#2276. Use of Miniaturized Bacterial Mutagenicity Assays for Drug Candidate Screening and Impurity Evaluation

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Abstract

Mutagenicity evaluation is required by regulatory agencies prior to first in human trials and/or pharmaceutical marketing. Early screening of drug candidates for mutagenic potential is beneficial to reduce the risk of major hazards before clinical trials. The 5- and 24- well plate Ames assays require significantly less amount of test article and are similar to the plate incorporation method using full plates. The purpose of this validation study is to demonstrate a comparable sensitivity of the miniaturized versions to the full plate assay. A total of 10 known Ames negative (anthenic, M-ethylmercapturic and L-methionine) and positive (2-aminofluorene, benzaldehyde, 7,12-dimethylbenz(a)anthracene, ICR-191, 2-nitrofuran, 4-nitroquinoline-N-oxide, sodium azide) compounds as well as two pharmaceutical-related starting materials/impurities (1-H-pyrazole-4-boronic acid and 4-methylcarboniliphosphonic acid) were used in the validation studies. The Ames assay was performed simultaneously using full, 6-well and 24-well plates for each validation test article in the presence and absence of rat liver induced metabolic activation system. Overall study conclusions were compared between the miniaturized formats and the full plate assay. Additionally, a comparison of the miniaturized assays was evaluated by comparing outcome from each test strain/test condition and also by comparing corresponding test compound concentrations between 6- and 24-well plates formats with the full plate assay results. Comparison of study data showed that both miniaturized formats correctly predict the mutagenicity outcome of all test compounds in the full plate assay and with a high concordance (~95%). The results indicate that the miniaturized formats provide an opportunity to conduct evaluation of drug candidates and drug impurities for mutagenic potential at an earlier stage in discovery and with a reduced compound requirement.

Introduction

Testing for genotoxic activity of new chemical entities is used as an early surrogate for carcinogenic potential. Testing for mutagenicity is required by regulatory agencies to conduct first in human trials and/or pharmaceutical marketing. To reduce mutagenicity-related late stage attrition, early screening is beneficial to maximize identification with minimal resources. The 5- and 24-well plate formats are miniaturized versions of the standard plate Ames assay that require significantly less compound and are similar to the standard plate incorporation Ames assay. The plating is performed onto wells of 24- or 6-well plate containing approximately 1.5 or 5 mL of Vogel-Bonner agar (compared to a standard full plate containing 20-25 mL of agar) respectively. The amounts of bacterial cell culture, 50-μL or buffer solution, and test article are reduced 5- and 20-fold from that employed for a standard plate assay, respectively. The reduced compound requirement for the miniaturized formats allows mutagenic evaluation of NCEs in early discovery phase. In case of a positive outcome with a lead candidate, additional structurally related compounds can be evaluated to assist medicinal chemistry efforts. These formats can also be deployed during early discovery phases for compounds with structural mutagenicity concern identified by expert chemists and/or in alkyl structure-activity-relationship programs. The purpose of the validation study was to assess the comparability of the miniaturized versions to known Ames negative and positive compounds to that of the standard full plate assay.

Materials and Methods

The Ames assay was conducted simultaneously using standard plates (90 mm), 6-well plates and 24-well plates for each validation compound with and without metabolic activation (S9) system. Six tester strains (TA100, TA102, TA98, TA1537, WP2uvrA and WP2uvrA[pKM101]) were tested in the 24-well plate format, whereas seven strains (TA100, TA98, TA102, TA1537, WP2uvrA and WP2uvrA[pKM101]) were evaluated in the 6-well and standard plate formats. TA1537 was excluded from the 24-well plate Ames assay due to low spontaneous revertant counts in this strain and was replaced with TA97a tester strain. Viable and positive controls were included with each study. Twelve, four and two replicates of vehicle control were included in the 24, 6-well and standard plate assay, respectively. Positive controls and test article concentrations were plated in triplicate for 24-well plate and in duplicate for 6-well and standard plate formats.

Results

90-mm vs 24-Well Plates, ICR-191, TA97a, No S9

- 2-fold
- 5- and 60-fold

- 50% vs 24 Well Plates
- 24 Well Plates

- 6-well vs 90-mm plates

- 6-well vs 90-mm plates

Summary and Conclusions

The 24- and 6-well format Ames assay correctly predicts the overall outcome in the standard 90-mm plate Ames assay.

Conclusions

Our findings from the validation study with 10 compounds indicate:

- The 24- and 6-well format Ames assay can be used with high confidence to evaluate drug candidates for genotoxicity with low compound requirements.
- The miniaturized Ames formats can also be used with high confidence to evaluate drug impurities for mutagenic potential.