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BACTERIAL MUTAGENESIS AND HOST CELL DNA DAMAGE BY CHEMICAL CARCINOGENS IN THE SALMONELLA/HEPATOCYTE SYSTEM

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SUMMARY

Coincubation of isolated and intact rat hepatocytes and Salmonella typhimurium, (Salmonella/hepatocyte system) strain TA 98 was employed to determine both bacterial mutagenicity and DNA damage in the hepatocytes as measured by alkaline elution, following treatment with 2-acetylaminofluorene (AAF), 2-aminofluorene (AF) and N-hydroxy-2-acetylaminofluorene (N-OH-AAF). Both the mutagenicity and the rate of DNA elution were dose-dependent for all three compounds. N-OH-AAF was 5 times more mutagenic and caused 80-100 times more DNA damage in the hepatocytes than AAF and AF when compared on a molar basis. The Salmonella/hepatocyte system may provide a more comprehensive evaluation of the potential genotoxic effect of chemicals than the currently used microbial mutagenesis sytems.

INTRODUCTION

The Salmonella (Ames) mutagenesis system is among the most widely used short-term tests for mutagenicity and potential carcinogenicity of chemicals (1-3). Since most chemical carcinogens require metabolic activation before their mutagenic and/or carcinogenic potential is expressed, it is necessary to incorporate into the assay the enzyme systems that are capable of activating these compounds. This is most commonly achieved by coincubating the bacteria and the compound with subcellular liver fractions (S-9, microsomes, etc.) from rodents (1). However, this combination does not, in most instances, allow for the detoxification processes that act upon chemicals in the intact organism, and therefore does not reflect the balance between metabolic acti-

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vation and detoxification that ultimately determines the toxic effect in vivo. In order to redress the balance between metabolic activation and detoxification of chemicals in the microbial mutagenesis sytems, several investigators (4,5), including ourselves (6), have employed isolated and intact hepatocytes as a source of metabolic activation (Salmonella/hepatocyte system). In this system the mutagenic species derived from the promutagen and/or procarcinogen must escape the detoxification processes of the hepatocyte in order to mutate the bacteria. It is, however, likely that these reactive metabolites would cause toxicity, including genotoxicity, in the hepatocytes. Also, the more reactive chemical species that are generated via metabolism might not escape from the cell, and would, in spite of the detoxification processes, exert their toxic effect solely on the hepatocyte. We have, therefore, concomitantly determined in the Salmonella/ hepatocyte system both the mutation frequency in the bacteria and the genotoxic effect in the hepatocyte, as measured by DNA alkaline elution technique (7,8), after exposure to the known carcinogens 2-acetylaminofluorene (AAF), 2-aminofluorene (AF) and N-hydroxy-2-acetylaminofluorene (N-OH-AAF).

MATERIALS AND METHODS

AAF and AF were obtained from Eastman Organic Chemicals Co. (Rochester, N.Y.); collagenase (C65II; 136 units/mg) was purchased from Worthington Biochemical Corp (Freehold, N.J.); Salmonella tester strain TA 98, and N-OH-AAF were generous gifts of Dr. Bruce N. Ames, University of California Berkeley, Berkeley, Calif. and Dr. Elizabeth K. Weisburger, National Cancer Institute, Bethesda, Md., respectively. Male Sprague-Dawley rats (180-200 g) were provided by the National Institutes of Health Animal Supply and maintained as previously described (9). The isolation of hepatocytes and the mutagenesis assay were performed as previously described (6). The alkaline elution of DNA was measured according to Kohn et al (7,8), and the DNA was quantitated, after the alkaline elution, by the fluorometric method of Erickson et al (10).

RESULTS AND DISCUSSION

The incubation of rat hepatocytes with the tester strains derived from Salmonella typhimurium in the presence of promutagens, can be used to detect the formation and the release of the mutagens into the incubation medium. Fig. 1 shows the mutation frequency of histidine independent revertants after

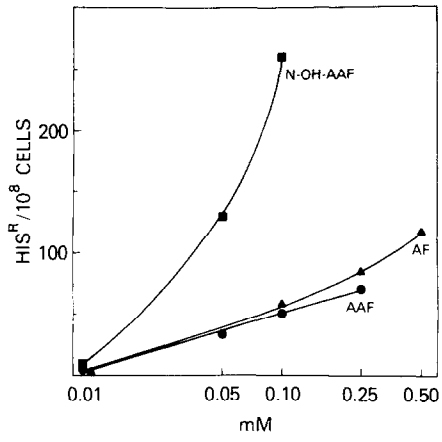

Fig. 1. Mutagenicity of AAF, AF, and N-OH-AAF in the Salmonella/hepatocytes system. The data points (●—● AAF, ▲—▲ AF, and ■—■ N-OH-AAF were determined after 1 hr. incubation and are means of three experiments.

one hour incubation with increasing concentrations of AAF, AF and N-OH-AAF. The N-OH-AAF was 5-6 times more mutagenic than either AAF or AF. The mutagenic effect of AF was slightly greater than that of AAF (Fig. 1), possibly due to greater formation of the hydroxylamine from AF than from AAF (11).

The alkaline elution technique has proven to be a powerful and sensitive method for the measurement of a variety of DNA lesions produced in mammalian cells by various types of DNA damaging agents (7,8). In Fig. 2 are shown the alkaline elution curves for DNA damage in rat hepatocytes after one hour incubation with AAF (0.10 and 0.25 mM), AF (0.10 and 0.50 mM) and N-OH-AAF (0.01 and 0.10 mM). The DNA damage induced by these three compounds showed the same pattern as was observed for the mutagenicity, namely, N-OH-AAF being the most potent whereas AAF and AF caused similar damge (Fig. 1 and 2).

