Evaluation of a MicroAmes Screen that is Fully Predictive for the Regulatory Salmonella/E. Coli Mammalian Microsome Mutagenicity Test

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Abstract

In the pharmaceutical industry there is a need for rapid genotoxicity screens that mimic regulatory assays. Predictive screens are key for assessing genotoxic risk of low-yield isolated impurities, and are important to ensure positive genotoxic responses are not found during regulatory testing, after considerable time and resources have been allocated to a drug candidate. A unique miniaturized rapid throughput version of the Ames test has been developed that requires only 10 mg. The strength of this microAmes screen is the use of regulatory Ames tester strains and classical endpoints (colonies, cytotoxicity, precipitates). To assess the robustness of this screen, 20 compounds comprising diverse mechanisms and structures, many related to pharmaceuticals, were tested with 4 regulatory accepted tester strains of Salmonella (TA97a, TA98, TA100, TA1535) and one E. coli strain (WP2uvrApKM101). Positive and negative controls were used and included compounds previously shown to yield borderline results in the full Ames test. The microAmes screen correctly predicted Ames mutagenicity in all cases and correctly predicted Ames non-mutagens except for 2 controversial compounds, oxazepam and menadione which yielded positive responses in the microAmes. Menadione was positive in WP2 without S9, while oxazepam was positive in several strains with S9 but only at concentrations where precipitates were present. In the full Ames test, menadione displays small increases in WP2 colony formation (<2 fold), whereas strong elevations are seen in the WP2-OxyR deficient strain, a variant sensitive to oxidative mutagens. Although oxazepam is negative in the full Ames test, this compound is a known in vivo mutagen, in vitro clastogen, and carcinogen. These results indicate that the microAmes not only accurately predicts the full-plate Ames assay, but is sensitive enough to detect known mutagenic compounds. The microAmes screen is therefore an extremely valuable predictive tool that can be used with high confidence to evaluate drug candidates and impurities for genotoxicity.

Introduction

The microAmes screen uses an agar format in multi-well plates and is a modification of the standard Salmonella/E. coli mammalian microsome assay (Ames et al. 1975/1981, Brusick et al. 1980, Maron and Ames 1983, Watanabe et al. 1998). As per the "full-plate" regulatory Ames assay, this screen uses an agar format, evaluates precipitation and cytotoxicity, and uses an equivalent top concentration (ICH and OECD guidelines). Because this is a "micronized" Ames assay, compound requirements are low (approx 10 mg) and turnaround time reduced (report within 2 weeks of receiving test article).

Methods

Each tester strain was preincubated both with and without an Aroclor-induced rat liver S9 fraction in duplicate with 6 concentrations ranging from 0.25 to 250 µg/well using various classes of compounds with diverse structures and mechanisms relevant to the pharmaceutical industry. The maximum concentration approximates the bacteria to compound ratio of the maximum recommended concentration in the full Ames test. Four concentrations of each positive control mutagen and multiple replicates of vehicle (DMSO) controls per multiwell plate were used.

MicroAmes results were compared to full plate Ames results obtained from NTP genetox database (Zeiger et al. or, in the case of missing or noncomparable data, from published literature and/or from in house data using the "full plate" preincubation assay. For comparisons, positive criteria for both microAmes and full plate Ames were 2-fold over vehicle control for TA97a (or TA97), TA100 and WP2uvrApKM101 (or WP2uvrA); and 3-fold over vehicle control for TA98, TA1535, and TA1537.

Results and Discussion

The microAmes was concordant with the full Ames test using known and weak mutagens with structures common to the pharmaceutical industry (Figure 1). Two compounds worthy of discussion are menadione which is positive in the microAmes, weakly positive in the full Ames test, and strongly mutagenic in the WP2-OxyR deficient strain which is a variant sensitive to oxidative mutagens (Urios et al. 1995). Oxazepam was positive in several strains with S9 but only at concentrations where precipitates were present. Oxazepam has been reported negative in the full Ames test; however, this compound is a known, in vitro clastogen, in vivo mutagen, and carcinogen (Giri and Banerjee 1996).

