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Review

Mutagens formed from β-carbolines with aromatic amines

Y. Totsuka, T. Takamura-Enya, R. Nishigaki, T. Sugimura, K. Wakabayashi*

Cancer Prevention Basic Research Project, National Cancer Center Research Institute, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan

Abstract

Norharman, widely distributed in our environment such as cigarette smoke and cooked foods, is not mutagenic to *Salmonella* strains, but becomes mutagenic to *Salmonella typhimurium* TA98 and YG1024 with S9 mix in the presence of aromatic amines, including aniline and *o*-toluidine. Therefore, we have designated norharman as a "co-mutagen". Since, humans are simultaneously exposed to norharman and aromatic amines in daily life, it is important to clarify the mechanisms of its co-mutagenic action to further understanding of the potential genotoxic effects in humans. Regarding the mechanisms of this action of norharman with aniline, a mutagenic compound, 9-(4′-aminophenyl)-9*H*-pyrido[3,4-*b*]indole[aminophenylnorharman (APNH)] is produced by their interaction, and converted to the hydroxyamino derivative which eventually forms the DNA adduct, dG-C8-APNH through possible ultimate reactive forms with esterification, and this induces mutations. Also other aminophenyl-β-carboline compounds, such as 9-(4′-amino-3′-methylphenyl)-9*H*-pyrido[3,4-*b*]indole[amino-3′-methylphenylnorharman (3′-AMPNH)], 9-(4′-amino-2′-methylphenyl)-9*H*-pyrido[3,4-*b*]indole [amino-2′-methylphenylnorharman (2′-AMPNH)], 9-(4′-aminophenyl)-1-methyl-9*H*-pyrido[3,4-*b*]indole[aminophenylharman (APH)] and 9-(4′-amino-3′-methylphenyl)-1-methyl-9*H*-pyrido[3,4-*b*]indole[amino-3′-methylphenylharman (AMPH)], have been found on reaction of norharman or harman with aniline or toluidine isomers. These compounds showed mutagenic and clastogenic actions in bacterial and mammalian cells. Among them, APNH demonstrated the most potent activity, and it was most extensively studied. When APNH was administered as a single dose to F344 rats, severe testicular toxicity was observed after 6 days. Moreover, liver preneoplastic lesions (GST-P-positive foci) in the liver clearly developed in animals fed 10–50 ppm of APNH in the diet for 4 weeks. Since, APNH was detected in 24 h urine of rats upon simultaneous administration with norharman and aniline by gavage, it is likely to be also produced from norharman and aniline in the human body. From these findings, it is suggested that aminophenyl-β-carboline derivatives may be classified as one of the novel types of endogenous mutagens and carcinogens.

Keywords: Reviews; Mutagens; β-Carbolines; Norharman; Aniline

Contents

Abbreviations: HAs, heterocyclic amines; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido [4,3-*b*]indole; Norharman, 9*H*-pyrido[3,4-*b*]indole; Harman, 1-methyl-9*H*-pyrido[3,4-*b*]indole; APNH, 9-(4′-aminophenyl)-9*H*-pyrido[3,4-*b*]indole; 3′-AMPNH, 9-(4′-amino-3′-methylphenyl)-9*H*-pyrido[3,4-*b*]indole; 2′-AMPNH, 9-(4′-amino-2′-methylphenyl)-9*H*-pyrido[3,4-*b*]indole; APH, 9-(4′-aminophenyl)-1-methyl-9*H*-pyrido[3,4-*b*]indole; AMPH, 9-(4′-amino-3′-methylphenyl)-1-methyl-9*H*-pyrido[3,4-*b*]indole; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; SCE, sister chromatid exchange; CHL, Chinese hamster lung; N-OH-APNH, hydroxyaminophenylnorharman; PHA, phenylhydroxylamine; dG-C8-APNH, $N^{4'}$-(2′-deoxyguanosin-8-yl)-9-(4′-aminophenyl)-9*H*-pyrido[3,4-*b*]indole; GST-P, glutathione-*S*-transferase placental form

* Corresponding author.

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1. Introduction

More than 20 years ago, Nagao co-workers reported that the β-carboline compound, norharman (9*H*-pyrido[3,4-*b*]-indole), itself not mutagenic to *Salmonella* strains with or without S9 mix, becomes mutagenic to *Salmonella typhimurium* TA98 and YG1024 with S9 mix, when mixed with aromatic amines, such as aniline and toluidine isomers [1–3]. On the bases of these observations, we designated norharman is a "co-mutagen". Harman (1-methyl-9*H*-pyrido[3,4-*b*]indole), another β-carboline compound, has similarly co-mutagenic activity with aniline or *o*-toluidine [3].

