Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 1, 2012

Research Article

COMPATIBILITY OF DIFFERENT SOLVENTS WITH SALMONELLA TYPHIMURIUM MUTANT STRAINS IN BACTERIAL REVERSE MUTATION ASSAY

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Received: 24 Aug 2011, Revised and Accepted: 16 Nov 2011

ABSTRACT

Ames bacterial reverse mutation assay is one of the basic screening tools to evaluate the mutagenicity of chemicals. The solubility of test compounds plays a major role in deciding the highest concentration for the Ames assay. The selection of a solvent for new chemical entities, which is compatible with *Salmonella typhimurium,* poses a challenging task for genetic toxicologists in pharma industries. We have selected Dimethyl sulfoxide, absolute ethanol, acetone and acetonitrile to evaluate its compatibility with Salmonella as compared to water using plate incorporation method. Observations in the present study revealed that all these solvents were found to be compatible as indicated by no significant changes in the bacterial revertatnt colony counts and hence can be continued as vehicles in Ames bacterial reverse mutation assay.

Keywords: Ames assay, Compatibility, *Salmonella typhimurium*, Solvent

INTRODUCTION

The Salmonella Ames assay is one of the most widely used short-term basic regulatory tests to assess the mutagenic potential of new chemical entities[1]. The solubility of the chemicals plays a very pivotal role in deciding the concentration for the Ames mutagenicity assay. Poorly soluble or water insoluble drugs are often a challenging task for formulators in the pharma industry. Apart from universal solvent (water), there are many organic solvents like DMSO, ethanol, acetone acetonitrile, etc which can be used as a vehicle in Ames assay[1]. Very few literatures depict the compatibility of the various solvents in Ames salmonella reverse mutation assay. By considering the lack of literature, the present experiment was planned to assess the compatibility of different solvents (DMSO, absolute ethanol, acetone and acetonitrile) with salmonella mutant strains.

MATERIALS AND METHODS

The *Salmonella typhimurium* tester strains TA97a and TA98 used in the present study were procured from Bruce Ames laboratory, USA. All the chemicals, reagents and positive controls used in the present study were obtained from sigma Aldrich, USA. Rat liver S9 fraction was prepared in house from male Sprague Dawley rats.

The method used in present study followed as per the recommendations of Maron and Ames (1983)[2] and OECD guideline 471 (1997)[3]. Briefly, bacterial culture (100µL) was exposed to different solvents (100µL; Water, absolute ethanol, DMSO, acetone and acetonitrile) both in presence (500 µL 5 % S9) and absence (500 µL of phosphate buffer) of metabolic activation systems by plate incorporation method. The plates were incubated at 37 ^{0}C for 72 hours and revertant colonies were counted manually. The data was evaluated and interpreted biologically and no statistical analysis was performed.

RESULTS & DISCUSSION

In general, in neither of the test compounds, no significant changes (increase or decrease) in the mean revertant colony counts were observed in both the tester strains (TA97a and TA98) as compared to negative control (water) in presence and absence of metabolic activation system. The revertant colony counts for different solvents are shown in the table 1.

Table 1: Bacterial reverse mutation assay using *Salmonella typhimurium*: *his$^+$* revertant colony counts

Without metabolic activation system																	
Strain / Revertant colony counts per plate		**Water**			**DMSO**			**Absolute ethanol**			**Acetone**			**Acetonitrile**			
TA97a	Individual Plate Count (n=3)	136	129	135	128	116	147	125	149	125	128	123	132	158	122	141	
	Mean ± SD	133.33 ± 3.79			130.33 ± 15.63			133.00 ± 13.86			127.67 ± 4.51			140.33 ± 18.01			
TA98	Individual Plate Count (n=3)	17	17	21	17	25	18	23	12	19	29	21	23	25	27	21	
	Mean ± SD	18.33 ± 2.31			20.00 ± 4.36			18.00 ± 5.57			24.33 ± 4.16			24.33 ± 3.06			
With metabolic activation system (5 % S9 v/v)																	
Strain / Revertant colony counts per plate		Water			DMSO			Absolute ethanol			Acetone			Acetonitrile			
TA97a	Individual Plate Count (n=3)	135	130	141	135	130	129	140	135	132	140	106	136	141	138	130	
	Mean ± SD	135.33 ± 5.51			131.33 ± 3.21			135.67 ± 4.04			127.33 ± 18.58			136.33 ± 5.69			
TA98	Individual Plate Count (n=3)	31	28	26	31	24	28	26	24	26	29	24	26	25	27	25	
	Mean ± SD	28.33 ± 2.52			27.67 ± 3.51			25.33 ± 1.15			26.33 ± 2.52			25.67 ± 1.15			

