STUDIES ON THE ACTIVATION OF ORGANIC ARSENICAL COMPOUNDS (PART II)

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1. Introduction

Concerning the mode of action of organic arsenical compounds it has been considered, heretofore, that they invade SH-enzymes of the parasite and the human body tissue in conformity with the chemotherapeutic interference of SH compounds such as Glutathione and cysteine hydrochloride. However, a phenomenon which

Table 1
*The influence of various second components against the anti-trypanosoma
action and the toxicity of Oxo-HCl*

name of various second components	minimal effective dose (mg/kg)	maximal tolerative dose (mg/kg)
1. Oxo-HCl alone	2.08	33.3
2. Oxo-HCl+glucose	1.78	38.4
3. Oxo-HCl+vitamin B ₁	1.66	35.7
4. Oxo-HCl+vitamin B ₂	2.5	—
5. Oxo-HCl+vitamin B ₆	1.78	33.3
6. Oxo-HCl+vitamin C	1.56	38.4
7. Oxo-HCl+sodium thiosulfate	1.78	33.3
8. Oxo-HCl+glycine	1.78	35.7
9. Oxo-HCl+leucine	1.78	33.3
10. Oxo-HCl+cystine	1.78	33.3
11. Oxo-HCl+methionine	1.78	35.7
12. Oxo-HCl+taurine	2.5	—
13. Oxo-HCl+tyrosine	1.47	35.7
14. Oxo-HCl+histidine	1.66	35.7
15. Oxo-HCl+sodium glutamate	1.43	35.7
16. Oxo-HCl+tryptophan	1.38	35.7
17. Oxo-HCl+anthranilic acid	1.38	35.7
18. Oxo-HCl+benzoic acid	2.5	—
19. Oxo-HCl+salicylic acid	2.5	—
20. Oxo-HCl+p-aminobenzoic acid	1.78	35.7
21. Oxo-HCl+procaine	1.78	—
22. Oxo-HCl+tutocaine	1.78	—

contradicts these chemotherapeutic interference, namely, the existence of a substance which will increase the efficiency of organic arsenical compounds, has hardly been recongnized.

The author, therefore, in Part I of this report series⁽¹⁾, upon examining the effects of 21 substances against Oxophenarsine Hydrochloride (Oxo-HCl) and attaining the result as of table 1, has discovered that aromatic amino acid, especially tryptophan, anthranilic acid and sodium glutamate possess a nature which increases the anti-trypanosoma-action of Oxo-HCl.

In this report, the author, therefore, has again continued to examine protein, especially plasma protein fractions and observed its effects against the anti-trypanosoma-action and toxicity of Oxo-HCl, and moreover, has made obvious the inhibitory action of Oxo-HCl against plasma and red blood cell cholinesterase, and also made clear that the toxicity of Oxo-HCl is caused by this inhibitory action against cholinesterase.

Thus, the author has noted the anti-cholinesterase-action of anti-trypanosoma-action and the toxicity of Oxo-HCl, and has reached some new knowledge which will be given below:

2. Concerning the effect of plasma protein against the anti-trypanosoma-action and the toxicity of Oxo-HCl. Method of experiment:

For plasma, normal horse plasma was used, divided further into albumin, eu-globulin, and pseudo-globulin by using ammonium sulfate. This plasma and plasma protein fractions were mixed with Oxo-HCl and used. For animal, mice of 13-15 g in weight were used; for parasite, *Trypanosoma gambiense* was used. To decide its efficiency, the minimal effective dose was determined after 14 day's observation for the toxicity, minimal tolerative dose was decided after 4 days observation.

As shown in Table 2, plasma increases the efficiency of Oxo-HCl at times and decreases it at others. This is related to the quantity of plasma used: when small amount is used, it increases the efficiency of Oxo-HCl: when too much is used, it will, on the contrary, lower the efficiency of Oxo-HCl.

From this fact, it can be deduced that there exists in the plasma a fraction which increases the efficiency of Oxo-HCl and fractions which decrease it. The fraction which increases the efficiency of Oxo-HCl is eu-globulin and fractions which decrease it are albumin and pseudo-glodulin.

