Rev. Inst. Med. trop. São Paulo
32 (4): 269-274, julho-agosto, 1990

SCREENING THE MUTAGENIC ACTIVITIES OF COMMONLY USED ANTIPARASITE DRUGS BY THE SIMULTEST, A SIMPLIFIED Salmonella/MICROSOME PLATE INCORPORATION ASSAY[1]

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SUMMARY

The mutagenic activities of 16 anti-parasite drugs were screened by the Simultest in both qualitative (spot test) and quantitative (plate incorporation) assays with a **Salmonella typhimurium** pool composed by the indicator strains TA97, TA 98, TA100 and TA102. Four anti Chagas' disease drugs (nifurtimox, benznidazole, CL 64,855, and MK 436) and two anti-amebae drugs (metronidazole and tinidazole) gave positive results in qualitative tests and incorporation of rat liver microsomes did not alter the results. Comparative dose response curves of the mutagenic activities of CL 64,855, metronidazole and benznidazole obtained by the simultest and by individual **Salmonella** indicator strains demonstrated that both approachs have similar sensitivities. The results corroborate the validity of the Simultest, as a simplified, fast and economic version of the Ames test in preliminary screening of potential mutagenic drugs.

KEY WORDS: Simultest; Anti-parasite drugs; Ames test.

INTRODUCTION

The high incidence in tropical countries of parasitic diseases as amebiasis, leishmaniosis, Chagas' disease, filariasis, schistosomiasis, malaria and helminthiasis, leads to an enormous consumption of a broad variety of anti-parasite drugs, sometimes used even for preventive purposes (NETO & BALDY, 1989).

Many papers described the mutagenic activities of several anti-parasite drugs of human use as metronidazole, nifurtimox and benznidazole in bacterial short-term tests as the Ames test (EBRINGER & BENCOVA, 1980; FERREIRA & FERREIRA, 1986; SPECK et al., 1986). The evidence of considerable positive correlation between mutagenic activity in the Ames test and carcinogenic effects in mammals emphasizes the relevance of testing the genotoxic activities of the anti-parasite drugs of human use (AMES, 1989; McCANN et al., 1975).

The Simultest was recently developed by NESTMANN et al. (1987) as a simplified version of the **Salmonella** mutagenicity test where the

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indicator strains are used in combination in the same plate. This approach proved to reduce considerably the workload associated with mutagenic screenings of compounds using several isolated **Salmonella** strains (AMES et al., 1975).

This study was performed to evaluate the usefulness and sensitivity of the Simultest as a fast and simplified approach to screening the mutagenic activities of several anti-parasite drugs commonly used in Brazil, without the need of testing every chemical with each of the recommended standard indicator **Salmonella** strain.

MATERIALS AND METHODS

Bacterial strains

The **Salmonella typhimurium** indicator strains TA97, TA98, TA100 and TA102 were used throught. All strains have been previously described (McCANN el al., 1975a; MARON & AMES, 1983).

Chemicals tested

All sixteen anti-parasite drugs analysed in this work are listed on Table 1. The following drugs were obtained at drugstores under their commercial formulations: Ascaridil (Janssen); Facyl (Campinas Chemical Laboratories); Fasigyn (Pfizer); Falmanox (Winthrop); Flagyl (Rhodia); Rochagan (Roche); Lampit (Bayer); Helmindrax (Haller); Octelmin (Schering); Panfugan (BYK); and Zentel (SmithKline). Oxamniquine and diethylcarbamazine were obtained at CEME (Central de Medicamentos) and Farmanguinhos (Fundação Oswaldo Cruz), respectively. CL 64,855 and MK 436, two drugs successfully used in the treatment of experimental Chagas' disease were kindly donated by Dr. Z. Brener and Dr. Z. Andrade, respectively (FILARDI et al., 1982; ANDRADE et al., 1987). All compounds were dissolved in water except for CL 64,855 and MK 436 which were solubilized in dimethyl sulfoxide. Aflatoxin B1 was purchased from Sigma.

Mutagenic assays

The **Salmonella** plate incorporation assay

TABLE 1
List of drugs used in this work.