In the present study, ethanol was found to be non-mutagenic, non-cytotoxic and proved to be a compatible solvent with Salmonella bacteria. Our findings were found similar to that obtained by other authors [4,5]. Oxidative metabolite (acetaldehyde) of ethanol is a proven mutagenic and carcinogenic in mammalian cells, but not in bacterial system as evinced by negative mutagenicity results in Salmonella and E.coli bacterial reverse mutation assays[6] and our findings were also in concordance with their findings. In mammalian cells, acetaldehyde induces genotoxicity by DNA-DNA and DNA-protein cross linking mechanism. In the present study, the absence of mutagenicity of ethanol even in the presence of metabolic activation system containing *alcohol dehydrogenase* essential for oxidation of ethanol might be attributed to the lack of formation of DNA cross linking unlike in mammalian cells.

DMSO being used as most common solvent after water in many laboratories since long time[7]. According to earlier published reports, DMSO could be used as alternate solvent when chemicals do not dissolve in water[1]. But DMSO had induced mutagenicity in TA1537 and TA2637 strains of *Salmonella typhimurium* both in the absence

and in the presence of rat liver S9 after 20 minutes of pre-incubation and plating[8]. But in the present study, DMSO (Di-methyl sulfoxide) has not induced mutagenicity both in the presence and absence of metabolic activation system and can be continued as vehicle in Ames bacterial reverse mutation assay.

Acetone was found to be negative for reverse mutations at 100 µL /plate. Results obtained here were analogous to the earlier published findings [9, 10].

Acetonitrile has not induced any mutagenicity in TA97a and TA98 tester strains tested at 100 µL /plate concentration which justified the findings of earlier works [11, 12].

Hence, it can be concluded that all the solvents used in the present study can be continued as alternative vehicle after water as they were found to be compatible with *Salmonella typhimurium* tester strains depending on the solubility of test compounds.

REFERENCE

1. Mortelmans K, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. Mutat Res 2000; 455: 29-60.
2. Maron DM, Ames BN. Revised Methods for the Salmonella Mutagenicity Test. Mutat Res 1983; 113: 173-215.
3. Bacterial Reverse Mutation Test. OECD guideline for testing of chemicals 471, 1997.
4. McCann J, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proc Natl Acad Sci U S A 1975; 72 (12): 5135–5139.
5. Hellmer L, Bolcsfoldi G. An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay II. In vivo results for 36 compounds tested in the mouse. Mutation Research 1992; 272 (2): 161-173.
6. Phillips BJ, Jenkinson P. Is ethanol genotoxic? A review of the published data. Mutagenesis 2001; 16 (2): 91-101.
7. Paramesh M, Niranjan MS, Sarfaraj Niazi, Shivaraja S, Rubbani MS. Synthesis and antimicrobial study of some of chlorine containing chalcones. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2 (2): 113-117.
8. Hakura A, Mochida H, Yamatsu K. Dimethyl sulfoxide (DMSO) is mutagenic for bacterial mutagenicity tester strains. Mutat Res 1993; 303: 127–133.
9. Kier LD, Brusick DJ, Auletta AE, Von Halle ES, Brown MM, Simmon VF, Dunkel V, McCann J, Mortelmans K, et al. The Salmonella typhimurium/mammalian microsomal assay. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 1986; 168: 69-240.
10. De Flora S, Zanacchi P, Camoirano A, Bennicelli C, Badolati GS. Genotoxic activity and potency of 135 compounds in the Ames reversion test and in bacterial DNA-repair test. Mutat. Res 1984; 133: 161-198.
11. Schlegelmilch R, Krug A, Wolf HU. Mutagenic activity of acetonitrile and fumaronitrile three short term assays with special reference to autoinduction. J Appl Toxicol 1988; 8: 201-209.
12. Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E. Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environ. Mutagen 1986. 8 (Suppl 7): 56-119.