The efficiency-increasing action of eu-globulin upon Oxo-HCl is extremely

Table 2

The influence of plasma and plasma protein fractions against the anti-trypanosoma-action and the toxicity of Oxo-HCl

Plasma and plasma protein fraction		minimal effective dose	maximal tolerative dose
name	dose=mixed with 2.3 mg Oxo-HCl	mg/kg	mg/kg
1. Oxo-HCl alone		2.5 mg	33.3
2. Oxo-HCl+plasma	5.0 cc	5.0	
3. Oxo-HCl+plasma	1.0 cc	1.38	
4. Oxo-HCl+albumin	40.0 mg	10.0	
5. Oxo-HCl+pseudo-globulin	10.0 mg	5.0	
6. Oxo-HCl+eu-globulin	1.0 mg	1.11	
7. Oxo-HCl+eu-globulin	2.5 mg	2.5	
8. Oxo-HCl+eu-globulin	5.0 mg	2.5	40.0

Fig. 1. The time required to extinguish *Trypanosoma gambiense* from the blood stream of mice after the injection of Oxo-HCl alone or Oxo-HCl + eu-globulin.

strong and the time required to extinguish trypanosoma gambiense from the blood stream of mice after the injection of Oxo-HCl+eu-globulin is much shorter than Oxo-HCl alone (Fig. 1). The increasing action of eu-globulin upon the efficiency of Oxo-HCl requires some time after mixing for reaction: no marked activation can be observed immediately after mixing, 5 minutes after mixing or 10 minutes after mixing, but by leaving it to react for 15 minutes this action of eu-globulin can be observed obviously.

From this fact comes the deduction that a loose combination occurs between NH group of Oxo-HCl and separate COOH group of amino acids which construct protein.

The action of eu-globulin which lowers the toxicity of Oxo-HCl is slightly weak compared to the action which increases its efficiency and M.L.D. is reduced a little. As a result, the chemotherapeutic index of Oxo-HCl increases nearly three times by the addition of eu-globulin.

3. The inhibitory action of Oxo-HCl against blood cholinesterase and the toxicity decreasing action of anti-histamine drug against Oxo-HCl.

It has already been determined by Gordon and Quastel⁽²⁾ that Oxo-HCl is the inhibitor against cholinesterase, but the author has used human red blood cell cholinesterase for specific cholinesterase, and horse plasma or human plasma cholinesterase for nonspecific cholinesterase, and thereby examined the inhibitory action of Oxo-HCl.

Method of experiment:

The activity of red blood cell cholinesterase was measured according to Warburg's manometer⁽³⁾: the activity of plasma cholinesterase was measured by Holl and Lucas's method⁽⁴⁾. The effect of anti-histamine drugs and SH compounds against the anti-trypanosoma action and the toxicity of Oxo-HCl was determined as described above. Result of experiment:

The inhibitory action of Oxo-HCl against red blood cell cholinesterase does not show any exceptional action when 3 mg or 6 mg of Oxo-HCl is mixed with 2.0 cc of human red blood cell suspension, but when 12 mg is mixed, about 22%, and when 24 mg is mixed, up to 50% lowering of the activity may be determined. The inhibitory action of Oxo-HCl against plasma cholinesterase when 3 mg of Oxo-HCl is mixed with 2.0 cc of plasma is 17%, with 5 mg, 26%, with 10 mg, 27%, with 20 mg, 29%, with 30 mg, 35%, with 40 mg, 40%, lowering of the activity may be determined (Table 3).

From the above, it can be considered that Oxo-HCl shows some degree of

Table 3
The inhibitory action of Oxo-HCl against blood cholinesterase
 (A) *in vitro* test.

Cholinesterase	Dose of Oxo-HCl added to plasma (or RBC) 2.0 cc	activity of enzyme		inhibitory percent (%)
plasma cholinesterase	normal plasma alone	3.11 (cc)		
"	40.0 mg	1.94		48
"	30.0 mg	2.04		34.4
"	20.0 mg	2.22		28.9
"	10.0 mg	2.26		27.3
"	5.0 mg	2.29		26.3
red blood cell cholinesterase		normal test		
	24 mg	382 cmm	194 cmm	46
"	12 mg	354	274	22
"	6 mg	214	198	7.5
"	3 mg	364	364	0

