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CL 64,855, A POTENT ANTI-*TRYPANOSOMA CRUZI* DRUG, IS ALSO MUTAGENIC IN THE *SALMONELLA*/MICROSOME ASSAY

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The nitroimidazole-tiadiazole derivative CL 64,855 (2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole, a potent anti-trypanosomal drug, was assayed in a short-term bacterial mutagenicity test with Salmonella typhimurium *strains TA 98, TA 100 and TA 102. Results indicate that CL 64,855 is a potent frameshift mutagen detected by strains TA 98 and TA 102. CL 64,855 was able to revert the indicators strains at concentrations as low as 0.1 μg/plate. Metabolic activation experiments with rat liver microsomal fractions did not increase the mutagenic action of CL 64,855.*

Key words: Ames test – anti-trypanosomal drug – mutagenesis

The nitroimidazole derivative CL 64,855 is a potent broad spectrum antimicrobial agent acting on a variety of bacteria and parasites (Berkelhammer & Asato, 1968; Burden & Recette, 1968; Burden, Shumacker & Kelly, 1968). Recently, Filardi & Brener (1982, 1984) reported a marked curative action of CL 64,855 on experimental infections with several strains of *Trypanosoma cruzi*.

In this paper we report the mutagenic effects of this drug in the most widely accepted and experimentally standardized short-term bacterial test, the *Salmonella* (Ames) assay (Ames, McCann & Yamasaki, 1975). Employment of specialized plasmid-harbouring genetically constructed *S. typhimurium* strains has allowed the identification of a great number of mutagens and carcinogens (McCann et al., 1975). The results obtained with this test revealed that CL 64,855 is a strong frameshift mutagen.

MATERIALS AND METHODS

Bacterial strains: *S. typhimurium* indicator strains used were TA 98, TA 100, and TA 102 (Table I). All strains harbour pKM 101, a plasmid which increases the susceptibility to mutagenesis (McCann et al., 1975). TA 102 is a new tester strain developed to detect a wide range of frameshift and oxidative mutagens (Levin et al., 1982). TA 98 and TA 100 detect, preferentially, frameshift and base pair substitution mutagens, respectively.

Drug used: Stock solutions (1 mg/ml) of CL 64,855 [2-amino-5-(1-methyl-5-nitro-2-imidazol)-1,3,4-thiadiazole] were prepared by dissolving the drug in dimethylsulfoxide. Further dilutions were made using the same solvent.

Mutagenicity assays: The procedure of Ames, McCann & Yamasaki (1975) was followed. Qualitative experiments (spot tests) were done with small drops containing 10μl of a 1 mg/ml solution of CL 64,855 applied on the surface of selective plates without histidine. His^+ revertants were scored after 48 hours incubation at 37°C. Quantitative experiments (dose-effect curves) were done on selectives plates with different concentrations of the drug mixed in the overlay agar.

Rat liver microsomal fractions (S9 mix) were prepared as described elsewhere (submitted to publication).

RESULTS

Table II shows the mutagenic effect of CL 64,855 on the indicator strains in qualitative experiments. CL 64,855 was able to induce mutations only with the frameshift indicator strains TA 98 and TA 102 (Fig. 1). The base pair substitution mutagen tester strain, TA 100, was not reverted by the drug even in the presence of metabolic activation supplied by the S9 mix (Table II).

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TABLE I

Bacterial tester strains used in this work

Strain	Genotype	Source
TA 98	his D3052 rfa ΔuvrB bio/pKM 101	B.N. Ames
TA 100	his G46 rfa ΔuvrB bio/pKM 101	B.N. Ames
TA 102	his Δ(G) 8476 galE 503 rfa/pA Q/pKM 101	B.N. Ames

TABLE II

Mutagenic activity of CL 64,855 on the indicator strains TA 98, TA 100, and TA 102 in qualitative spot test experiments

