A New Concept of Co-mutagenicity from a Phenomenon Forgotten for the Past Two Decades: Is It More Important than Previously Expected?

In 1977, during the course of studies on the mutagenic principle in tryptophan pyroles, it was incidentally observed that the mutagenic activity towards Salmonella typhimurium TA98 in the presence of S9 mix was drastically reduced or almost lost at certain fractionation steps. Interestingly, combining some nonmutagenic fractions with those that showed only very weak or no mutagenicity resulted in restoration of the original level of mutagenic activity. One of the fractions contained norharman and harman, both of which are nonmutagenic β-carboline derivatives to S. typhimurium TA98 in the presence of S9 mix, while the others contained unknown mutagenic compounds or heterocyclic amines at lower levels (1). This phenomenon, in which nonmutagenic β-carbolines augmented the mutagenicity of nonmutagenic compounds or drastically enhanced the weak mutagenicity, was termed co-mutagenicity. The co-mutagenicity was only demonstrated when β-carbolines were incubated with other compounds in the presence of S9 mix.

The most clear-cut demonstration of co-mutagenicity was made by incubation of norharman with aniline in the presence of S9 mix. Aniline alone showed no mutagenic activity to S. typhimurium TA98. Typical data are shown in Table 1 (2). The case of toluidines (aminotoluene) is also remarkable. Among three toluidine isomers, o- and m- isomers showed mutagenicity in the presence of norharman, whereas p-toluidine remained inactive (see Table 2) (2,3). Moreover, yellow OB and N-nitrosodiphenylamine, which are themselves nonmutagenic, were shown to be mutagenic to S. typhimurium TA98 with S9 mix only in the presence of norharman (4,5). Although less dramatic, the mutagenicity of 4-dimethylaminoazobenzene was also enhanced by norharman (4).

The mechanism and significance of co-mutagenicity has awaited clarification since the time of its first discovery over two decades ago. Recently Totsuka et al. (6) found that norharman can conjugate with aniline to form a third compound (1) in the presence of S9 mix (6).

The compound (1) was purified and the structure was determined, as shown in Figure 1. It is very important that the chemically synthesized compound (I), 9-(4'-aminophenyl)-9H-pyrido[3,4-b]indole (aminophenylnorharman), still required the metabolic activation by S9 mix to exert mutagenicity to S. typhimurium TA98. This compound is easily organically synthesized in high yield with norharman and p-bromonitrobenzene as starting materials. Furthermore, DNA extracted from S. typhimurium TA98 incubated with norharman plus aniline or from S. typhimurium TA98 incubated with aminophenylnorharman, both in the presence of S9 mix, demonstrated the same DNA adduct pattern when assessed using the 32P-postlabeling method (6).

Norharman and harman are present at relatively high concentrations in natural and industrial environments; for example, cigarette tar contains norharman and harman at levels of 3.3–14.1 µg/cigarette. They can also be formed in vivo from tryptamine, a decarboxylated form of the amino acid tryptophan. Tryptamine nonenzymatically reacts with formaldehyde or the C1-unit metabolite and acetaldheyde, abundant after ethanol ingestion and in diabetic patients, to yield norharman and harman, respectively. Ushiyama et al. (7) found that the urine of inpatients receiving only parenteral alimentation contained norharman and harman, suggesting in vivo formation of β-carbolines. Therefore, it is likely that co-mutagenic reactions may occur in the human body.

The structure of aminophenylnorharman formed from norharman and aniline has an N–C bond between the indole nitrogen and C-4 position of aniline. Hydroxylation at the C-4 position of aniline is possibly catalyzed by a member of the cytochrome P450 family. However, norharman and p-aminophenol did not yield any mutagenicity to S. typhimurium TA98 when incubated in the presence of S9 mix; thus, p-aminophenol is presumably not an ultimate metabolite for the formation of aminophenylnorharman.

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**Table 1. Mutagenicity of norharman (200 µg/plate) and aniline (100 µg/plate) in Salmonella typhimurium TA98 in the presence of S9 mix**

| Revertants/plate | Norharman and aniline | Norharman | Aniline |
|------------------|-----------------------|------------|---------|
|                  | 3,400                 | 0          | 0       |

**Table 2. Mutagenicity of norharman (200 µg/plate) with toluidine isomers (100 µg/plate) in Salmonella typhimurium TA98 in the presence of S9 mix**

| Revertants/plate | Norharman + o-toluidine | Norharman + m-toluidine | Norharman + p-toluidine |
|------------------|-------------------------|-------------------------|-------------------------|
|                  | 6,990                   | 62                      | 0                       |

No mutagenic activity was observed with any of the compounds alone.

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**Figure 1.** The formation of compound I from the co-mutagen norharman with aniline in the presence of S9 mix and the structure of compound I.
Aminophenylnorharman is genotoxic and causes cellular damage in rats; it is also likely to be carcinogenic in vivo. Hepatic degeneration, erosive changes in the urinary bladder with hematuria, and testicular toxicity were observed in rats administered 20 mg/kg body weight of the compound by gavage six times during 1 week. In this context, the International Agency for Research on Cancer report suggesting carcinogenicity of aniline in rats and o-toluidine in rodents is also of interest (8).

The science of co-mutagenesis opens new vistas for organic chemistry, metabolism of foreign substances, DNA adduct formation, DNA repair, co-carcinogenicity, in vitro effects on cultured mammalian cells, in vivo toxicity, pharmacological action, and in vivo carcinogenicity. The significance of co-mutagenesis may hitherto have been very much underestimated and clearly warrants detailed attention.

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