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Pharmacokinetic and toxicological studies of antimony dextran glycoside (RL-712)

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Summary

1. The absorption, tissue distribution and excretion of antimony dextran glycoside (RL-712) has been studied in normal rodents.

2. Some organs in the body, especially liver and spleen, retain large amounts of antimony for considerable periods of time. Excretion of antimony in the urine was low and only about 10–12% of the dose administered was excreted within the first 48 hours.

3. Blood levels were maintained for at least 3 days after a single intramuscular dose to rabbits, corresponding to 14 mg Sb/kg body weight.

4. Toxicity studies and tests on foetal toxicity in mice and rats, respectively, showed no abnormalities.

5. The possible value of RL-712 in the prophylaxis and treatment of leishmaniasis is discussed.

Introduction

It is well known that the pentavalent antimonials are effective in the treatment of leishmaniasis. Intramuscular and intravenous injections of these compounds produce antimony blood levels of short duration and antimony is excreted rapidly in the urine. This evidence indicates that the intramuscular or intravenous doses of antimonials are not fully utilized in the body for the desired function. Most of the administered dose may pass from the site of injection into the blood stream at a rate faster than that required to produce the maximal pharmacological response and is rapidly eliminated.

The possibility of achieving a more efficient use of the administered dose of antimony has been the leading idea in the development of a pentavalent antimonial drug (RL-712) which is retained in the organism for a longer period than the traditional antimonials. Chemically RL-712 is a stable non-ionic complex of antimony hydroxide and a partially depolymerized dextran glycoside (molecular weight about 5,000). The preparation has a high molecular weight (above 40,000) and that fact may account in part for the lower excretion rate of the drug in laboratory animals. The toxicity is low, on intravenous injection in mice the LD50 is over 500 mg Sb/kg body weight.

RL-712 has been shown to possess antileishmanial activity in hamsters infected with a strain of *Leishmania donovani* (Mikhail, Khayyal & Girgis, 1969) which compared favourably with sodium stibogluconate (pentostam).

An antimony dextran complex has been used with success by Sen Gupta, Chatterji & Mukherjee (1961) in the treatment of kala-azar. There are few details

known about the preparation, but it seems that after injection in experimental animals the antimony was deposited in the spleen, liver and lymph nodes.

There is some risk of overdosing in the administration of short-acting drugs. In order to produce a desirable duration of action, a drug may be given at excessively high doses or at too short intervals. Pentavalent antimonials are injected daily in courses of 10–12 days in the treatment of kala-azar. With RL-712 it may be possible to maintain the rate of diffusion of the drug from depots (site of injection, liver), at a constant level prolonging the elimination half-life.

Methods

Absorption and tissue distribution of RL-712 in normal mice

Two groups of 10 mice (female, strain NMRI) weighing 18–22 g were injected intramuscularly either with 0·04 ml of an RL-712 solution containing 26·0 mg Sb/ml (52 mg Sb/kg body weight) or with 0·0125 ml of a solution of *N*-methyl-glucamine-antimonate (glucantime) containing 80·5 mg Sb/ml (50·3 mg Sb/kg body weight).

The mice were killed with chloroform at the following times after injection: 6, 24, 48 and 72 h, 1 week, 2, 3, 4, 5 and 6 weeks for the RL-712 and 24 h, 1 week and 4 weeks for the glucantime.

Immediately after death the injected leg, non-injected leg (skeletal tissue), kidneys, heart, spleen and liver were removed, weighed and analysed for their content of antimony.

The antimony content of tissues, serum and urine was determined by a variation of the method of Gellhorn, Krahl & Fertig (1946a) which can measure 0·5 μg Sb/ml serum, urine or gramme wet tissue.

Antimony blood levels in rabbits

Three rabbits weighing 3·5–4·0 kg were injected intramuscularly in the gluteus maximum muscle with 2 ml of RL-712 solution containing 26·0 mg Sb/ml, corresponding approximately to 14 mg Sb/kg body weight. Blood samples were taken before and 1, 3, 5, 7, 24, 48 and 72 h after injection, allowed to clot and centrifuged to separate serum from blood cells. The serum was kept at −18° C until the analysis was performed.

Excretion in mice and rats

Mice were dosed as described above and the urine collected in a metabolism cage during the periods: 0–6 h, 6–24 h, 24–30 h and 30–48 h after injection for RL-712 and 0–24 h and 24–48 h after injection for glucantime. The total amount of antimony injected per 10 mice was 10·40 mg (RL-712) and 10·06 mg (glucantime).