Strains	Induction of His^+ mutants	
	– S9	+ S9
TA 98	+	+
TA 100	–	–
TA 102	+	+

– S9 = without S9 mix; + S9 = with S9 mix

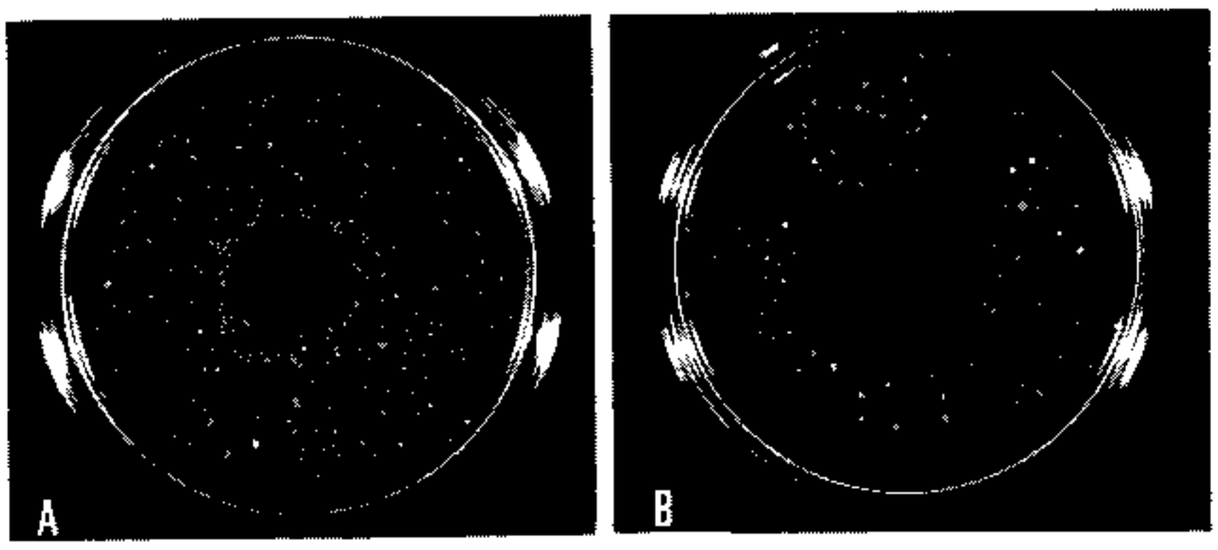

Fig. 1: Qualitative (spot test) plates indicating the mutagenic action of CL 64,855 on the frameshift *S. typhimurium* tester strains. A – TA 102 indicator strain; B – TA 98 indicator strain.

Fig. 2 illustrates the dose-effect curve of CL 64,855 with the *S. typhimurium* strains TA 98 and TA 102. Concentrations as low as 0.1 μg/plate can be easily detected as mutagenically active in quantitative assays. TA 102 proved to be the most sensitive strain, at least 60% more sensitive than TA 98 as determined by the number of revertants per plate at the tested concentration range of CL 64,855. TA 102 was also more resistant to the lethal action of the drug than TA 98, an excision repair deficient strain (data not shown).

Metabolic activation experiments were performed with the strains TA 98 and TA 102 (Table III). Aflatoxin B1, a potent indirect mutagen and carcinogen which is activated by mammalian microsomal fractions, was used as control. The mutagenic activity of aflatoxin B1 was detected only with TA 98 tester strain. A slight increase in the number of revertants per plate was usually observed in plates treated with S9 mix. This effect can be attributed to a slight mutagenic action of S9 mix even in the absence of any added mutagen (Peak, Dornfeld & Venters, 1982).

