Preparation and Pharmacological Properties of 4-Hydroxymethyl-2-(3'-amino-4'-hydroxyphenyl)-1:3-dithia-2-arsacyclopentane (Mapharside-BAL Compound)

By R. A. PETERS AND L. A. STOCKEN
Department of Biochemistry, Oxford

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As an extension to the work on the provision of an antidote to the arsenical war gases it has been shown in the United Kingdom (Stocken & Thompson, 1941; Stocken, Thompson & Whittaker, 1944) and in the United States (Eagle, 1943) that injections of 2:3-dimercaptopropanol (BAL) will save animals poisoned with lethal amounts of mapharside, if injected within 7 min. There is also evidence that BAL has a marked therapeutic effect in patients suffering from the toxic complications of arsenical therapy (Longcope, Wintrobe & Luetscher, 1943; Carleton, Peters, Stocken, Thompson & Williams, 1944). Nevertheless, it was thought advisable to investigate directly the toxicity of the compound of BAL and mapharside, which has been prepared in this laboratory and will be here called Maph-BAL. Previous experiments have shown clearly that the lewisite-thioarsinite has a toxicity of less than one-fifth that of the parent compound. On the other hand, to our surprise, Maph-BAL under certain conditions is more toxic than mapharside; in agreement with the lewisite experiments, however, its toxicity is diminished to one-fifth that of mapharside in the presence of extra BAL.

EXPERIMENTAL

Preparation of mapharside-BAL

Maph-BAL was prepared by dissolving 1 mol. of mapharside and 1 mol. BAL in ethanol (20 times

the vol. of BAL) and evaporating the solution to crystallization in a vacuum desiccator. The first crop of crystals was 70% of the theoretical, m.p. 172–3° (uncorr.) The mother liquor was rejected since precipitation by ether gave a sticky, coloured product. (Found: As, 22.7%; S, 18.9%; Cl, 10.3%. $C_8H_{13}O_2NClS_2As$ requires: As, 22.76%; S, 18.75%; Cl, 10.4%.) On standing, the compound remained unchanged for several weeks when kept in the dark, but after 2 years became discoloured.

Injection experiments

In order to obtain a comparison of the toxicities of mapharside with Maph-BAL, groups of rats were injected intramuscularly in the hind leg with solutions of the compounds in a 1:2 mixture of water and propylene glycol. The solutions were made up immediately before use. Table 1 gives the results obtained under comparable conditions and other additional results obtained either as controls for later experiments or at times when the two substances were not injected on the same day into rats of the same weight. Throughout this paper the dosage level of Maph-BAL given in the tables is calculated from the mapharside content; to obtain the absolute amount of Maph-BAL injected the figures must be multiplied by the factor 1.45.

From Table 1 it will be seen that whereas the LD₅₀ dose for mapharside is approximately

Table 1. *Direct comparison of toxicity for mapharside (Maph) and mapharside-BAL (Maph-BAL)*

(a) Experiments done by comparison at same time and with rats of same weight

Date	Dose (mg./kg.)	No. of animals	Maph		No. of animals	Maph-BAL	
			Survivors			Survivors	
			No.	%		No.	%
24. xi. 44	20	20	7	35	20	0	0
11. xii. 44	15	10	5	50	10	0	0
29. i. 45	10	10	10	100	10	0	0
29. i. 45	7	10	10	100	10	0	0

(b) Other experiments

6. xi. 44	40	6	0	0	—	—	—
9. xi. 44	30	20	3	15	—	—	—
Dec., Jan. and Feb.	14–15	—	—	—	20	2	10
Dec. and Jan.	10	10	8	80	6	2	33
1. ii. 45	4	—	—	—	10	4	40
1. ii. 45	2	—	—	—	10	10	100

15 mg./kg., that for Maph-BAL may be as low as 4 mg./kg. The animals were kept under observation for at least 1 month.

It was observed that the rats died very much more quickly from the effects of the thioarsinite than from the mapharside, seldom in less than 6 hr. after injection of the mapharside, but were usually dead in 2 hr. after the Maph-BAL. Only one case of a late death (3rd day after injection) took place and this was with the 4 mg./kg. dosage of Maph-BAL.

Although these results show an increased toxicity due to combination of the mapharside with BAL, further experiments proved that when an excess of BAL was present, the animals were able to tolerate several lethal doses of the Maph-BAL compound. Different amounts of Maph-BAL were injected intramuscularly into one hind leg and 10 min. later 5% (w/v) BAL in benzyl benzoate-arachis oil mixture (Eagle, 1943) into the other leg. The results obtained are presented in Table 2.

Table 2. *Protective effect of BAL against the toxicity of Maph-BAL in rats*

No. of rats	Dosage of Maph-BAL (mg./kg.)	Dosage of BAL (mg./kg.)	Survivors	
			No.	%
5	20	50-70	5	100
5	40-45	"	5	100
10	63-71	"	8	80
11	90-95	"	7	64
10	108-113	"	3	30
5	126-138	"	0	0

Having established that an excess of BAL could suppress the toxicity of several lethal doses of Maph-BAL, experiments were carried out to discover the minimum amount of BAL required to abolish the effects of 1 LD₅₀ dose of Maph-BAL. These tests were done in the same way as described above and as will be seen from Table 3 one equivalent of BAL is adequate for complete protection.

