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DIFFERENCES IN EFFECTS OF NORHARMAN WITH VARIOUS CLASSES OF CHEMICAL MUTAGENS AND AMOUNTS OF S-9

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SUMMARY: The mutagenicities of aniline, o-toluidine and yellow OB were demonstrated only in the presence of the β-carboline compound, norharman. The effect of norharman increased linearly with increase in the amount of S-9. The mutagenicity of 4-dimethylaminoazobenzene was greatly enhanced by the presence of norharman, and again dose-dependency on the amount of S-9 was observed. In the presence of a large amount of S-9, norharman caused several fold enhancement of the mutagenicities of N-2-fluorenylacetamide, benzo(a)-pyrene, and 1,4-dimethyl-3-amino-5H-pyrido(4,3b)indole, isolated from a trypto-phan pyrolysate. However, norharman suppressed the mutagenicities of these compounds in the presence of a small amount of S-9. The mutagenicity of kaem-pferol, a flavonoid, was inhibited by norharman with either a large or small amount of S-9.

INTRODUCTION

We have previously reported that addition of norharman to a mixture of o-toluidine or aniline, S-9 Mix and Salmonella typhimurium TA98 resulted in the appearance of mutagenicity of aniline or o-toluidine (1). This phenomenon was not observed with m-toluidine or p-toluidine (1). Yellow OB, which is carcino-genic, but is not mutagenic with S-9 Mix only, showed mutagenicity when nor-harman was added to the incubation mixture (2). The mutagenicities of 4-dimethylaminoazobenzene (DAB), N-2-fluorenylacetamide (FAA), 1,4-dimethyl-3-amino-5H-pyrido(4,3b)indole (Trp-P-1), 1-methyl-3-amino-5H-pyrido(4,3b)indole (Trp-P-2) and benzo(a)pyrene (BP) were enhanced under certain incubation

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Abbreviations used are: DAB, 4-dimethylaminoazobenzene; FAA, N-2-fluorenyl-acetamide; Trp-P-1, 1,4-dimethyl-3-amino-5H-pyrido(4,3b)indole; Trp-P-2, 1-methyl-3-amino-5H-pyrido(4,3b)indole; BP, benzo(a)pyrene; PCB, polychlo-rinated biphenyl; MC, methylcholanthrene; DMBA, 7,12-dimethylbenz(a)anth-racene

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condition in the presence of norharman (3,4,5). In our experiments, S-9 fraction was prepared from the liver of rats that had been treated with polychlorinated biphenyl (PCB).

Levitt et al. reported that norharman inhibited the metabolism of BP and reduced the mutagenicity of BP in a system containing S-9 fraction from the liver of mice that had been treated with methylcholanthrene (MC) (6). The present report shows that the effect of norharman on mutagenicity depends on the class of chemical, and on the amounts of S-9 fraction.

MATERIALS AND METHODS

Chemicals: Norharman was obtained from Aldrich Chemical Co., DAB, FAA, BP and aniline were kindly purified and given by Dr. M. Nakadate, of the National Institute of Hygienic Sciences, Tokyo. o-Toluidine was purified and given by Dr. Y. Hashimoto, Tohoku University. Trp-P-1 and Trp-P-2, synthesized by the method of Akimoto et al. (7), were gifts from Dr. H. Nomura, Takeda Pharmaceutical Co. Ltd., Osaka. Yellow OB was purchased from Tokyo Kasei Kogyo Co. Ltd., Tokyo.

Mutation test: Salmonella typhimurium TA98 was used (8). The mutation test was performed by the method of Ames et al. (8) with the modifications described previously (9), including a step of preincubation of the test substance with S-9 Mix and S. typhimurium TA98 for 20 minutes at 37°C. S-9 Mix contained 2 μmoles NADH, 2 μmoles NADPH, 2.5 μmoles G6P, 2.5 μmoles ATP in 0.5 ml. His^+ revertant colonies on the plates were counted after 2-day incubation at 37°C. The background lawn was checked carefully under the stereomicroscope to confirm that the compound did not have a lethal effect. S-9 fraction was prepared from the liver of rats injected with PCB.