Name	Active Compound	Major Application
Ascaridil	2,3,5,6,-tetrahidro-6-phenylimidazol-[2,1-b] thiazole	Ascaridiasis
Cloroquina (chloroquine)	7-chloro-4-(4-diethyl-amino-1-methyl-butylamino-quinolone diphosphate	Malaria
Dietilcarbamazina (diethylcarbamazine)	citrate 1-diethylcarbamoyl-4-methyl-piperazine dihydrogen citrate	Filariasis
Facyl and Fasigyn (tinidazole)	1-[2-(ethyl-sulfonyl)-ethyl)-2-methyl-5-nitro-imidazole	Amebiasis
Falmonox (teclosan)	N,N'(p-phenylendime-thylene)-bio(2,2-dicloro-N-dicloro-N (2-etoxiethyl) acetamide	Amebiasis
Flagyl (metronidazole)	(1-2(hydroxiethyl)-2-methyl-5-nitronidazole	Amebiasis
C1 64.855	2-(amino-5(1-methyl-5-nitro 2-imidazolyl)-1,3,4-thiadiazole.	Chagas' Disease
Lampit (nifurtimox)	(4-(5-nitro-furfurilidene-amino)-3-methyl-thiomorpholine,1,1-dioxide	Chagas' Disease
Rochagan (benznidazole)	N-benzyl-2-nitro-imidazole-1-acetamide)	Chagas' Disease
MK436	3-(1-methyl-5-nitro-imidazol-2yl)3a,4,5,6,7,7a-hexahydro-1,2-benzidazole	Chagas' Disease
Helmindrax Octelmin (thiabendazole and mebendazole)	2(4'thiazolyl)-benznidazole	Helminthiasis
Panfugan (mebendazole)	methyl-[5(6)-benzoyl-2-benzmidazolyl] carbamate	Schistosomiasis
Oxamniquine	6-Quinolinemethanol,1,2,3,4-tetrahydro-2-[[(1-methylethyl) amino] methyl]-7-nitro-6-quinolinemethanol	Schistosomiasis
Zentel (albendazole)	methyl-5-(propyl-thio)1-H-benzmidazole-2-yl) carbamate	Helminthiasis

MELO, M. E. B. & FERREIRA, L. C. S. — Screening the mutagenic activities of commonly used anti-parasite drugs by the simultest, a simplified **Salmonella**/microsome plate incorporation assay. **Rev. Inst. Med. trop. S. Paulo, 32(4)**: 269-274, 1990.

was performed with each of the indicator strains as decribed by MARON & AMES (1982). The qualitative assays (spot tests) were carried out by careful placing 10 μl drops containing either 10,50 or 100 μg of each drug on plates previously poured and seeded with one of the indicator strains.

The Simultest was performed essentially as described by NESTMANN et al. (1987). Overnight cultures of the four tester strains were mixed throughly in equal amounts in a sterile tube. Aliquots of 100 μl of the strain pool were added to 2 ml of molten top agar kept at 45°C. After addition of the tested compound the tubes were poured in selective plates without histidine (MARON & AMES, 1983) and incubated for 48 hr at 37°C. Whenever necessary 250 μl of a 10% S9 mixture prepared with Aroclor-activated rat liver extracts, were incorporated to the plates. Qualitative assays were performed as described above for the standard **S. thyphimurium** assay except by the use of the strain pool instead of a single indicator strain. Data presented are the average of two experiments in which duplicates plates were used. Mutagenic activity was determined by counting the number of induced revertants colonies for histidine auxotrophy (His^+).

RESULTS

Seven out of sixteen anti-parasites drugs tested in spot test with the Simultest gave positive mutagenic results (Table 2). All four anti-Chagas' disease drugs were detected as mutagens. Two anti-amoeba drugs, metronidazole (Flagyl) and tinidazole (Facyl and Fasigyn), were also able to induce the reversion of histidine prototrophic mutants (His^+) in the **Salmonella** strain pool. Addition of S9 mixture to the mutagenic assays did not altered the results.

Three mutagenic anti-parasite drugs, CL 64,855; benznidazole and metronidazole were further analised in quantitative mutagenic assays. Comparison of the Simultest and the standard assay with the most responsive **Salmonella** strain, determined by considering the fold increase of mutation frequency over the spontaneous background reversion, showed that both procedures can detect the mutagenic activities of the drugs as a linear dose response curve (Figures 1, 2 and 3). When metronidazole was used the strain pool gave a lower number of His^+ revertantes per plate than the TA100 strain, the most responsive indicator strain for this compound (Figure 1). The mutagenic activity of CL 64,855 detected by the simultest was intermediary to results obtained with the TA102 and TA98 strains, two frameshift mutation indicator strains (Figure 2). Finally, in the case of benznidazole, the Simultest was slightly less sensitive than the responsive strain TA100 in the concentration range tested (Figure 3).

TABLE 2
Mutagenic activity of the anti-parasite drugs tested with the Ames test and the Simultest in qualitative assays (spot test).