Half an hour after injection of the Maph-BAL the animals showed signs of arsenical intoxication, many being in a state of collapse with diarrhoea and lachrymation. We have obtained some indication of the limiting time which could be allowed to elapse before administration of the antidote to ensure

Table 3. *The protection of rats against the toxic effect of Maph-BAL by minimal amounts of BAL*

No. of rats	Dosage of Maph-BAL (mg./kg.)	Dosage of BAL injected 10 min. after the Maph-BAL (mg./kg.)	No. equivalents BAL	Survivors	
				No.	%
5	15	22	2.8	5	100
5	16	12	1.4	5	100
10	15-21	10	0.9-1.25	10	100
10	14	6	0.8	9	90
7	15-16	4	0.5	0	0

survival of the animal. It will be noticed from Table 4 that increasing the dose at 30 min. diminishes the mortality rate but that after longer intervals there is no effect.

Table 4. *The effect of the time interval elapsing before injection of BAL on the survival rate of rats given Maph-BAL and BAL*

No. of rats	Dosage of Maph-BAL (mg./kg.)	Time interval (min.)	Dosage of BAL (mg./kg.)	Survivors	
				No.	%
14	15-20	30	10	5	35
9	15-20	60	8-10	5	55
7*	15-20	90	8-10	2	28
10	15	30	50	10	100
5	61-64	30	52	5	100
10	15	45	40-43	4	40
9	15	60	60-65	2	22

* In this group more than seven had been injected but some had died before the BAL was due to be given.

Since it was found that a single dose of 4 mg./kg. BAL was inadequate to protect animals against a lethal dose of Maph-BAL, we tried the effect of repeated doses. Five rats were injected with 15 mg./kg. of Maph-BAL and 4 mg./kg. BAL, given 10, 130, 250 and 370 min. afterwards; all the animals survived. When, however, the amount of BAL was decreased to 2 mg./kg. and given under the same conditions no protection was obtained. Doses of 4 mg./kg. BAL at intervals of 4 hr. have been tolerated by men (Eagle, 1944; Sulzberger, Baer, Kanof & Eagle, 1944).

Table 5. *A comparison of the therapeutic efficiency against the toxicity of Maph-BAL of the injection of two monothiols and of BAL*

Compound	Dosage of Maph-BAL (mg./kg.)	Dose of thiol (mg./kg.)	No. equivalents thiol	No. of rats	Survivors	
					No.	%
Untreated	15	—	—	6	1	17
Glutathione	15	75	1.9	6	1	17
Cysteine ester HCl	15	46.5	1.95	6	0	0
*Cysteine ester HCl	15	475	20	6	4	66
BAL	15	10	1.25	5	5	100

* Given by intraperitoneal injection.

Glutathione (GSH) and cysteine ethyl ester HCl were also given to see whether a monothiol could suppress the toxicity of Maph-BAL. Some effect was shown with 40 mol. of the cysteine ester but neither glutathione nor the cysteine ester approaches BAL in therapeutic value. The results are shown in Table 5.

Experiments upon Protozoa

To obtain some information about the protoplasmic toxicity experiments were carried out with the ciliate *Colpidium colpoda* which has been under continuous subculture in this laboratory for many years (Peters, 1921, 1929; Walker, 1928). The technique adopted in these toxicity experiments was rather different from that described by Walker, owing to the relative non-toxicity of the mapharside. Observations were made at room temperature over periods up to 48 hr., and the toxicity was judged from the amount of the arsenical required to kill in this period. This method is not so accurate as that of rapid observation, but the differences in toxicity were large. All solutions were brought to c. pH 7.4 before use. Mapharside was toxic at a concentration of $m/1000$ – $m/1500$; Maph-BAL was much more toxic namely at $m/24,000$; BAL alone was not toxic at concentrations of $m/6400$ or $m/12,800$; but at these concentrations it did not decrease or increase the toxicity of Maph-BAL. The interpretation of these observations is not clear; possibly the whole Maph-BAL molecule is toxic to the ciliate, and the fact that the temperature of this experiment was c. 20° lower than that of the animal experiments is consistent with this; but it may also be that under these conditions the time of observation is so long that the BAL in the dilute solutions has time to oxidize. It is hoped to elucidate these points in further work.

DISCUSSION

The difference in the independent toxicities of lewisite-BAL and Maph-BAL is indeed remarkable; such facts make generalization the despair of biological workers. Close examination of the two compounds under the same conditions of pH (pH 9–9.5) gives evidence of a slight nitroprusside reaction for the mapharside compound and none for the lewisite compound; this would indicate a slight dissociation of the former under conditions in which the lewisite compound remains intact.

