteine (Fig. 5b). DTT treatment of the enzyme inhibited by cysteine resulted into two-fold recovery in kinase activity (Fig. 5a). At the same time DTT treatment of thymidine kinase inhibited by isocysteine did not change the degree of inhibition ratio (Fig. 5b). Similar results were obtained after DTT treatment of partially-purified preparations of thymidine:cytosine deoxyribosyl transferase inhibited by cysteine and its isomer (Fig. 5c, d).

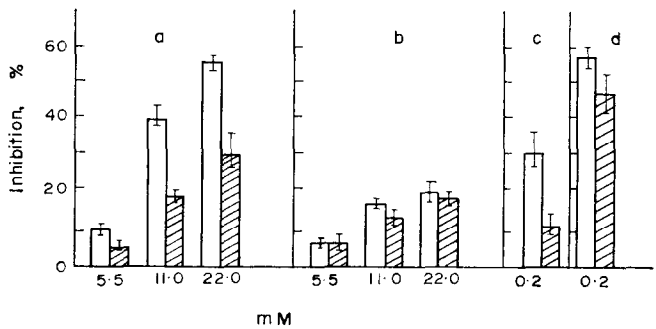

FIG. 5. Effect of different concentrations of cysteine and isocysteine on thymidine kinase and thymidine:cytosine deoxyribosyl transferase before (open columns) and after (shaded columns) DTT treatment. (a) Thymidine kinase + cysteine, (b) Thymidine kinase + isocysteine, (c) Thymidine: cytosine deoxyribosyl transferase + cysteine, (d) Thymidine:cytosine deoxyribosyl transferase + isocysteine.

Finally one may assume that if the inhibitory effect of cysteine on the activity of the enzymes studied is due partly to thiol interaction with enzyme resulting in mixed disulfide formation, such interaction in the case of isocysteine probably does not take place to an appreciable extent.

DISCUSSION

Although the isomer of cysteine, isocysteine, was prepared by Schöberl and Braun in 1939, its evaluation from a radiobiological point of view remains contradictory. According to Langendorff *et al.*, DL-isocysteine administered into mice at a dose of 200 mg/kg 5 min before irradiation was ineffective.[12] At the same time increasing the dose of drug up to 400 mg/kg caused a statistically significant radiosensitizing effect to X-rays.[13,14] In more recent papers isocysteine is regarded as an ineffective drug.[15–17] In a paper published in 1968, Braun considers isocysteine as an ineffective isomer of cysteine,[18] but in a recent communication, this author considers isocysteine as a radiosensitizer.[19]

Therefore we tested DL-isocysteine HCl prepared by us according to the method of Schöberl and Braun. Our data indicates that isocysteine does not possess radiosensitizing action when administered before the irradiation at doses of 300, 600 and 1000 mg/kg (Table 1).

According to Langendorff a radiosensitizing effect is exerted by the L-isomer of isocysteine,[13] and this effect is evident after the administration of drug at a dose of 200 mg/kg (or 400 mg/kg of the racemic compound). Our results shows that the administration of isocysteine at a dose of 300 mg/kg (600 mg/kg of L-isomer did not result in sensitization of the animals. The same result was obtained regardless of the increase in the dose of racemic compound up to 1000 mg/kg (or 500 mg/kg of L-isomer). The reason for the discrepancy between our results and those of Langendorff is not yet completely understood.

DL-Isocysteine does not possess a radiosensitizing power, and may be regarded as ineffective isomer of the radioprotector cysteine. For instance according to Smith

et al. L-cysteine being injected into rats at doses of 400, 600 and 1000 mg/kg before the irradiation, protects 40, 70 and 90 per cent of animals respectively.[20]

Isocysteine ineffectiveness may be due to some peculiarities in its tissue distribution. Therefore we determined the content of isocysteine in radiosensitive and radioresistant organs of the rat.

Although after intraperitoneal administration of isocysteine at a dose of 400 mg/kg the drug was found in the liver only, this may reflect the low sensitivity of the method used for its quantitative assay. However, 15 min after isocysteine injection at a dose of 1000 mg/kg, which is equally ineffective in a radiobiological sense, the drug was found in the thymus, spleen and liver (Table 3). The picture is similar 15 min after i.p. administration of L-cysteine at the same dose.[1] The highest concentration of isocysteine 15 min after the injection occurred in the liver, the lowest in the thymus. As one may see from the data obtained on the liver, the increase in time interval between the injection of drug and decapitation of the animals leads to a steady decrease of isocysteine content in the organs (Table 3).

