Ann. Soc. belge Méd. trop.
1983, 63, 319-324

TRYPANOSOMA CRUZI: A PHARMACOLOGICAL COMPARISON OF SOME BOLIVIAN ISOENZYMIC STRAINS

by

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Summary — The drug susceptibility of 3 isoenzymic strains of *Trypanosoma cruzi* was studied. Two drugs (SQ 18, 506 and Ro 7-1051) and 6 stocks were used. The drug test was carried out on epimastigote cultures. Significant susceptibility differences were found for Ro 7-1051 between the 3 strains. For SQ 18, 506, there were differences between strains 1 and 2, and 2 and 3, but no differences between 1 and 3. Strain 2 seemed more sensitive to SQ 18,506 than to Ro 7-1051. For strain 1, the opposite was observed. There was no difference of susceptibility for the two drugs in the case of strain 3. A great intrastrain variability was observed, and the interstrain differences were not clearly proportional to the genetic distances Nevertheless, drug susceptibility was correlated in some degree to the biochemical classification.

KEYWORDS : *Trypanosoma cruzi*; Isoenzymic; Pharmacological Comparison.

Introduction

Drug resistance variability among different *Trypanosoma cruzi* strains has been proved on several occasions (Brener & Chiari, 1967; Brener *et al.*, 1976; Andrade & Figueira, 1977; Avila *et al.*, 1981; Cover & Gutteridge, 1981). However, these different strains were not related to any zoological classification.

The presence of distinct isoenzymic strains of *T. cruzi* was established by Miles *et al.* (1977). Ready & Miles (1980) proposed a numerical taxonomy of *T. cruzi* zymodemes, based on an intuitive interpretation of the zymograms. Genetic interpretation of the zymograms (Tibayrenc *et al.*, 1981 a & b) allowed a classification based on the use of genetic distances : average number of codon differences per gene between two populations (Nei, 1972; Tibayrenc, 1980). On these theorical bases, two main strains were observed in Bolivia (Tibayrenc & Desjeux, 1983), as well as some lesser strains (Tibayrenc *et al.*, 1983).

In this work, we compared drug susceptibility for 3 isoenzymic strains encountered in Bolivia.

Material and Methods

— Origin of the stocks :

We initially used at least two different stocks for each strain. But after the end of experiments, we observed by electrophoresis that stock C24 (theorically strain 1) had been mixed accidentally with laboratory reference

strain Tulahuen, which is closely related to strain 2. So we did not take in account the results concerning this stock and strain 1 was represented by only one stock. Table 1 shows the origin of the six *T. cruzi* stocks which were finally used. Matrix 1 indicates their classification by genetic distances (Tibayrenc *et al.*, 1983). All the stocks, isolated from *Triatoma infestans* (Tibayrenc *et al.*, 1982) were adapted by weekly passage in GLSH monophasic culture medium, at 28 °C (Le Ray, 1974), during at least two months prior to the experiments. We used only one cloned stock (SC43cl1) but Tibayrenc & Miles (1983) showed the genetic homogeneity of the uncloned stocks used in the present work.

MATRIX 1

Genetic distances among the strains studied (Tibayrenc *et al.*, 1983), estimated on 12 enzymic loci.

	Strain 1	Strain 2
Strain 2	1.71	0
Strain 3	1.77	0.41

TABLE 1

Origin of the stocks

Isoenzymic strain :	Stock (laboratory code) :	Place :
Strain 1	C37	Chiwisivi
Strain 2	C50	Chiwisivi
	Y5	Yungas
	SC43cl1	Santa Cruz
Strain 3	TU15	Tupiza
	TU18	Tupiza

Chiwisivi : a river valley at 60 km SE La Paz. Altitude 2700 m.
Yungas : Subtropical valleys at 100 km E La Paz. Altitude 1600 m.
Santa Cruz : (suburb Cotoca) Subtropical town at 400 km SE La Paz. Altitute 400 m.
Tupiza : A river valley at 1000 k m S La Paz. Altitude 2700 m.

— Drugs used :

a) 5-nitrofuran (trans-5-amino-3-(2-(5-nitro-2-furyl) vinyl) 1,2,4 oxadiazole; SQ 18, 506), from the SQIBB Institute for Medical Research, Princeton, New Jersey, U. S. A.

b) 2-nitroimidazole (N-benzly-2-nitro-1-imidazole acetamine); Ro 7-1051 benzimidazole; Rochagan®; Ragonil®; from ROCHE Laboratories, Buenos Aires, Argentina.

