EXPERIMENTAL CHEMOTHERAPY OF TRYPANOSOMIASIS

I. Effect of p-Phenylene Diguanidine and Related Compounds Against Experimental Infections with *Trypanosoma equiperdum*

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INTRODUCTION

A search was begun in our laboratories in 1944 for a compound which might possess advantages over existing agents for the treatment of trypanosomiasis, and particularly for one which would be effective when administered orally. Very early in the course of this screening program it was found that p-phenylene diguanidine possessed curative properties against *Trypanosoma equiperdum* in mice when administered either orally or intraperitoneally. It was not as powerful a trypanocide as Bayer 205 or several other known compounds, but it possessed several interesting properties which encouraged extensive investigations. The parasitological data obtained with p-phenylene diguanidine and several related compounds in experimental animals is presented in this paper, and the chemistry of the group is discussed in detail elsewhere (1).

The effectiveness of guanidine compounds in experimental trypanosomiasis was first demonstrated by British investigators. King, Lourie and Yorke (2) showed that a series of homologues of Synthalin possessed trypanocidal properties. The trypanocidal action rose as the number of methylene groups in the alkyl chain was increased; the decane, dodecane and tetradecane derivatives were the most active. Activity was then found in alkylene diamidines in which two guanyl groups were attached to the ends of the methylene chain without the intervention of other groups. Lourie and Yorke (3) then proceeded to aromatic diamidines and selected 4,4′-diamidino stilbene (Stilbamidine) as the most active member of the group. St. A. Heathcote (4) gives a recent summary of the pharmacology and therapeutic uses of Stilbamidine and related compounds.

The guanidine compounds described in the present report, and more particularly p-phenylene diguanidine, are not so strikingly effective in laboratory infections with trypanosomes as are the aromatic diamidines. p-Phenylene diguanidine, however, has a relatively simple structure, and it cures *T. equiperdum* infections in mice in well-tolerated doses when administered orally.

Material and methods. Mice infected experimentally with *T. equiperdum* were used as laboratory hosts throughout the major part of these investigations. Each mouse was inoculated intraperitoneally with a saline suspension containing approximately 50,000 to 100,000 trypanosomes, or an average of 2.5 million trypanosomes per kilogram body weight in 20 gram mice. This parasite produces highly consistent infections in untreated mice, with death occurring almost invariably within three to five days after inoculation with the above

known trypanocides in laboratory animals. Although it is far less effective than Bayer 205, Stilbamidine, Melarsen, and p-arseno-phenylbutyric acid when administered parenterally, it does possess curative properties at relatively low oral dosages. It differs from the arsenicals, Bayer 205, and the aromatic diamidines, in that very large single intraperitoneal doses (100 mgm. per kgm.) are required to produce cures in mice infected with *T. equiperdum*. The total curative intraperitoneal dose when administered for seven doses (4 mgm. per kgm. twice daily, a total of 28 mgm. per kgm.) is approximately one-fourth of

TABLE 5

Compounds related to p-phenylene diguanidine which show trypanocidal activity

COMPOUND NO.	NAME	APPROXIMATE P-PHENYLENE DIGUANIDINE EQUIVALENT	
		I.P.	Oral
350-L	p-Phenylenedibiguanide monohydrate	0.08	<0.05
253-L	2-Chloro-1,4-phenylene diguanidine dihydrochloride	0.16	0.05
369-L	2-Chloro-1,4-phenylene dibiguanide dihydrochloride	0.16	<0.1
291-L	2-Methyl-1,4-phenylene diguanidine dihydrochloride	0.08	0.05
251-L	1,6-Diisopropyl-p-phenylenediguanidine dihydrochloride	0.08	<0.05
83-L	Diphenylurea-4,4'-diguanidine carbonate	1.0 plus	0.2
318-L	1,3-Bis(4-guanidophenyl)-guanidine trihydrochloride	1.0 plus	0.3
331-L	Bis-(4-guanidophenyl)- sulfide carbonate	1.0 plus	1.0
353-L	Bis-(4-guanidophenyl)-sulfoxide dihydrochloride	0.16	<0.1
325-L	Bis-(4-guanidophenyl)-sulfone dihydrochloride	<0.08	<0.05
340-L	3,3'-Dimethyl-4,4'-diguanido biphenyl	1.0 plus	0.3

the single dose needed to produce cures. The chemotherapeutic indices given for p-phenylene diguanidine when administered in multiple doses (table 6) are lower than those which have been reported for Melarsen (5), p-arseno-phenylbutyric acid (6) and Stilbamidine (3) when administered in a single dose.