RESULTS AND DISCUSSION

Fig. 1-a and b shows the effect of norharman on the mutagenicities of o-toluidine and yellow OB. These two compounds were not mutagenic with S-9 Mix only, but on the further addition of 200 μg of norharman they showed mutagenicity, which increased linearly with increase in the amount of S-9. A similar appearance of mutagenicity in the presence of norharman has been observed with aniline (1). The role of norharman is unknown, but the following three possibilities may be considered. [1] Norharman may inhibit the process of inactivation of these chemicals or enhance the process for their activation; [2] Intercalation of norharman into double-stranded DNA has been demonstrated (10) and this intercalation may increase the susceptibility of DNA to damage by metabolites of these chemicals. DNA modified by intercalation of norharman

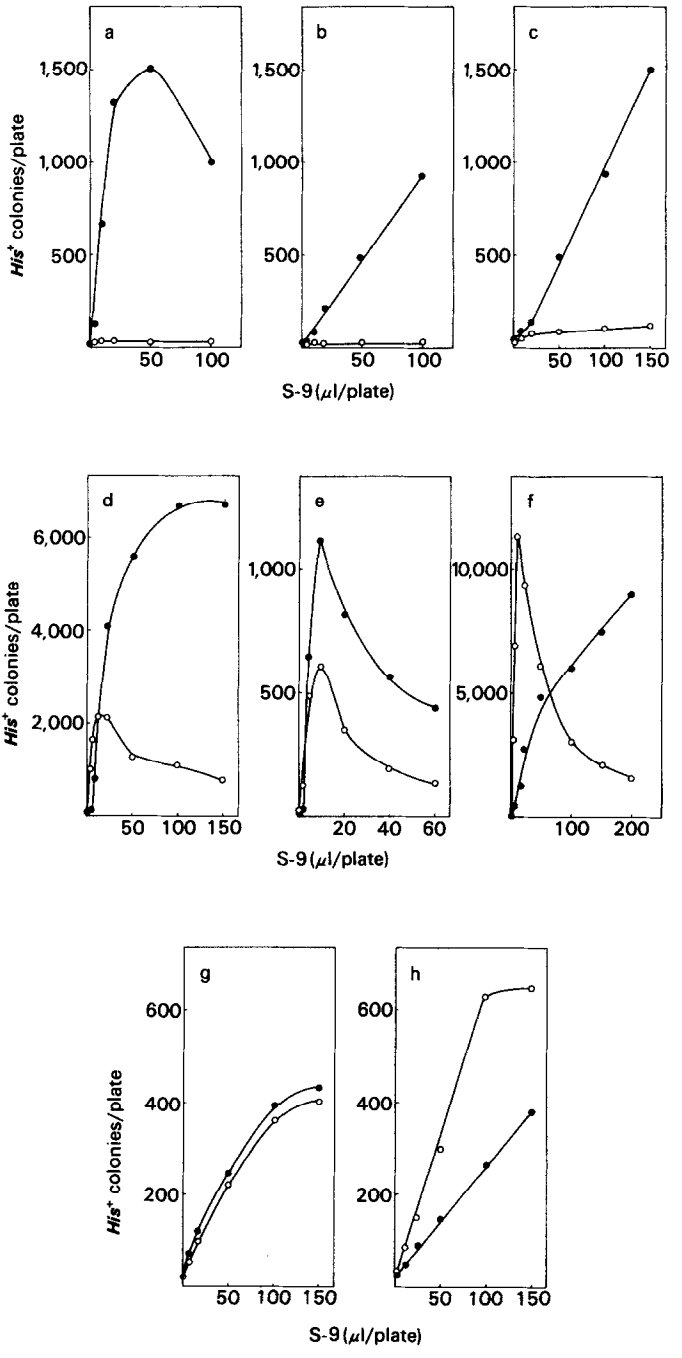

Fig. 1. Effect of norharman on the mutagenicities of different classes of chemicals with various amounts of S-9. (a) 50 μg o-toluidine. (b) 50 μg yellow OB. (c) 50 μg DAB. (d) 25 μg FAA. (e) 1 μg BP. (f) 0.5 μg Trp-P-1. (g) 50 μg DMBA. (h) 40 μg kaempferol. ●—●, with 200 μg norharman; o—o, without norharman.