— Drug test :

Fresh media were inoculated with 8 days old epimastigote forms in order to obtain the same final parasite concentration for an endvolume of 10 ml. The drugs, sterilized by filtration (Millipore®, 0.22 μm Ø pores) were immediately added in 50 μl of DMSO (dimethyl sulfoxide), to a final concentration of 1 $\mu g.ml^{-1}$ for SQ 18, 506, and 15 $\mu g.ml^{-1}$ for RO 7-1051 according to Baker & Selden (1981). A control was made for each test : two inoculations were made at day 0, one with the drug, one without the drug, with DMSO. Two trials at least (see table 2) were made for each stock and

each drug. Preliminary experiments had shown that the differences of drug susceptibility between the stocks were more pronounced from day 5 to day 7. So we used a comparison at day 6. The living parasites were diluted in Hanks Wallace isotonic solution and then counted in a haemocytometer at a 400x magnification. In order to estimate the drug effect, we used the following ratio (see table 2) : $\frac{\text{control concentration of living epimastigotes}}{\text{test concentration of living epimastigotes}}$.

It was homogeneous (mean of 0.09 ± 0.07) for all the tests at day 0. The initial concentration of living epimastigotes was 2.81 ± 0.54.10^6 parasites.ml^{-1} for all the tests.

TABLE 2

Results obtained at day 6. Drug susceptibility for all stocks studied, with the two drugs used.

		With Ro 7-1051 at 15 µg.ml^{-1}			With SQ 18,506 at 1 µg.ml^{-1}		
Isoenzymic strain	Stock	Ratio (1)	Stock mean	Strain mean	Ratio (1)	Stock mean	Strain mean
Strain 1	C37	15.24	15.73 ± 3.09 (2)	15.73 ± 3.09	5.07	5.60 ± 0.74	5.60 ± 0.74
	C37	16.96			6.12		
	C37	11.70			—		
	C37	19.00			—		
Strain 2	C50	2.71	2.46 ± 0.35	3.13 ± 1.26	18.00	17.65 ± 0.49	12.62 ± 4.73
	C50	2.21			17.30		
	Y5	1.99	2.20 ± 0.30		6.77	7.88 ± 0.97	
	Y5	2.41			8.38		
		—			8.50		
	SC43cl1	4.75	4.74 ± 0.02		13.00	14.70 ± 2.40	
	SC43cl1	4.72			16.40		
Strain 3	TU15	9.89	9.85 ± 0.06	8.23 ± 1.87	7.53	5.62 ± 1.85	4.77 ± 1.74
	TU15	9.80			5.48		
	TU15	—			3.84		
	TU18	6.73	6.61 ± 0.18		3.49	3.51 ± 0.03	
	TU18	6.48			3.53		

(1) Ratio : $\frac{\text{Control concentration of living epimastigotes}}{\text{Test concentration of living epimastigotes}}$, on day 6.

(2) Standard deviation.

Results and Discussion

The results are summarized in tables 2 and 3. Table 2 shows the results obtained at day 6.

1) *Comparisons interstrain, intradrug*

For Ro 7-1051, we used the statistical distribution-free test of Mann & Whitney : there were significant differences between the 3 strains. For SQ 18,506, we used the same test to compare strains 2 and 3, which were significantly different. To compare strain 1 with the 2 others, we had to use the T test, the variances being not significantly different, and supposing that the distributions were normal : strains 1 and 2 showed significant differences ($T = 6.55$, $ddl = 7$) but this was not the case for strains 1 and 3 ($T = 0.73$; $ddl = 5$). These differences are correlated in some degree to the biochemical classification. However, they are not clearly proportional to the genetic distance. This may be explained by the fact that the two phe-

nomena have not the same genetic determinism : drug resistance (which is problably an adaptative character) is perhaps under the dependance of regulatory genes (Ninio, 1979; Tibayrenc *et al.*, 1983). On the other hand, biochemical taxonomy is based on the study of protein loci, often considered as being selectively neutral (Wilson *et al.*, 1977).

TABLE 3

Comparison for drug sensibility interstock, intradrug

(= : no significant différence; ≠ : significant difference)

Ro 7-1051 (drug 1)

	C37	C50	Y5	SC43	TU15
C50	≠	—			
Y5	≠	=	—		
SC43	≠	=	=	—	
TU15	≠	≠	≠	≠	—
TU18	≠	=	≠	=	=

SQ 18.506 (drug 2)

	C37	C50	Y5	SC43	TU15
C50	≠	—			
Y5	=	≠	—		
SC43	≠	≠	=	—	
TU15	=	=	≠	≠	—
TU18	=	≠	≠	≠	=

2) *Comparisons interstock, intradrug*

We used the variance analysis, which showed that the whole population of the stocks was not homogeneous for drug resistance considering each drug. Then we used for individual comparisons the T test, the variance being estimated on the whole range of stocks. The results are summarized in table 3. They show clearly in some cases an intrastrain variability. For example, within strain 2, there is a variability for drug 2. We did not observe any variability within strain 3 for the 2 drugs.

