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Review

Bacterial mutagenicity screening in the pharmaceutical industry

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ABSTRACT

Genetic toxicity testing is used as an early surrogate for carcinogenicity testing. Genetic toxicity testing is also required by regulatory agencies to be conducted prior to initiation of first in human clinical trials and subsequent marketing for most small molecule pharmaceutical compounds. To reduce the chances of advancing mutagenic pharmaceutical candidates through the drug discovery and development processes, companies have focused on developing testing strategies to maximize hazard identification while minimizing resource expenditure due to late stage attrition. With a large number of testing options, consensus has not been reached on the best mutagenicity platform to use or on the best time to use a specific test to aid in the selection of drug candidates for development. Most companies use a process in which compounds are initially screened for mutagenicity early in drug development using tests that require only a few milligrams of compound and then follow those studies up with a more robust mutagenicity test prior to selecting a compound for full development.

This review summarizes the current applications of bacterial mutagenicity assays utilized by pharmaceutical companies in early and late discovery programs. The initial impetus for this review was derived from a workshop on bacterial mutagenicity screening in the pharmaceutical industry presented at the 40th Annual Environmental Mutagen Society Meeting held in St. Louis, MO in October, 2009. However, included in this review are succinct summaries of use and interpretation of genetic toxicity assays, several mutagenicity assays that were not presented at the meeting, and updates to testing strategies resulting in current state-of the art description of best practices. In addition, here we discuss the advantages and liabilities of many broadly used mutagenicity screening platforms and strategies used by pharmaceutical companies. The sensitivity and specificity of these early mutagenicity screening assays using proprietary compounds and their concordance (predictivity) with the regulatory bacterial mutation test are discussed.

Contents

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1. Introduction

In the pharmaceutical industry, genetic toxicity testing is used as an early alternate for carcinogenicity testing. Regulatory agencies require genetic toxicity testing is conducted prior to initiation of first in human (FIH) clinical trials and subsequent marketing for most small molecule compounds. A compound's potential to induce genotoxic damage is usually evaluated using a 3- or 4-test battery with individual tests detecting specific endpoints indicative of DNA damage. A typical standard test battery consists of a gene mutation assay conducted in bacteria, an *in vitro* mammalian chromosome damage test, and an *in vivo* test for structural and/or numerical chromosome damage, all conducted in compliance with Good Laboratory Practices (GLPs) [1]. Bacterial mutagenicity tests are widely used in the pharmaceutical industry during drug discovery as part of a compound selection strategy. The *Salmonella*-reverse-mutation assay or Ames test is the gold standard for mutagenicity testing and has been shown to be the most predictive *in vitro* assay for rodent and human carcinogenicity [1,2].

1.1. Standard bacterial reverse mutation tests (Ames assays) for drug development

The Ames assay was developed by Bruce Ames and colleagues in the mid 1970s [2] and has been subsequently revised over the years to improve sensitivity to many types of mutagens [3–5]. This assay serves as an important initial assay to determine a compound's mutagenic potential. Multiple strains of *Salmonella typhimurium* and *Escherichia coli* have been created that carry specific distinct mutations in either the histidine or tryptophan synthetic pathway, respectively, that result in the requirement for an exogenous supply of those amino acids for growth (auxotrophy). In a typical regulatory-compliant assay [5] these bacterial strains are grown in agar-filled 100 mm Petri dishes along with the compound under investigation. Compounds that are mutagenic lead to the reversion of the mutation back to the wild type such that exogenous amino acids are no longer required for growth. These revertant colonies are enumerated on agar Petri dishes after a growth period of 48–72 h [2]. The test compound is considered mutagenic, or "positive" in the assay, if the fold-increase in revertant colonies in test compound-treated dishes exceeds typically 2 to 3-fold that of vehicle-treated controls or, less commonly, if the increase is statistically significant. The bacteria used in the test are engineered to be highly sensitive to a variety of mutagens through reduction of DNA repair capability and enhanced cell wall permeability to test articles. This assay also has the ability to determine the molecular nature of mutations by employing tester strains carrying base-pair substitutions or frameshift mutations. A large proportion of compounds require metabolic activation to the ultimate mutagenic form. In these cases, a variety of exogenous metabolic activation sources are built into the standard test design (for example, rat or hamster Aroclor-induced liver S9). Typically, only phase I metabolism is simulated