Compounds	MUTAGENIC ACTIVITY[a]			
	Strain[b]		Pool[c]	
	−S9	+S9	−S9	+S9
Ascaridil	—	—	—	—
Chloroquine	—	—	—	—
Dietilcarbamazine	—	—	—	—
Facyl	+	+	+	+
Fasygin	+	+	+	+
Flagyl	+	+	+	+
Falmonox	—	—	—	—
CL 64,855	+	+	+	+
Oxamniquine	—	—	—	—
Pantelmim	—	—	—	—
Helmindrax	—	—	—	—
Panfugan	—	—	—	—
Rochagan	+	+	+	+
MK 436	+	+	+	+
Zentel	—	—	—	—
Aflatoxin B1[d]	—	+	—	+

a — mutagenic activities were evaluated by the presence (+) or not (−) of His^+ colonies around the point of drug deposition on seletive plates.
b — isolated indicator strain (TA97, TA98, TA100, TA102).
c — pool of the four indicator strains.
d — amount used: 1 μg/plate.

DISCUSSION

Detection of genetoxic activity of drugs was considerably improved by the introduction of the Ames Test. At least 3,000 research and industrial laboratories are currently using the **Salmonella** histidine reversion assay all over the world (AMES, 1984). The most important attributes of this test are its simplicity, rapidity and low cost compared to other approachs as animal testing and mammalian cell cultures (MARON &

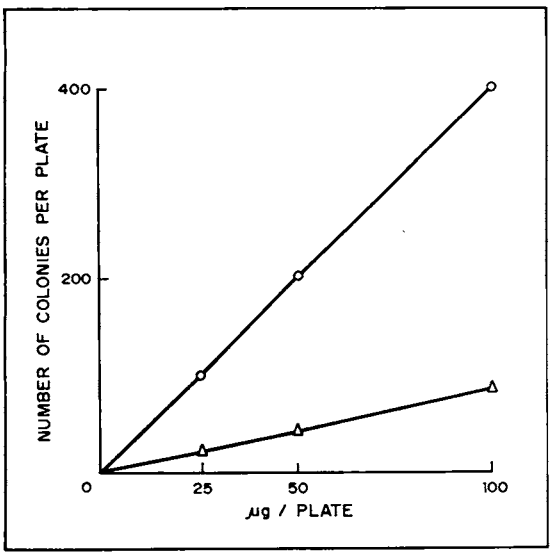

Fig. 1 — Dose response curve of metronidazole in plate incorporation assays. The mutagenic activities were based on the number of His ' colonies per plate determined by the TA100 strain (O) and the strain pool (Δ) without addition of S9 mix.

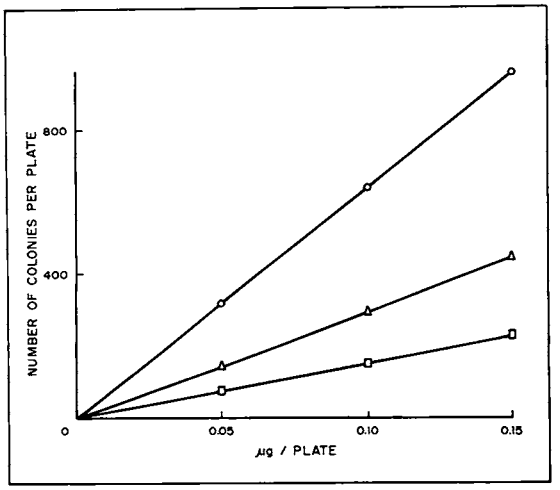

Fig. 2 — Dose response curve of CL 64.855 in plate incorporation assays. The mutagenic activities were based on the number of His ' colonies per plate determined by the TA102 strain (O), TA98 strain (□) and the strain pool (Δ) without addition of S9 mix.

AMES, 1984). Nonetheless, the workload and costs of screening programs with the **Salmonella** test turned out to be still elevated especially for third world laboratories.

ZIEGER et al. (1985) suggested a more cost-efficient strategy for identifying mutages. This approach involved initial testing of all chemicals