Pairs of female albino rats (Wistar strain), 240–260 g, were anaesthetized with ether and injected intramuscularly with 0·51 ml of a solution of RL-712 containing 26·0 mg Sb/ml, corresponding to approximately 50 mg Sb/kg body weight. The urine was collected for 48 hours.

Toxicity studies

Four groups of 5 mice (female, strain NMRI) were injected intramuscularly alternately in the right and left hind legs three times a week for 17 weeks. In the first 4 weeks the dose was 0·04 ml, in the next 7 weeks 0·06 ml and in the last 6 weeks 0·1 ml of a solution of RL-712 containing 26·0 mg Sb/ml. A total of 80 mg antimony was injected per mouse.

Other groups were given smaller doses of 0·01, 0·02 and 0·04 ml for the same periods, totalling 28·2 mg antimony per mouse.

Evaluation of foetal toxicity

Rats (Wistar strain, weighing 200 g) were divided into 3 groups: a control group given 0·9% NaCl w/v (saline) and 2 test groups given 125 and 250 mg Sb/kg body weight of an RL-712 solution containing 38·3 mg Sb/ml, administered by intramuscular injection under ether alternately into the right and left hind legs, on five occasions between the 8th and 14th day of gestation. On day 20 the number of foetuses, resorptions and implantations were recorded. The foetuses were examined for external malformations and either fixed or autopsied in the fresh state. The fixed foetuses were examined for internal abnormalities, half of them by clearing methods for skeletal structure, the remaining by the freehand razor-blade method of sectioning rat foetuses.

Results

Absorption and tissue distribution of RL-712 and glucantime

The results are expressed as μg Sb wet weight. As can be seen from Tables 1 and 2, RL-712 is absorbed from the injection site and deposited mainly in the liver and spleen, while glucantime is deposited to a very small extent in the same organs.

TABLE 1. *Antimony levels in tissue or organs of mice expressed in μg of antimony per g wet tissue (organ). Administered dose: 52 mg Sb/kg body weight as RL-712*

Time after injection	Skeletal muscle	Kidneys	Heart	Spleen	Liver
6 hours	1·3	15·3	24·4	65·1	156·8
24 hours	17·6	14·3	26·0	67·6	—
48 hours	33·8	11·7	23·5	39·7	124·0
72 hours	—	13·6	22·2	—	105·0
1 week	—	12·7	21·1	32·7	80·6
2 weeks	—	9·1	8·8	7·4	68·0
3 weeks	—	4·4	6·8	7·5	66·7
4 weeks	—	0·5	1·3	6·6	33·6
5 weeks	—	1·0	2·0	5·0	34·0
6 weeks	—	1·4	1·5	4·6	20·7

TABLE 2. *Antimony levels in tissue or organs of mice expressed in μg of antimony per g wet tissue (organ). Administered dose: 50·3 mg Sb/kg body weight as glucantime*

Time after injection	Skeletal muscle	Kidneys	Heart	Spleen	Liver
24 hours	1·0	3·5	4·5	4·5	7·5
1 week	—	2·5	2·0	2·5	7·5
4 weeks	—	⩽0·5	⩽0·5	⩽0·5	3·0

Blood levels of RL-712 in rabbits

Figure 1 shows serum levels of antimony after the intramuscular administration to rabbits of 14 mg Sb/kg body weight as RL-712. The maximum level is attained 5 h after injection.

Excretion in mice and rats

Of the injected antimony from RL-712 only 12% is excreted in the urine of mice within 48 hours, and 10% in rats, while 88·6% of the antimony is excreted in the urine of mice given glucantime.

The total recovery of antimony after a single intramuscular injection to mice of RL-712 corresponding to 50 mg Sb/kg was 100·4% after 6 h, 79·8% after 24 h and 78% after 48 hours. The total recovery for glucantime at the same dosage level was 90·2% after 24 hours.

Toxicity in mice

The mice given a total of 2,377 mg Sb/kg body weight as RL-712 grew as well as non-injected mice. Histological examination 2 weeks after the last injection showed a normal appearance of the injected areas and also of the heart, spleen, liver and kidneys. The amount of antimony present in these organs is given in Table 3.

