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THE CURATIVE POWER OF 4-(2:6-DIAMINOPYRIMIDIN-4-YLAMINO)PHENYLARSINE OXIDE (COMPOUND 12,065) IN INFECTIONS OF *TRYPANOSOMA RHODESIENSE* IN LABORATORY ANIMALS

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The toxicity to laboratory animals of 4-(2:6-diaminopyrimidin-4-ylamino)phenylarsine oxide (compound 12,065), and its ability to cure laboratory infections of *Trypanosoma rhodesiense* were investigated. The intramuscular LD50 to mice was 22 mg./kg.; rats were more susceptible. Monkeys tolerated two daily doses of 6 mg./kg., but not two daily doses of 10 mg./kg. The intramuscular CD50 to mice was about 6 mg./kg. with two strains of *T. rhodesiense* recently isolated from human patients, and 2 mg./kg. with a strain of *T. rhodesiense* which had been maintained by cyclical transmission through tsetse flies in the laboratory for about 17 months. These results are compared with those reported by Ainley and Davey (1958) for a strain of *T. rhodesiense* which had been maintained in the laboratory by cyclical transmission for about 12 years and then by syringe transmission for about 10 years. The syringe-transmitted strain was at least 20 times more susceptible to the drug than the recently isolated strains. The potencies of compound 12,065 and melarsen oxide/BAL in curing *T. rhodesiense* infections in mice were compared: the CD50 for compound 12,065 was 6.5 mg./kg. and for melarsen oxide/BAL was 3.7 mg./kg. The ratios LD50/CD50 for the drugs were about 4 and 6 respectively.

Ainley and Davey (1958) and Davey (personal communication) have shown that 4-(2:6-diaminopyrimidin-4-ylamino)phenylarsine oxide (compound 12,065) cured infections of *Trypanosoma rhodesiense* in mice and monkeys, even when the central nervous systems of the monkeys were involved.

Ainley and Davey (1958) used a branch of the Tinde strain of *T. rhodesiense* from Kahama, Tanganyika, which they had maintained by syringe passage in mice for about 10 years, after 12 years of cyclical transmission at Tinde, Tanganyika. Prolonged syringe passage has been shown to alter the susceptibility of trypanosome strains to arsenical drugs (Murgatroyd and Yorke, 1937). Preliminary tests of the compound were therefore made in laboratory animals infected with strains of *T. rhodesiense* which had been maintained by cyclical transmission through tsetse flies and with strains recently isolated from sleeping sickness patients.

MATERIALS AND METHODS

Strains of Trypanosome.—Strain "Lugala II" was isolated from *Glossina pallidipes* in a white rat in September, 1955 (East African Trypanosomiasis Research Organization, 1956). It was maintained, after one initial syringe passage, by cyclical transmission through *G. morsitans* and laboratory guinea-pigs or (occasionally) white rats.

Strain "SS 21" and strain "SS 115" were isolated from human patients by inoculation of blood into white rats (Robertson and Baker, 1958). Experimental animals were inoculated directly from the first isolation rat.

The three strains of *T. rhodesiense* originated from the area on or near the north-east shore of Lake Victoria in the Eastern Province of Uganda.

Experimental Animals.—The animals used were albino rats and mice of the closed colonies of the East African Trypanosomiasis Research Organization at Tororo, and adult monkeys (*Cercopithecus aethiops johnstoni*) trapped in Nzega District, Western Province, Tanganyika, and kept in captivity at Tororo for at least one year.

Drugs.—Compound 12,065 was supplied by Imperial Chemical Industries Limited, Pharmaceuticals Division, as the solid isethionate dihydrate. All doses are given in mg. of the base, one unit of weight of which is equivalent to 1.56 units of the isethionate dihydrate.

Melarsen oxide/BAL (May and Baker), or 4-(3-hydroxymethyl-1-arsa-2:5-dithia*cyclo*pentyl)

benzene (Friedheim, 1950), was supplied as a 3.6% solution in propylene glycol, equivalent to a 2.5% solution of the base.