(B) *in vivo* test (Rabbit)

	dose (mg/kg)	before injection	after injection							
			1-2 minute	5 min.	15 min.	30 min.	45 min.	60 min.	90 min.	120 min.
Oxo-HCl	16 mg	1.01 cc			0.64			0.66	0.56	0.57
"	"	0.97			0.69		0.59	0.61	0.63	0.63
"	"	0.99		0.69	0.66			0.66		0.64
"	"	0.87	0.51	0.40		0.45		0.45		
"	"	0.80	0.45	0.40						
Oxo-HCl	16 mg	0.78	0.67	0.73						
+ Cysteine 200 mg/kg	"	0.86		0.63	0.62					
"	"	0.99		0.88	0.85					
Oxo-HCl	16 mg	0.81	0.73	0.73	0.75		0.74			
+ Benadryl 4 mg/kg	"	0.81	0.74	0.74	0.77		0.76			
"	"	0.79	0.70	0.69	0.75		0.74			

inhibitory action against blood cholinesterase, and that not necessarily all, but perhaps a considerable part of the side reaction of Oxo-HCl is caused by the inhibition of cholinesterase.

Secondly, when testing the inhibitory action of Oxo-HCl against cholinesterase by mixing cysteine and Benadryl, it was found out that in the case of cysteine, the inhibitory action of Oxo-HCl against cholinesterase is contradicted and in the case of Benadryl this action of Oxo-HCl is contradicted seven more severely than with cysteine (Table 3). Therefore, it is inferable that the toxicity of Oxo-HCl can, by using anti-histamine drug, be lowered much more powerfully than by

using cysteine. The anti-histamine drugs used in this experiment were Benadryl and pyribenzamine and as SH compounds, BAL, Cysteine and glutathione were used. The dose of Benadryl and pyribenzamine is small compared to BAL, cysteine and Glutathione, yet the action which lowers the toxicity of Oxo-HCl is, in the case of both benadryl and pyribenzamine, matched with that of BAL and better than in the case of cysteine and glutathione (Table 4). Moreover, the efficiency of Oxo-HCl is markedly lowered by the mixture of SH compounds, whereas with anti-histamine drug, although it lowers the efficiency of Oxo-HCl to some extent, when compared with the former by chemotherapeutic index, ultimately, it can be said that anti-histamine drug is superior to SH compounds as the toxicity reducing drug.

Table 4
The influence of anti-histamine drug and SH compound against the anti-trypanosoma action and the toxicity of Oxo-HCl

name of second components	dose (mg/kg)	minimal effective dose (mg/kg)	maximal tolerative dose (mg/kg)
1. Oxo-HCl alone		2.5	33.3
2. Oxo-HCl+BAL	40	10.0	50.0
3. Oxo-HCl+Cysteine	200	2.5	40.0
4. Oxo-HCl+Glutathione	100	5.0	40.0
5. Oxo-HCl+Benadryl	4.0	5.0	50.0
6. Oxo-HCl+Pyribenzamine	3.0	2.5	50.0

4. Summary

(1) By studying the action of plasma against Oxo-HCl, it has been discovered that plasma increases or decreases its efficiency according to the quantity used.

(2) This fact allows the presumption that there exists, in plasma protein fraction, a part which increases its efficiency and parts which decrease its efficiency. This efficiency increasing part is eu-Globulin; the efficiency decreasing parts are albumin and pseudoglobulin.

(3) With the addition of eu-globulin the efficiency of Oxo-HCl is increased, the toxicity is decreased slightly, and the chemotherapeutic index grows 3 times as that of Oxo-HCl alone.

(4) This action of eu-globulin requires some time for reaction after mixing. From this fact it is inferable that a compound is found by a loose combination between Oxo-HCl and the separate acid group of the amino acid constructing eu-globuline.

(5) Oxo-HCl obstructs red blood cell cholinesterase, and also inhibits plasma cholinesterase. From this fact, it is deduced that one cause for side reaction of Oxo-HCl might lie in the inhibitory action against cholinesterase.

(6) When cysteine or Benadryl is mixed with Oxo-HCl, the inhibitory action against cholinesterase is decreased. From this, it is thought that anti-histamine drug possesses an action which markedly lowers the toxicity of Oxo-HCl.

(7) The action of anti-histamine drug (Benadryl and pyribenzamine) which lowers the toxicity of Oxo-HCl is superior to that of cysteine and glutathione, and is equal to that of BAL. And, as the action which lowers its efficiency is less than that of SH compounds, the author thinks, the anti-histamine drug are to be given proper attention as the toxicity reducing drug against Oxo-HCl.

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