A proved explanation of the toxicity of Maph-BAL cannot yet be given; nevertheless, the fact that reversal is possible when the animal is showing all the usual signs of arsenical intoxication makes it fairly certain that the toxicity of the compound is due to dissociation. This is supported by the nitro-

prusside experiment mentioned; further, if toxicity in the whole animal was due to the undissociated molecule, it should be increased (not decreased) by the presence of extra BAL which would tend to decrease the dissociation. It is probable that the more rapid toxic effects of the compound are due to a penetration to the sites which are dangerous to the animals, by virtue of its physical properties which are different from those of mapharside. In this way the arsenic would be liberated locally where it could exert its maximum toxic effect; the increased toxicity would then be explained as due to decreased chance of elimination, as compared with mapharside, before toxic effects ensued. It is to be noted that mapharside itself has high water solubility, which will encourage elimination from the system.

From work reported with the pyruvate oxidase system (Stocken, Thompson & Whittaker, 1944) 1 mol. BAL in excess was required to reverse the toxicity of mapharside; it is interesting to note that similarly one extra mol. is required to protect the whole animal in the case of the rat. As regards the therapeutic application of BAL in man against poisoning by substances forming arsenoxides *in vivo*, there is now a strong argument for giving the maximum amounts of BAL which can be tolerated, because excess of BAL protects against any toxicity of Maph-BAL as well as of mapharside. Though caution in interpretation is necessary, there is no present reason for thinking that any Maph-BAL reaching the circulation by liberation of As in the tissues will not have with it sufficient uncombined BAL to prevent the development of any toxic action.

SUMMARY

1. Toxicity experiments have been carried out in rats with the compound mapharside-BAL, mapharside, and mixtures of Maph-BAL and BAL.
2. In presence of 1 mol. excess BAL, Maph-BAL has about one-fifth the toxicity of mapharside.
3. Injected alone, Maph-BAL is more toxic than BAL.
4. Repeated doses of BAL (4 mg./kg.) are sufficient to save animals from the toxic effect of Maph-BAL.
5. Maph-BAL is more toxic to the ciliate *Colpidium colpoda* than mapharside.
6. The clinical implications of these facts are discussed.

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* Information available on application to Ministry of Supply, London.

An Experimental Investigation of the 'Ring Hypothesis' of Arsenical Toxicity

By V. P. WHITTAKER, *Department of Biochemistry, Oxford*

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Earlier papers in this series (Stocken & Thompson, 1946*a, b*) described the development between 1940 and 1942 of British Anti-Lewisite (BAL) as an antidote for arsenical poisoning. It is now believed that the most important way in which the toxic arsenicals interfere with the normal life of the cell is by inhibiting an essential stage, that of pyruvate oxidation, in the series of chemical reactions by which it is supplied with energy (Peters, Sinclair & Thompson, 1946). The subsequent discovery (Stocken & Thompson, 1946*a*) that the arsenical protein formed by the interaction of lewisite and kerateine contains over 70% of its arsenic in combination with sulphur in the ratio of 1As:2S, suggested that the vesicant arsenicals act by reacting with two neighbouring essential thiol groups in enzyme proteins (e.g. those of the pyruvate system) with the formation of a relatively stable cyclic thioarsinite. The introduction into this system of a dithiol with which the arsenic can form a cyclic thioarsinite more stable than that which it forms with the protein, causes the arsenic to leave the protein and reverses its toxic effect on the cell. Monothiols, which, in the case of arsenical vesicants, are useless as antidotes, form open-chain thioarsinites which are less stable than the protein thioarsinites and are thus unable to remove the arsenic.

A confirmation of this hypothesis, which may be termed the 'ring hypothesis' of arsenical toxicity, has been sought in a study of the relative effectiveness as arsenical detoxicants of a series of $\alpha\omega$ -dithiols and some monothiols with comparable physical properties. If the formation of a small stable ring is the underlying reason for the ability of

dithiols to detoxicate arsenic *in vivo*, clearly the size of the ring is one of the most important factors in determining their therapeutic effectiveness. Attention was first directed to 1:2 and 1:3-dithiols because the corresponding cyclic thioarsinites are five- and six-membered rings, and by analogy with other types of ring compounds, rings of this size would be expected to be the easiest to form and among the most stable when formed; but it was thought desirable to undertake a study of the variation of antidotal efficiency attendant upon a successive increase in the spacing of thiol groups one from the other in the dithiol molecule in order to investigate the validity of the ring hypothesis still further. The relative effectiveness of the series of $\alpha\omega$ -dithiols was assessed by measuring their ability to reactivate the pyruvate oxidase system after poisoning with lewisite. The results obtained are discussed later in the light of known facts about the stereochemistry of large rings and are considered to support the ring hypothesis.

EXPERIMENTAL

Synthesis of $\alpha\omega$ -dithiols

The starting-point for the synthesis of most of the $\alpha\omega$ -dithiols was the succinic acid series of dicarboxylic acids. These were esterified and reduced by the Bouveault-Blanc method (using Manske's procedure, 1934) to the corresponding diols, which were converted to the dithiols via the dibromides by heating the latter with 6 mol. ethanolic sodium or ammonium hydrosulphide (Stocken, 1947) at 80–90° in a sealed bottle for 24 hr. On cooling, the reaction mixture was acidified (<pH 4), the solvent removed *in vacuo* and the dithiol extracted from the solid residue with chloroform. After drying, the chloroform was removed