Infection, Examination, and Treatment of Experimental Animals.—Mice and rats were infected by the intraperitoneal inoculation of infected blood either diluted with citrated normal saline or heparinized and diluted with isotonic glucose solution. Animals in each experiment received equal volumes of diluted blood from the same donor, so that the number of trypanosomes inoculated was approximately equal within each experiment.

Fresh preparations of blood collected from the tails of infected animals were examined daily (except on Sundays). Twenty fields of these preparations were examined under the 4 mm. objective of the microscope and, if no trypanosomes were seen, the animals were classed as negative.

Both drugs were given by intramuscular injection. Infected mice were treated when about five or more trypanosomes/microscope field were seen in the fresh blood preparations, six to eight days after inoculation. Compound 12,065 was dissolved in sterile distilled water to give solutions of from 0.017% to 0.8%. In Expt. No. 6 the concentrations of the drug solutions were adjusted so that each mouse received a volume of 0.05 ml., the melarsen oxide/BAL solution being diluted with propylene glycol. Monkeys and rats were treated on the basis of their individual body weights; mice were treated on the basis of the average body weight of the groups. The criterion of cure adopted was that mice, after the initial clearance of trypanosomes from their blood brought about by the drug, should not relapse within 42 days of treatment.

Treatment of Results.—The lethal and curative doses for 50% of the animals (LD50 and CD50) were calculated by the method of Thompson (1947). In determining the CD50, the mice which died without parasitaemia within 42 days of their treatment were discarded because it was not known whether their deaths were due to sub-patent trypanosomiasis or extraneous causes.

RESULTS

Toxicity Tests.—Seven groups of ten uninfected mice, average weight about 20 g., received single doses of from 2.6 to 25.6 mg./kg. of compound 12,065. The LD50 was about 22 mg./kg. The intramuscular LD50 for mice calculated from the results of Davey (personal communication) was about 39 mg./kg.

Two adult male monkeys, weighing 5 kg., survived total doses of 4 and 12 mg./kg. respectively of compound 12,065 (given in two equal daily doses) with no apparent ill effects. One adult male monkey, weighing 4.5 kg., died 10 days after receiving 20 mg./kg. in two equal daily doses. It

TABLE I

THERAPEUTIC ACTIVITY OF COMPOUND 12,065 AND MELARSEN OXIDE/BAL IN *TRYPANOSOMA RHODESIENSE* INFECTIONS IN WHITE MICE

* Denotes that one of these mice showed trypanosomes in its blood on the 65th day after treatment but was still alive without further parasitaemia on the 100th day.
† Indicates that the mice received 0·05 ml. of normal saline.
‡ Denotes that the mice were given 0·05 ml. of propylene glycol.
§ Indicates that some of these mice (2 receiving 4 mg./kg. and 1 receiving 8 mg./kg.) relapsed between 74 and 92 days after their treament and died with parasitaemia; they are also included in the column headed "Died with Parasitaemia."

Expt. No.	Strain of *T. rhodesiense*	Drug	Dose (mg./kg. of Base)	Number of Mice				CD50 (and Range; P=0·95) (mg./kg.)
				In Group	Died with Parasitaemia	Died without Parasitaemia Less than 42 Days After Treatment	Survived at Least 42 Days After Treatment Without Parasitaemia (Presumed Cured)	
4	Lugala II	Compound 12,065	0	5	5	0	0	2·0 (0·9–4·6)
			1	4	1	3	0	
			2	5	2	1	2*	
			4	4	0	1	3	
			8	4	0	1	3	
5	SS 21	,,	0	10	10	0	0	5·7 (3·8–8·5)
			0·5	8	2	6	0	
			1	7	6	1	0	
			2	10	7	3	0	
			4	10	5	5	0	
			8	10	0	5	5	
6	SS 115	,,	0†	9	9	0	0	6·5 (3·9–10·8)
			2	8	6	2	0	
			4	9	5	1	3	
			8	7	4	0	3	
			12	10	0	5	5	
		Melarsen Oxide/BAL	0‡	10	10	0	0	3·7 (2·1–6·4)
			2	10	6	2	2	
			4	9	3	3	5§	
			8	10	1	3	7§	
			12	9	0	2	7	

had been ill, with diarrhoea and lassitude, for 5 days and, at autopsy, the kidneys were swollen and haemorrhagic.