Norharman and harman are produced by pyrolysis of L-tryptophan and have been reported to be present at much higher levels than mutagenic and carcinogenic HAs in cigarette smoke condensate and cooked foodstuffs [4]. The compounds were also detected in human urine samples [5,6]. Since, these β-carboline compounds were detected in both of urine samples collected from healthy volunteers eating normal diet and patients receiving parenteral alimentation, it is suggested that norharman and harman are probably produced endogenously in human body. Aniline and toluidine isomers are also present in cigarette smoke condensate and certain vegetables. These aromatic amines are ubiquitously utilized in various ways as industrial raw materials [7–9] and aniline is detectable in human urine and breast milk samples [10–12]. Therefore, it is likely that humans are simultaneously exposed to β-carbolines and aromatic amines in daily life and the clarification of the mechanisms of its co-mutagenic action is important in order to understand potential genotoxic effects in humans.

2. Structures of mutagens produced by norharman with aromatic amines

Since norharman showed mutagenic activity when mixed with aromatic amines and S9 mix, efforts were made to identify the mutagenic compound formed by reaction between norharman and aniline in the presence of S9 mix. After incubation, the reaction mixture was separated by HPLC on a semi-preparative ODS column with a gradient solvent system. Examination of mutagenicity in *S. typhimurium* YG1024 with S9 mix, revealed activity mainly in the fractions with retention times of 48–60 min (compound I). The mutagenic compound I was further isolated and purified by HPLC, and various spectral determinations performed including UV, mass and ^{1}H-NMR spectra. The UV absorption spectrum of the mutagenic compound I showed absorption maxima at 238, 287 and 356 nm, and its mass spectrum exhibited a molecular ion peak at *m*/*z* 259. With ^{1}H-NMR spectral data measured in acetone-d_6 indicated 7 of the 11 aromatic protons could be assigned to the norharman moiety and 4 protons to the aniline moiety. Based on spectral data, the mutagenic compound I was deduced to be a coupled compound of norharman and aniline, namely 9-(4′-aminophneyl)-9*H*-pyrido[3,4-*b*]indole [aminophenylnorharman (APNH)]. This assumption was confirmed by its chemical synthesis. The yield of APNH from norharman in the enzymatic reaction was around 0.01%. On the other hand, when the mutation assay of the each fractions collected from HPLC separation was carried out in absence of S9 mix, mutagenicity was detected in fractions with retention times of 60–68 min (compound II). Aromatic amine compounds need to be converted to an *N*-hydroxyamino derivative to cause mutagenicity [13] and compound II was tentatively considered to be hydroxyaminophenylnorharman (N-OH-APNH). To confirm its structure, N-OH-APNH was synthesized, and finally the structure of compound II, showing mutagenic activity without S9 mix, was concluded to be N-OH-APNH [14,15].

As mentioned above, the other aromatic amines, such as *o*- and *m*- but not *p*-toluidine, have also been shown to react with norharman to elicit mutagenicity in *S. typhimurium* TA98 and YG1024 in presence of S9 mix. Mutagenic compounds produced from norharman with *o*- or *m*-toluidine were isolated and their structures were determined. The mutagenic compounds produced from norharman with *o*-toluidine and *m*-toluidine were 9-(4′-amino-3′-methylphneyl)-9*H*-pyrido[3,4-*b*]indole [amino-3′-methylphenylnorharman (3′-AMPNH)] and 9-(4′-amino-2′-methylphenyl)-9*H*-pyrido[3,4-*b*]indole [amino-2′-methylphenylnorharman (2′-AMPNH)], respectively [16]. The yields of these compounds in the incubation mix were around 0.06 and 0.02%, respectively.

It already demonstrated that also harman, another β-carboline compound, causes co-mutagenicity in *S. typhimurium* TA98 in presence of S9 mix and aniline [3]. Therefore, it is likely that coupled mutagenic compounds of harman with aniline or *o*-toluidine are produced in the reaction mixtures. Two mutagenic compounds were identified so far, namely 9-(4′-aminophenyl)-1-methyl-9*H*-pyrido[3,4-*b*]-indole [aminophenylharman (APH)] formed in the reaction of harman with aniline, and 9-(4′-amino-3′-methylphenyl)-1-methyl-9*H*-pyrido[3,4-*b*]indole [amino-3′-methylphenylharman (AMPH)] from the reaction with *o*-toluidine. The yields of these compounds were around 0.0025% for APH and 0.005% for AMPH. The chemical structures of the five aminophenyl-β-carboline derivatives, APNH, 3′-AMPNH, 2′-AMPNH, APH and AMPH are given in Fig. 1.