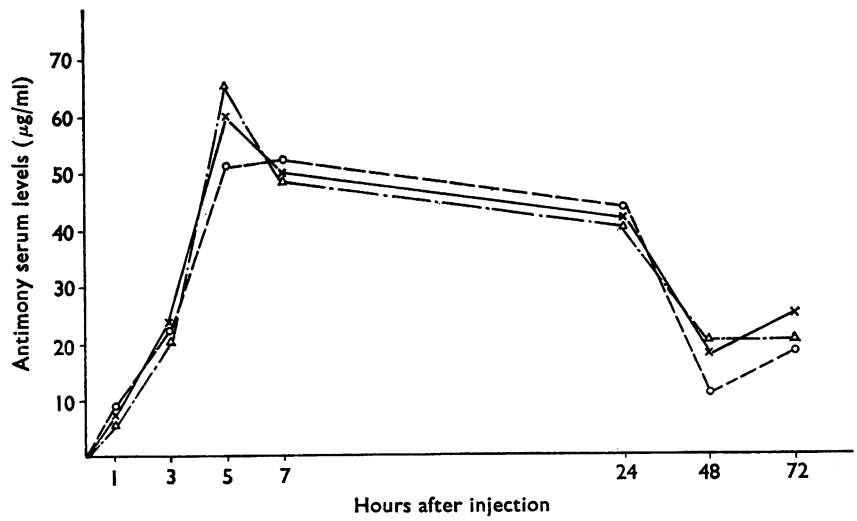

FIG. 1. Serum levels of antimony in three rabbits after intramuscular administration of 14 mg Sb/kg as antimony dextran glycoside (RL-712).

TABLE 3. *Antimony levels in organs of mice as a percentage of the total antimony injected during* 17 *weeks*

Organ	% of total antimony injected
Liver	0·54
Spleen	0·026
Heart	0·016
Kidneys	0·008

The growth of the mice injected with 860 mg Sb/kg body weight as RL-712 was the same as that of non-injected mice up to 26 weeks after the last injection and 43 weeks after the initiation of the trials. No signs of toxicity appeared during the injections or in the post-injection period.

Evaluation of foetal toxicity in rats

No abnormalities were seen in the foetuses from the test animals or the controls. To find out if the drug was able to penetrate the placenta, 2 foetuses of each group (including control) were analysed for antimony, but none could be detected in the 3 groups examined.

Discussion

Absorption and tissue distribution

Gellhorn, Tupikova & van Dyke (1946b) found 11–16% of the injected dose of antimony in the liver of hamsters 24 h after injection of trivalent antimonials and only 3–4% with pentavalent antimonials. With all drugs antimony was localized to the greatest extent in the liver. Browne & Schulert (1964) also found that most of the antimony was stored in the liver of the hamsters 24 h after a single injection of either antimony dimercaptosuccinate (astiban) or tartar emetic.

Abdallah & Saif (1962) concluded that in man there is a strong tendency for trivalent antimony to be retained in the tissues for long periods (up to 50 days) and in very high concentrations, especially in the liver. El-Halawani (1962) found that in man the trivalent antimonials are concentrated more in the liver, whereas the pentavalent are concentrated more in the spleen.

Recently Molokhia & Smith (1969a) with trivalent antimonials in mice recorded the highest antimony levels in the liver, gastrointestinal tract (from the bile), kidney and urinary bladder (urinary excretion).

In our studies, mice that had received a single intramuscular injection of glucantime corresponding to 50 mg Sb/kg showed after 24 h a low concentration of antimony in the kidneys, heart, spleen, and liver. The highest concentration was found in the liver and amounted to 7·5 μg Sb/g corresponding to 1% of the administered dose. Four weeks after injection there was 3·0 μg Sb/g (0·5% of the administered dose) in the liver and non-measurable amounts of antimony in kidneys, heart and spleen.

With RL-712 in mice there is a different picture. Forty-eight hours after a single intramuscular injection corresponding to 52 mg Sb/kg the liver was found to contain 15·4% of the administered dose (124 μg Sb/g), 26·1% was found in the skeletal muscle and 0·56% in the spleen. Four weeks after the injection 6·2% of the administered dose (33·6 μg Sb/g) was still found in the liver and 0·12% in the spleen. Thus RL-712 is deposited mainly in the liver and spleen and 4 weeks after the injection concentrations in the liver are more than ten times higher than those after glucantime (Tables 1 and 2).

Blood levels of antimony

Bartter, Cowie, Most, Ness & Forbush (1947) using ^{124}Sb-labelled tartar emetic given intravenously to man found preferential uptake of the drug by the red blood

cells. Otto, Maren & Brown (1947) gave pentavalent antimonials 2–3 mg Sb/kg intravenously to patients and the plasma concentration of antimony was much higher than in the cells at the same period. After 24 h very small concentrations of antimony were found in both plasma and red cells.