Tests of Curative Power.—In all experiments, the blood of treated animals was at least temporarily cleared of trypanosomes: all untreated control animals died with heavy parasitaemia within 37 days of inoculation.

An initial test with rats failed because the animals were very susceptible to the drug; all of those which received 4 mg./kg. or more died, apparently of poisoning.

The results of the tests with mice are set out in Table I. In Expt. No. 6, melarsen oxide/BAL, the drug at present in use for the treatment of advanced cases of *T. rhodesiense* infection (Apted, 1957), was compared with compound 12,065.

Discussion

The intramuscular CD50 of compound 12,065 in mice was about 6 mg./kg. with the two recently isolated strains SS 21 and SS 115, and 2 mg./kg. with the strain Lugala II which had been maintained in the laboratory by tsetse fly transmission for about 17 months. Ainley and Davey (1958) showed that, with their strain of *T. rhodesiense* which had been cyclically transmitted for 12 years and then syringe passaged for about 10 years, the CD50 of compound 12,065 lay below 0.25 mg./kg. for subcutaneous administration: it was probably below this value, and certainly below 0.5 mg./kg., when given intravenously (Davey, personal communication). Thus the recently isolated strains were three times as resistant as the strain cyclically transmitted for 17 months and at least 20 times as resistant as the strain which had been transmitted by cyclical and syringe passage for about 21 years.

The difference between these results and those of Ainley and Davey (1958) is unlikely to have been due solely to the different routes of administration of the drug, because the calculated LD50 (Davey, personal communication) differed by a factor of less than two whether given intravenously, subcutaneously or intramuscularly. The greater resistance of the strains used in the present work could be due to the fact that the mice were treated at rather higher levels of infection than were those of Ainley and Davey (1958) or to the fact that the strain used by these workers had become less resistant during its prolonged maintenance in animals other than man by tsetse fly and syringe transmission. The strain cyclically transmitted for 17 months (Lugala II) and the strains newly isolated from man were treated at about the same levels of infection in the mice. The results suggest that the intensity of infection does not fully explain the difference. Whether the loss of resistance of the strain of Ainley and Davey (1958) was due to the prolonged cyclical transmission or the ten years of syringe transmission is not clear from these results, although the latter procedure is likely to have been at least partly responsible. Syringe transmission has been shown to increase the susceptibility of trypanosome strains to arsenical drugs (Murgatroyd and Yorke, 1937).

The comparison of compound 12,065 and melarsen oxide/BAL shows that the drugs have a similar curative power in infections of *T. rhodesiense* in mice. The CD50 of melarsen oxide/BAL was lower than that of compound 12,065, but the difference was not statistically significant.

The LD50 of compound 12,065 to mice was 22 mg./kg., so the ratio LD50/CD50 (a modification of the therapeutic index, Lourie and Yorke, 1939) was about 4. The toxicity of melarsen oxide/BAL was not investigated, but the results given by Davey (personal communication) show the intravenous LD50 to mice to have been about 22 mg./kg. also; the ratio LD50/CD50 for the latter drug was therefore about 6. A more crucial comparison of the two drugs would be their relative ability to cure monkeys whose central nervous systems were infected with a strain of *T. rhodesiense*. Such tests were attempted in collaboration with Dr. D. H. H. Robertson, but failed because the infected monkeys died almost as soon as involvement of the central nervous system was demonstrable, even when suppressive treatment with suramin was used. A strict parallel between the behaviour of the drugs in mice and men cannot be assumed, but compound 12,065 and melarsen oxide/BAL are likely to be of similar potency in treating advanced cases of human *T. rhodesiense* infection. If so, the possibility of giving the former drug intramuscularly might be an advantage in clinical practice. However, the toxicity of compound 12,065 in man would need careful investigation before clinical use; Apted (personal communication) has obtained some evidence that it may be more toxic to man than melarsen oxide/BAL.

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