The Salmonella/hepatocyte system allows for comparison of the mutation frequency in the bacteria and the DNA damage in the hepatocytes when potentially mutagenic and/or carcinogenic chemicals are tested. This system offers several complementary features to those systems that utilize only subcellular liver fractions to metabolically activate the test compounds. Since the intact hepatocyte is used as the activating system, the bacterial

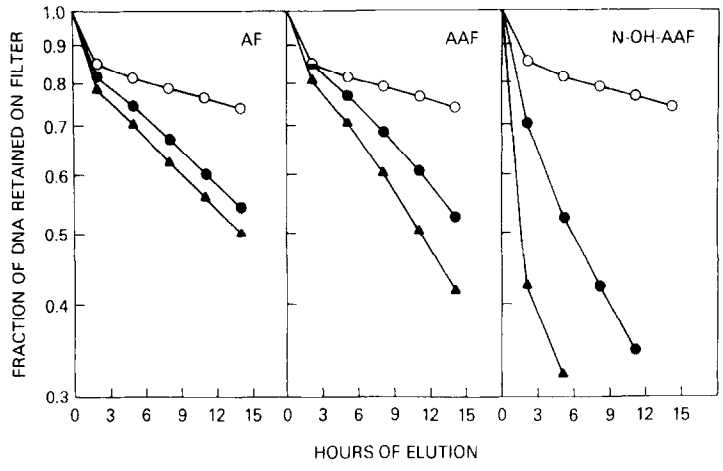

Fig. 2. Kinetics of DNA alkaline elution in hepatocytes after 1 hr incubation with AF, AAF, and N-OH-AAF in the Salmonella/hepatocyte system. The data illustrate representative experiments, o—o, control; ●—● and ▲—▲, are respectively 0.10 and 0.50 mM for AF; 0.10 and 0.25 mM for AAF and 0.01 and 0.10 mM for N-OH-AAF.

mutagen that is formed must not only escape the cellular detoxification processes but also be stable enough to diffuse into the incubation medium and into the bacteria in order to induce the mutation. This information would, therefore, provide important data upon which prediction about the in vivo toxicity of a compound might be based. The DNA damage in the hepatocyte that is caused by the reactive metabolite(s) is quantitated and can be compared to the mutation frequency in the bacteria. The difference in sensitivity between the bacterial mutagenicity and DNA damage by N-OH-AAF is clearly illustrated in Figs. 1 and 2. A substantial amount of DNA damage is observed at 0.01 mM N-OH-AAF whereas no bacterial mutagenicity is found. Since the metabolic activation of N-OH-AAF may proceed via several enzyme systems (11), it seems likely that more than one of the reactive metabolites formed could damage the hepatocyte DNA. However, the mutagenic activation of N-OH-AAF, in the classical Salmonella system in which either liver microsomal or S-9 fractions are used, has been shown to proceed almost exclusively via deacetylation (11). The difference in the sensitivity between the hepatocyte DNA damage and the bacterial mutagenicity of N-OH-AAF may therefore reflect

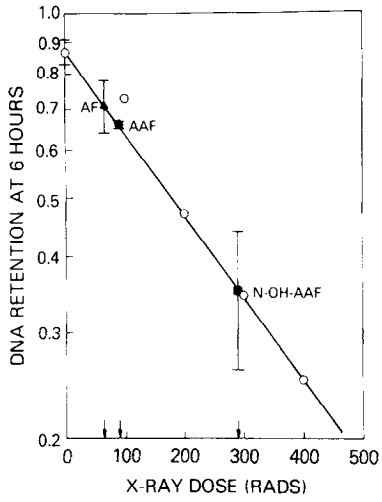

Fig. 3. Comparison of DNA damage after X-ray and AF, AAF and N-OH-AAF treatment of hepatocytes. The data is expressed as mean ± S.D. of three or four experiments.

selectivity in the bacterial mutagenicity of this compound. Whether the deacetylation of N-OH-AAF is an important activation process, as is the case for the bacterial mutagenicity, for damaging the hepatocyte DNA remains to be established.

The alkaline elution technique allows for quantitation of chemically induced DNA damage to be compared with radiation induced DNA damage. This is shown in Fig. 3, in which hepatocytes were exposed to increasing dose of X-rays, and the alkaline elution measured. Since the DNA break frequency for X-rays is 2.7×10^{-12} breaks per dalton per rad (7), the approximate number of breaks for AF (0.5 mM), AAF (0.25 mM) and N-OH-AAF (0.01 mM) is 1.7×10^{-10}, 2.4×10^{-10} and 7.8×10^{-10} per dalton, respectively. Swenberg et al (12) employing Chinese hamster lung fibroblast (V79) found increased elution of DNA when these cells were exposed to AAF and N-OH-AAF at concentrations similar to those used in our study. However, no attempts were made to estimate the break frequency (12). Since the endonuclease activity of dead cells can result in increased DNA elution, the viability of the hepatocytes was measured (trypan blue exclusion) before and after the incubation with the compound. The DNA elution profiles were in all instances independent of the

changes in cell viability (data not shown). This is possible due to the loss of DNA from the filter when the non-viable cells are lysed.

At present we do not know whether the breaks are due to a direct action of the compounds on DNA, or result from repair processes (nuclease action) of the hepatocyte. However, the Salmonella/hepatocyte system can also be used to study the DNA repair in the hepatocytes and thereby complement both the bacterial mutagenesis and DNA elution data when genotoxicity of chemicals are evaluated. Work along these lines is in progress.

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