The fact that few guanidines related to p-phenylene diguanidine showed significant trypanocidal activity was rather surprising. Although all possible deviations in structure were not investigated, a sufficient number of related derivatives were tested to demonstrate that p-phenylene diguanidine *per se* probably possesses the maximum potentialities in this particular group of guanidines.

Chronic toxicity studies will be helpful for evaluating the potentialities of p-phenylene diguanidine for clinical use in man. Some studies have been under-

TABLE 6

Comparison of four guanidines by the multiple dose technique. (Series E-417)

(T. equiperdum *in mice*)

COMPOUND	DOSAGE	APPROXIMATE CURATIVE DOSE (CD_{50}) MGM./KGM.		APPROXIMATE LETHAL DOSE (LD_{50}) MGM./KGM.		CHEMOTHERAPEUTIC INDEX $\frac{LD_{50}}{CD_{50}}$	
		I.P.	Oral	I.P.	Oral	I.P.	Oral
p-Phenylene diguanidine	B.I.D. 3½ days	4.0	20.0	40.0	175.0	10	8.7
1,3-Bis(4-guanidophenyl) guanidine tri HCL	B.I.D. 3½ days	2.0	60.0	20.0	400.0	10	6.6
Bis-(4-guanidophenyl)-sulfide CO_2	B.I.D. 3½ days	2.0	20.0	50.0	175.0	25	8.7
3,3'-Dimethyl-4,4'-diguanide biphenyl	B.I.D. 3½ days	2.0	60.0	20.0	80	10	1.3

TABLE 7

Comparison of Stilbamidine, Bayer 205 and four guanidine derivatives. Multiple dose technique. (T. equiperdum *in mice*)

COMPOUND	APPROXIMATE SUPPRESSIVE DOSE (M.Tm.D.) MGM./KGM. B.I.D. FOR 3½ DAYS		APPROXIMATE CURATIVE DOSE (CD_{50}) MGM./KGM. B.I.D. FOR 3½ DAYS		RATIO OF I.P. TO ORAL TREATMENT	
	I.P.	Oral	I.P.	Oral	M.Tm.D.	CD_{50}
Stilbamidine	0.0175	1.6	0.025	3.0	1:91	1:120
Bayer 205	0.15	50.0	0.2	100.0	1:333	1:500
p-Phenylene diguanidine	2.0	10.0	4.0	20.0	1:5	1:5
1,3-Bis(4-guanidophenyl) guanidine Tri HCL	1.0	45.0	2.0	60.0	1:45	1:30
Bis-(4-guanidophenyl)-sulfide CO_2	1.0	10.0	2.0	20.0	1:10	1:10
3,3'-Dimethyl-4,4'-diguanide biphenyl	1.0	20.0	2.0	60.0	1:20	1:30

TABLE 8

Comparison of three arsenicals by the single dose technique

(T. equiperdum *in mice*)

COMPOUND	PARENTERAL CURATIVE DOSE (CD_{50})	PARENTERAL LETHAL DOSE (LD_{50})	CHEMOTHERAPEUTIC INDEX $\frac{LD_{50}}{CD_{50}}$
	mgm./kgm.	mgm./kgm.	
Tryparsamide	940*	3,750*	4
	1000†	4,000†	4
Melarsen	0.5†	17.5†	35
p-Arsenoso-phenylbutyric acid	1.6*	33*	21

* Eagle *et al.*, 1944.

† Weinman, 1946.