Conclusion

The microAmes screen not only accurately predicts the full-plate Ames assay, but is sensitive enough to detect known mutagenic compounds that are weakly positive in the full Ames and are known clastogens. The microAmes screen is therefore an extremely valuable predictive tool that can be used with high confidence to evaluate drug candidates and impurities for genotoxicity using minimal compound and with fast turnaround.

References

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Brusick, D.J., Simmon, V.F., Rosenkranz, H.S., Ray, V.A., and Stafford, R.S. An evaluation of the Escherichia coli WP2 and WP2 uvrA reverse mutation assay. Mutation Research 76:169 190, 1980.

Maron, D.M. and Ames, B.N. Revised methods for the Salmonella mutagenicity test. Mutation Research 113:173 215, 1983.

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Urios A, Herrera G, and Blanco M. Detection of oxidative mutagens in strains of Escherichia coli deficient in the OxyR or MutY functions: Dependence on SOS mutagenesis. Mutat Res. 332:9-15, 1995.

Giri AK and Banerjee S.Genetic toxicology of four commonly used benzodiazepines: a review. Mutat Res. 340:93-108, 1996

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Figure 1
Summary of MicroAmes Results (Comparison to Full Plate Ames Test)

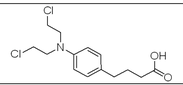
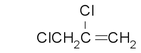
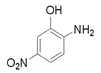
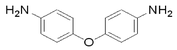
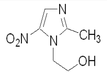
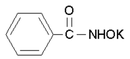
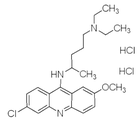
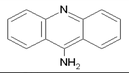
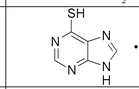
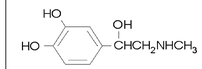
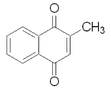
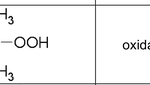
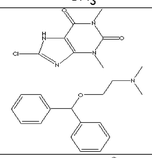
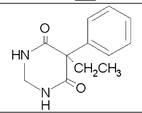
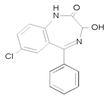
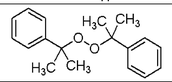
Compound Tested	Structure	Category	Micro-Ames	Full Plate Ames
Chlorambucil		alkylating	(+)	(+)
2,3-Dichloro-1-propene		alkylating	(+)	(+)
2-Amino-5-nitrophenol		adduct	(+)	(+)
4,4'-Oxydianiline		adduct	(+)	(+)
Metronidazole		adduct	(+)	(+)
Benzohydroxamic acid		adduct	(+)	(+)
Quinacrine		intercalating	(+)	(+)
9-Aminoacridine		intercalating	(+)	(+)
6-Mercaptopurine		inhibits DNA synthesis	(+)	(+)
DL-Epinephrine		oxidative	(+)	(+)
Menadione		oxidative (known mutagen)	(+)	(+)

Figure 1 (Cont)
Summary of MicroAmes Results (Comparison to Full Plate Ames Test)

Compound Tested	Structure	Category	Micro-Ames	Full Plate Ames
t-Butyl hydroperoxide		oxidative	(+)	(+)
Dimenhydrinate		pharmaceutical	(-)	(-)
Primidone		pharmaceutical	(+)	(+)
Oxazepam		pharmaceutical (clastogen, *in vivo* mutagen, carcinogen)	(+)	(-)
Diazepam (structurally similar to oxazepam)		pharmaceutical	(-)	(-)
Salicylamide (structurally similar to benzohydroxamic acid)		pharmaceutical	(-)	(-)
Allopurinol (structurally similar to 6-mercaptopurine)		pharmaceutical	(-)	(-)
Dicumyl peroxide (negative control for t-butyl hydroperoxide)		oxidative (negative control)	(-)	(-)
Dimethylsulfoxide		solvent (negative control)	(-)	(-)