may be subject to error-prone repair; [3] Norharman and aniline may be converted to a new mutagenic compound by S-9 Mix.

Fig. 1-c shows the effect of adding norharman to the incubation mixture of DAB. The mutagenicity of DAB was markedly enhanced, and increased with increase in the amount of S-9. The effects of norharman shown in Fig. 1-a, b and c could be expressed as a "comutagenic action" of norharman.

Fig. 1-d demonstrates the effect of norharman in the incubation mixture of FAA. With 25 μg of FAA and no norharman, 20 μl S-9 was optimal, inducing 2260 revertants/plate, and further addition of S-9 decreased the number of revertants. With less than 5 μl of S-9, norharman inhibited the mutagenicity of FAA, but with over 20 μl it enhanced the mutagenicity of FAA. In the presence of 100 μl S-9, 25 μg of FAA alone induced 1160 revertant colonies per plate and with norharman it induced 6828 revertants. This biphasic effect of norharman may be due to its interference with the balance between the activation and inactivation pathways of FAA. A similar biphasic effect of norharman was observed with BP, although the enhancing effect was less than with FAA, as shown in Fig. 1-e. Previously Levitt et al. (6) studied the effect of norharman on the mutagenicity of BP using S-9 from mice treated by MC. The amount of S-9 protein which they used was comparable to that used in our previous studies (3), and falls in the range shown in Fig. 1-e in which norharman had an enhancing effect. Therefore, the discrepancy between our previous (3) and present results, and their results (6) may be due to a difference in the activities of the various enzymes involved BP metabolism in S-9 from the livers of PCB-treated rats and MC-treated mice. As stated by Levitt et al., differences in experimental conditions may also have influenced the results; our method involved preincubation of the mixture of test substances, S-9 Mix and bacteria, but they used Ames' original method.

Fig. 1-f shows the mutagenicity of Trp-P-1 with and without norharman. Trp-P-1 was first isolated from a tryptophan pyrolysate (11). It is a new γ-carboline derivative of a very strong frameshift mutagen (12). As shown in

Fig. 1-f, with small amounts of S-9, norharman inhibited the mutagenicities of Trp-P-1 but with large amounts of S-9, it enhanced the mutagenicities of Trp-P-1. A similar phenomenon was observed with Trp-P-2.

Fig. 1-g shows the mutagenicity of 7,12-dimethylbenz(a)anthracene (DMBA) with and without norharman. Norharman had little effect. Fig. 1-h shows the inhibitory effect of norharman on the mutagenicity of kaempferol, a naturally occurring flavonol. The mutagenicities of flavonols have been reported (13,14, 15).

The mutagenicity observed in the Salmonella/microsomal system reflects a complex balance between metabolic activation (8) and metabolic inactivation (16), because the test chemicals and their metabolites are subjected to various metabolic activations and inactivations in the system. Norharman is known to inhibit arylhydrocarbon hydroxylase (6) and monoamine oxidase (17). Thus it is understandable that, depending on the nature of the test chemical, norharman may cause enhancement or decrease in mutagenicity or both. The overall effect also depends on the amount of S-9, but the action of norharman on aniline, o-toluidine and yellow OB and also on DAB can be called "comutagenic" because these compounds are mutagenic only in the presence of norharman, or their mutagenicities are increased by norharman with all amounts of S-9 used.

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