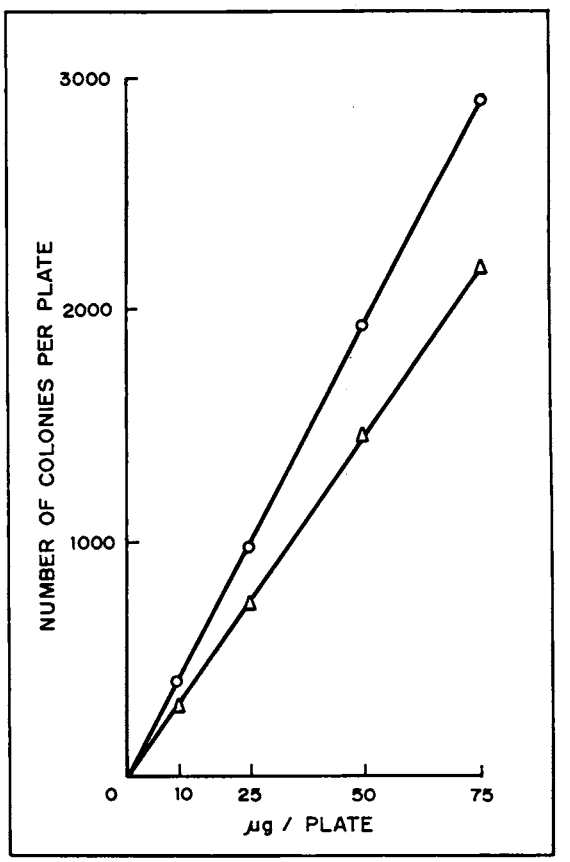

Fig. 3 — Dose response curve of benznidazole in plate incorporation assays. The mutagenic activities were based on the number of His ' colonies per plate determined by the TA100 strain (O) and the strain pool (Δ) without addition of S9 mix.

with the most sensitive strain for the highest number of mutagens, as TA100 or TA102, followed by a second and possibly a third test using successively less effective strains. This strategy can result in a 25% reduction in the final costs when compared to the conventional approach where the four strains and three activation system (hamster liver, rat liver, and no activation) are used in combination. On the other hand, screening programs performed with the Simultest using the plasmid-containing strain mixture can reduce the initial costs of the enterprise to only 20% of the standard procedure (NESTMANN et al., 1987).

One possible drawback on the use of the Simultest is the impossibility to distinguish between mechanisms of mutagenesis, for example, distinction between frameshift or base change

MELO, M. E. B. & FERREIRA, L. C. S. — Screening the mutagenic activities of commonly used anti-parasite drugs by the simultest, a simplified **Salmonella**/microsome plate incorporation assay. **Rev. Inst. Med. trop. S. Paulo, 32(4)**: 269-274, 1990.

mutagens. However, in most mutagen screening programs the most important result searched is the incidence of mutagenic compounds. Once a mutagen is detected with the Simultest, individual strains can be employed to identify the specific responsive strain.

The detection of mutagenic activities in anti-parasite drugs of human consumption sheds a considerable concern to the health conditions of the users. Recent reports demonstrated that several anti-parasite drugs of general human use can induce genetic damages detected by microbial systems (ESPINOSA-AGUIRRE et al., 1987; FERREIRA & FERREIRA, 1986; HARTMAN et al., 1975). In some cases, mutagenic metabolities are produced after reaction with ingested nitrites and/or nitrates, sheding additional concerns on the use of these drugs (ARRIAGA ALBA et al., 1988). When several possibilities for the quimiotherapeutic treatment of a parasitary disease are available, it would be advisable to adopt those without any mutagenic history. On the other hand, treatment of illnesses as the Chagas' disease offer no possibility for a therapeutic choice since all available alternatives, presently reduced to only benznidazole, nifurtimox and two promissing experimental drugs (MK 436 and CL 64,855) are mutagens. In these cases, a further effort on the search for new quimiotherapeutic alternatives are strongly recommended.

RESUMO

Rastreamento de atividade mutagênica de drogas anti-parasitárias de uso comum pelo Simultest, uma versão simplificada do teste Salmonella/**fração microssomal**

As atividades mutagênicas de 16 drogas com ação anti-parasitária foram avaliadas pelo Simultest em ensaios qualitativos (spot testes) e quantitativos (incorporação em placa) com uma mistura das linhagens indicadoras de **Salmonella typhimurium** TA97, TA98, TA100 e TA102. Quatro drogas anti-doença de Chagas (nifurtimox, benzonidazol, CL 64,855 e MK 436) e duas drogas anti-amebiase (metronidazol e tinidazol) deram resultados positivos em testes qualitativos e a incorporação de fração microssomal de fígado de rato não alterou os resultados. Curvas comparadas de efeito da dose da atividade mutagênica do metronidazol, benzonidazol e CL 64,855 detectadas pelo Simultest e linhagens indicadoras individuais demonstraram que as duas abordagens possuem sensibilidades semelhantes. Os resultados corroboram a validade do Simultest como uma versão simplificada, rápida e econômica do teste de Ames no rastreamento preliminar de drogas potencialmente mutagênicas.

ACKNOWLEDGMENTS

We acknowledge the excelent technical assistence of Mr. S. H. Almeida and Dr. Ames and Dr. Rosenkranz for the bacterial indicator strains.

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Recebido para publicação em 23/10/1989