These data permit us to conclude that the ineffectiveness of isocysteine was not connected with any peculiarities in its distribution in the organs.

Therefore the next problem was to study the biochemical effects in rat organs 15 min after i.p. injection of both isomers.

As may be seen from Table 4 the i.p. administration of both isomers at a dose of 400 mg/kg leads to moderate inhibition in thymidine kinase activity in the thymus and spleen 15 min after injection. The increase in the dose up to 1000 mg/kg in the case of cysteine leads to a significant increase in the inhibition of enzyme activity in the organs tested. The administration of isocysteine at an equal dose did not increase the inhibition. If, for example, injection of cysteine at a dose of 1000 mg/kg results in the inhibition of thymidine kinase in the thymus and spleen by 30–50 per cent after irradiation, and affords 90 per cent of survival,[20] the administration of isocysteine at the same dose leads to 12–15 per cent of inhibition of the enzyme, and was ineffective in the increase in survival of irradiated animals.

As it has been shown by us earlier that inhibition of some processes of DNA precursor synthesis developing after MEA injection is connected with mixed disulfides formation between the aminothiol and the appropriate enzymes.[1–3] Therefore it was of interest to see to what extent the mixed disulfides of cysteine and isocysteine are responsible for the observed inhibition of thymidine kinase activity. The results are represented in Fig. 5.

The addition of various amounts of cysteine to the extracts of thymus and to the partially purified thymidine:cytosine deoxyribosyl transferase preparations leads to a significant inhibition in the activity of both enzymes (Fig. 5a, c). The addition of equimolecular amounts of isocysteine to the extracts of thymus resulted in much lower inhibition of thymidine kinase (Fig. 5b). In the case of partially purified transferase preparations the inhibitory action of isocysteine on the activity of the enzyme was more pronounced than that of cysteine (Fig. 5d). After the cleavage of mixed disulfides with DTT the inhibitory effect of cysteine on the activity of both enzymes was twofold lower. On the other hand DTT treatment of the enzymes inhibited by isocysteine did not result in a drop of inhibition (Fig. 5b, d). This testifies to the fact that mixed disulfides formation between isocysteine and the enzymes studied does not take place. Consequently the inhibition of thymidine kinase

activity observed after isocysteine injection is not connected with mixed disulfides but may be the result of the formation of other types of bonds between the drug and the enzyme.

The analysis of data obtained indicates that the radioprotective action of cysteine is not due solely to the inhibition of DNA synthesis as such, but by the character of the bonds which determines this inhibition. The administration of both isomers at a dose of 400 mg/kg induces a similar degree of inhibition thymidine kinase activity in the thymus and spleen (Table 4). However, the injection of cysteine at the same dose results in 40–50 per cent of survival,[20] but the injection of isocysteine at a dose of 400 mg/kg is fully ineffective (Table 1). In our previous paper we demonstrated that of the types of bonds between MEA and proteins of cellular microstructures in various organs, only the mixed disulfides were correlated in time with radioprotective effectiveness of the aminothiol.[5]

It is evident that the ability of SH-containing compounds to form mixed disulfides with intracellular enzymes to an appreciable extent determines their action on the process of DNA precursors formation. It is expedient to stress that some peculiarities in the properties of the compounds structurally similar to radioprotectors but ineffective was already discussed in the literature.

By means of ESR-spectroscopy it has been shown that after X-irradiation the radical yield per 100 eV (G) for cysteine equals 0·7. However for isocysteine there is a much bigger radical yield (G = 5·1).[21]

Injection of DL-isocysteine into rats 15 min before the administration of a toxic dose of α-naphthylthiourea to the animal did not result in the increase of survival, although injection of cysteine at the same dose leads to 95 per cent of survival.[22] Unlike cysteine isocysteine does not activate inactive cathepsin samples.[14] Braun has studied the comparative effect of i.p. injection of cysteine and isocysteine on the diameter of mitochondria isolated from the intestinal crypts.[18] It turned out that if 5–10 min after the injection of cysteine a significant increase in the diameter of mitochondria (swelling) occurs, isocysteine administration did not induce similar effects.

The sum total of new and earlier data shows that the effectiveness of aminothiols as radio protective depends on their interaction with metabolic processes of the host. The leading role in the inhibition of enzymes activity in tissues, and consequently in radiobiological effectiveness of the aminothiols is determined by the formation and cleavage of mixed disulfides.

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