3) *Comparisons intrastrain, interdrug*

For strains 2 and 3, we used the distribution-free test of Mann & Whitney. Strain 3 had the same comportment for the 2 drugs. Strain 2 was more sensible to SQ 18,506 than to Ro 7-1051. For strain 1, we used the T test under the same conditions than in paragraph 1 : this strain appeared more sensible to Ro 7-1051 than to SQ 18,506 ($T = 4.32$, ddl $= 4$).

4) *Comparisons intrastock, interdrug*

We had to use an individual T test under the same conditions than in paragraph 1. Nevertheless, the variances showed significant differences for stocks SC43cl1 ($F'_1 = 12844$) and TU15 ($F'_1 = 844$). We observed differences of resistance for both drugs in all the stocks, except in stock TU15. For exemple, stock C50 was much more sensible to SQ 18,506 than to Ro 7-1051 (see table 2).

Conclusion

Biochemical taxonomy is useful to be taken into account for a pharmacological study, but it is not sufficient to foresee all the results, for the following reasons :

— lack of clear proportionality between pharmacological sensitivity and genetic distances;
— great intrastrain variability.

Nevertheless, we were able to see that drug resistance is correlated in some degree to the biochemical classification of *T. cruzi* : as a matter of fact, there were significant differences between the isoenzymic strains.

Further data are required with a greater number of stocks for each strain, and above all, with animal experiments.

The study of the genetic background of drug resistance constitutes in itself an original way of research, which should be given greater importance in the future.

Acknocledgments — *This work was supported by the French Ministry of Foreign Affairs and the Direction Générale à la Recherche Scientifique et Technique (Grant nº PVD/81.L-1423). We are grateful to Doctor Michael A. Miles (London School of Tropical Medecine and Hygiene), who cloned kindly SC43 stock.*

Trypanosoma cruzi : **une comparaison pharmacologique de quelques souches isoenzymatiques boliviennes.**

Résumé — La sensibilité médicamenteuse de 3 souches isoenzymatiques de *Trypanosoma cruzi* a été étudiée. Deux drogues (SQ 18,506 et Ro 7-1051) et 6 isolats ont été utilisés. Le test pharmacologique a été effectué sur cultures d'épimastigotes. On a observé des différences significatives entre les 3 souches pour le RO-7-1051. Pour le SQ 18,506 des différences ont été vues entre les souches 1 et 2, et 2 et 3, mais pas entre les souches 1 et 3. La souche 2 est apparue plus sensible au SQ 18,506 qu'au Ro-7-1051. La souche 1 a montré la situation inverse. La souche 3 n'a pas montré de différence de susceptibilité pour les 2 drogues. On a observé une grande variabilité intrasouche, et les différences intersouches n'ont pas montré de proportionalité nette avec les distances génétiques. Cependant, la susceptibilité médicamenteuse était corrélée dans une certaine mesure à la classification biochimique.

Trypanosoma cruzi : **een farmacologische vergelijking van enkele Boliviaanse isoenzymatische stammen.**

Samenvatting — De medicamenteuse gevoeligheid van drie isoenzymatische *T. cruzi* stammen werd bestudeerd. Twee preparaten (SQ 18,506 en Ro 7-1051) en zes isolaten werden daartoe aangewend. De farmacologische test werd uitgevoerd op epimastigote kulturen. Tussen de drie stammen werden significante verschillen vastgesteld voor Ro 7-1051. Voor SQ 18,506 werden verschillen waargenomen tussen stammen 1 en 2, en 2 en 3, doch niet tussen de stammen 1 en 3. Stam 2 bleek gevoeliger voor SQ 18,506 dan voor Ro 7-1051. Voor stam 1 was dit omgekeerd. Stam 3 vertoonde geen verschil in gevoeligheid voor beide preparaten. Een grote intrastam variabiliteit werd vastgesteld en de interstam verschillen waren niet duidelijk proportioneel t.o.v. de genetische afstanden. Niettemin was de medicamenteuse gevoeligheid in een bepaalde mate gecorreleerd aan de biochemische klassifikatie.

Received for publication on June 15, 1983.

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