Abdallah & Saif (1962) injected ^{124}Sb-labelled astiban intramuscularly into man in doses of 1·4–2·1 mg Sb/kg and could not measure blood levels after either single or multiple doses. El-Bassousi, Ata & Abd-El Al (1963) gave children with urinary schistosomiasis a single injection of various trivalent antimonials in doses of 5–7 mg Sb/kg. The highest blood level was attained 0·5–1 h after injection and fell rapidly within 24 hours. Using activation analysis Molokhia & Smith (1969b) found that the erythrocytes' uptake of the pentavalent compound, sodium stibogluconate (pentostam) was insignificant in contrast to tartar emetic and astiban.

It may be concluded from the work of Otto *et al.* (1947), Abdallah & Saif (1962) and El-Bassousi *et al.* (1963) that with both trivalent and pentavalent antimonials no substantial blood levels can be maintained. There may be two reasons for this ; the rapid excretion in the urine of antimony compounds, especially the pentavalent ones and the uptake of antimony by different tissues (liver) especially the trivalent antimonials.

Rabbits injected intramuscularly with RL-712 (14 mg Sb/kg) maintained blood levels of antimony for at least 72 hours after a single injection (Fig. 1). This probably indicates that RL-712 is absorbed slowly from the injection site and that absorption takes place mainly via the lymphatics.

Excretion

Gellhorn *et al.* (1946b) recovered 16% of the antimony in the urine and 52% in the intestine, 24 h after the injection of trivalent antimonials to hamsters ; after intraperitoneal injection of pentavalent antimonials they recovered 60–73% of the antimony in the urine and 3–7% in the intestine.

Otto *et al.* (1947) treated patients with trivalent or pentavalent antimonials and found that the bulk of both were excreted in the urine, the rate being higher for the pentavalent antimonials. The faecal excretion was much slower than the urinary excretion for either trivalent or pentavalent antimonials. El-Halawani (1962) concludes that young people usually excrete antimony more rapidly than older people and emphasizes that retention of antimony is essential to effect a cure. Drugs may be found effective *in vitro*, but ineffective in man, because they are rapidly excreted.

Browne & Schulert (1964) treated mice and hamsters with ^{124}Sb-labelled astiban. Sixty-five per cent of the injected dose was recovered in the urine of hamsters after 48 h and faecal excretion was about 6% in the same period.

Our data on the excretion of glucantime and RL-712 are consistent with the pattern of absorption and distribution of the two drugs. Glucantime being a low molecular weight antimonial is rapidly absorbed from the injection site and rapidly eliminated from the circulation into the urine, accumulation in tissues or organs being low. RL-712, on the contrary, being a larger molecule is absorbed slowly from the injection site, the blood level being maintained for several days and antimony being retained mainly in the liver and spleen. The excretion of antimony within 48 h of the injection is low (10–12%).

Table 4 shows the differences in absorption, tissue distribution and excretion for traditional trivalent and pentavalent antimonials and for RL-712. Like the trivalent antimonials, RL-712 is retained in the liver and, like the pentavalent antimonials, is retained in the spleen. RL-712 differs from both trivalent and pentavalent antimonials in that after injection blood levels are maintained for several days; it is slowly absorbed from the injection site in macrophages via the lymphatics, deposited in the same organs as the leishmanial parasite, i.e., mainly in liver and spleen, and excreted slowly in the urine.

In trials with laboratory animals RL-712 has been shown to have a prophylactic effect on leishmaniasis. Hamsters received a single dose of 300 mg Sb/kg body weight either as RL-712 or pentostam and 10 days later were infected with a strain of *L. donovani*. Hamsters receiving RL-712 survived the infection, while those receiving pentostam did not (Khayyal, 1971).

In conclusion, with a drug like RL-712 it may be possible to treat patients with lower doses of antimony and with less frequent injections. Because of the fact that both parasites and antimony are deposited in the same organs, the parasites will be exposed to antimony for a longer period, provided the antimony in the organs is present in an active state. RL-712 may be a drug with prophylactic value as well. If it is injected before a leishmanial infection, it will be deposited in the liver and spleen to some extent and may be able to destroy the parasites even though infection occurs several days or weeks after the injection. If both the drug and the parasites are taken up by the macrophages and deposited in the same organs it is theoretically possible to immunize people against leishmaniasis by giving the drug and infecting with an appropriate parasite at the same time or a few days later.

TABLE 4. *Absorption, distribution and excretion of antimonials in mice*

Drug	Absorption injection site	Antimony blood levels	Affinity red blood cells	Retention in			Excretion
				Spleen	Liver	Macrophages	
Trivalent antimonials	Rapid	Short duration	+	−	+	−	Rapid
Pentavalent antimonials	Rapid	Short duration	−	+	(+)	−	Rapid
RL-712	Slow	Long duration	−	+	+	+	Slow

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