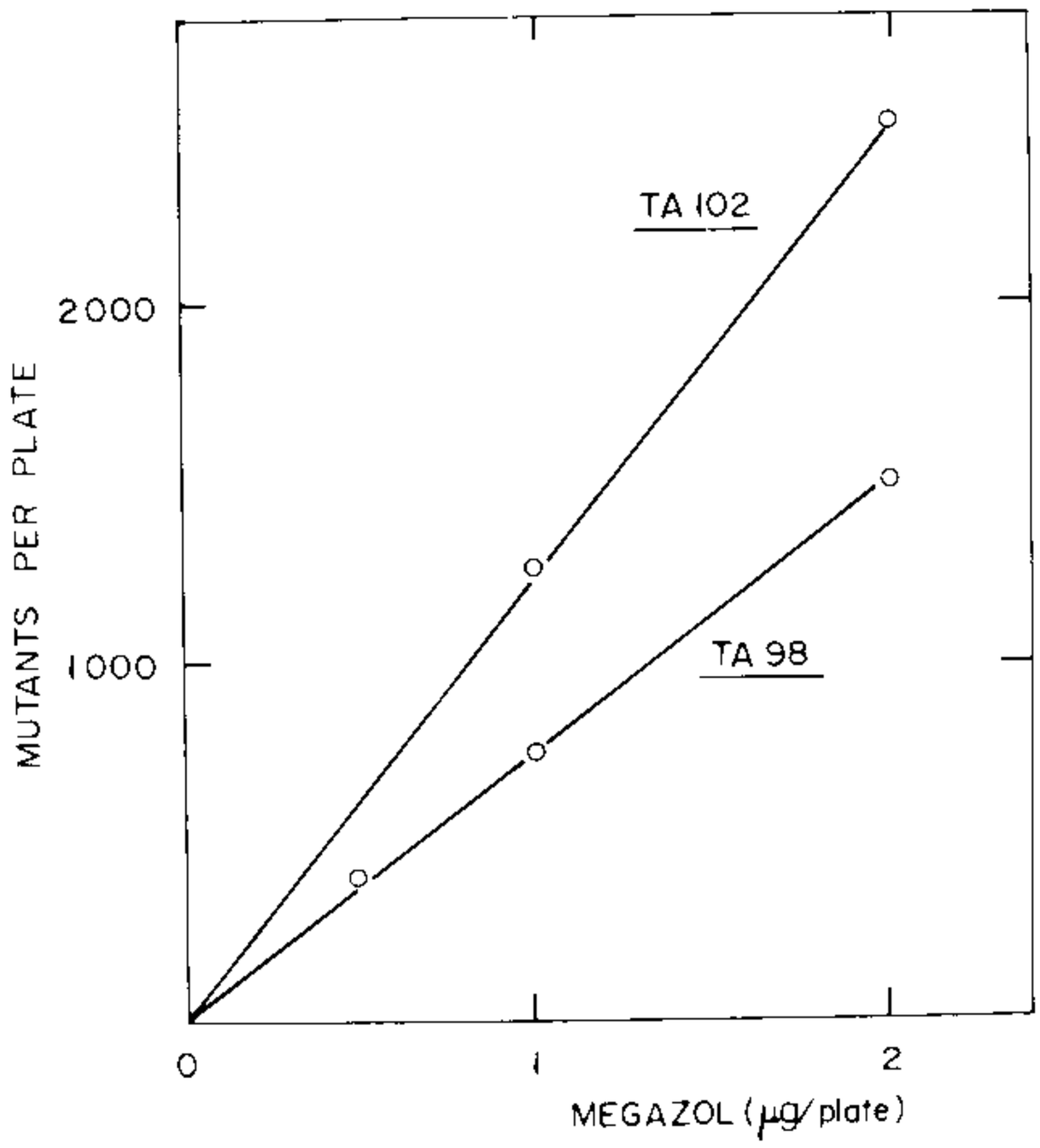

Fig. 2: Qualitative assay of the direct mutagenic activity of CL 64,855 on *S. typhimurium* tester strains TA 98 and TA 102.

TABLE III

Metabolic activation experiments mediated by S9 mix on mutagenicity of CL 64,855

Drug	Amount (µg/plate)	S9*	Strain	
			TA 102	TA 98
CL 64,855	--	–	340	45
	–	+	385	70
	0.2	–	580	220
	0.2	+	620	260
	0.5	-	1.100	490
	0.5	+	1.230	570
Aflatoxin B1	6	–	358	50
	6	+	350	1.810

*S9 mix present (+) or not ( )

DISCUSSION

The *Salmonella*/liver microsome test for mutagenicity is in current use in over 3.000 government, industrial, and academic laboratories throughout the world and it is the most reliable short-term test for detecting potential carcinogens (Ames, 1984). In this work we applied this powerful test to a nitroimidazole-thiadiazole derivative with curative action in experimental

T. cruzi infections (Filardi & Brener, 1982). Our results showed that CL 64,855 is a potent and specific mutagen to two out of three indicator strains used. Using specialized genetically constructed indicator strains it was possible to ascertain that CL 64,855 has a rather specific frameshift mutation induction ability. This result is in sharp contrast with our previous observations about the genetic activity of benznidazole and nifurtimox, widely used anti-trypanosomal drugs. Both drugs have specific base pair substitution mutation induction ability (unpublished observations). These data could mean that CL 64,855 and the other two anti-trypanosomal drugs exert their mutagenic actions through different DNA interaction pathways.

It could be concluded from our results that CL 64,855 is a direct mutagen, i.e., a mutagen that exerts its genetic effects without mediation by mammalian enzymes. No difference were observed in the number of His^+ revertants between selective plates treated or untreated with S9 mix. However, like other nitroderivatives it is also possible that CL 64,855 could be reduced by bacterial nitroreductases before being able to induce mutations in DNA (Rosenkranz & Speck, 1976). This possibility is now under current investigation.

In view of the high correlation index between mutagenicity and carcinogenicity in the Ames assay it would seem reasonable to submit CL 64,855 as well other anti-trypanosomal drugs to rigorous control to evaluate their possible carcinogenic side effects.

RESUMO

O derivado nitroimidazole-tiadiazol CL 64.855 (2-amino-5-(1-metil-5-nitro-2-imidazoli)-1, 3, 4-tiadiazol), um potente agente tripanomicida, foi submetido a um ensaio mutagênico bacteriano com as linhagens indicadoras de *Salmonella typhimurium* TA 98, TA 100 e TA 102. Os resultados indicaram que o CL 64.855 é um potente mutagênico tipo troca de referencial detectado pelas linhagens TA 98 e TA 102. O CL 64.855 foi capaz de reverter as linhagens indicadoras em concentrações tão baixas quanto 0,1 μg/placa. Ativação metabólica com frações microssomais de fígado de rato foram incapazes de aumentar a ação mutagênica do CL 64.855.

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