3. Genotoxic activities of aminophenyl-β-carboline derivatives

Chemically synthesized APNH, 3′-AMPNH, 2′-AMPNH, APH and AMPH caused higher mutagenicity in *S. typhimurium* TA98 and YG1024 (which detect frameshift mutations) than in the strains TA100 and YG1029 (which are sensitive to base pair mutations) in presence of S9 mix.

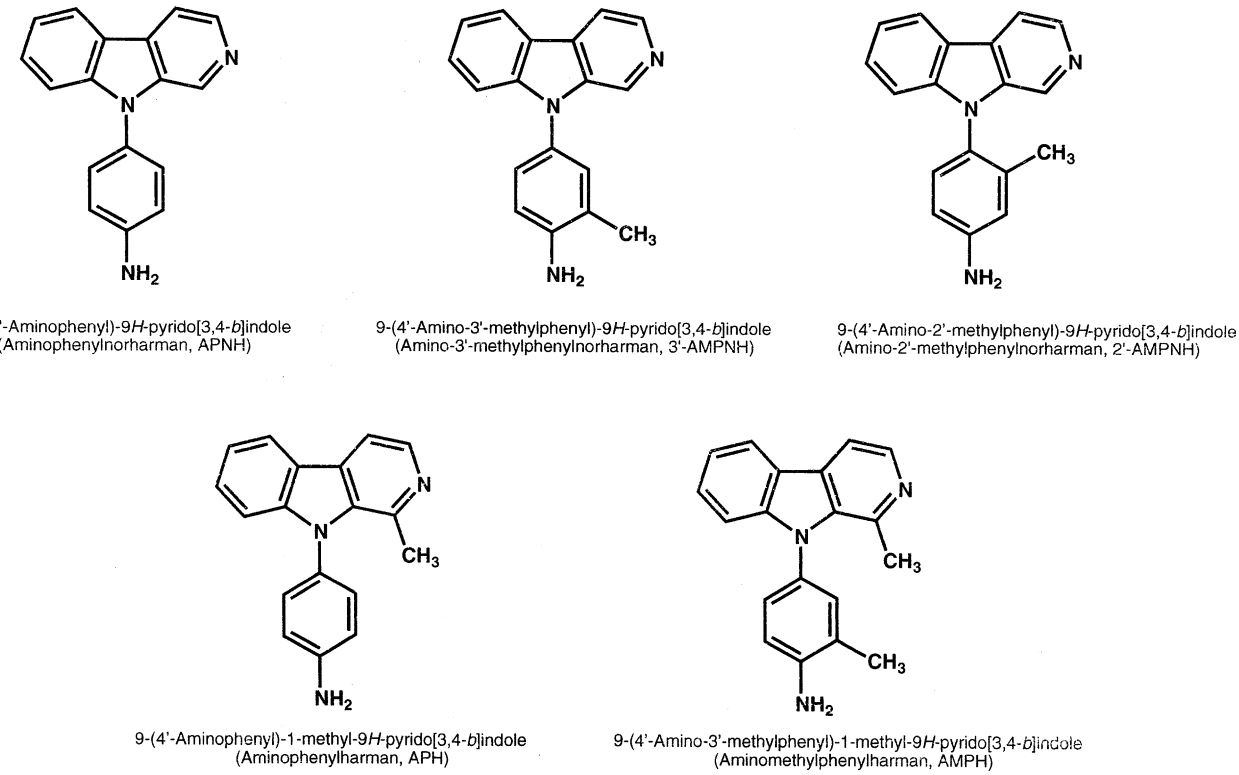

Fig. 1. Chemical structures of aminophenyl-β-carboline derivatives.

Moreover, YG1024, a TA98 derivative with high acetyltransferase activity proved more sensitive than the parent strain. This observation suggest that acetyltransferase is required for the mutagenicity of these aminophenyl-β-carboline derivatives. The mutagenic activity of APNH, 2′-AMPNH, 3′-AMPNH and three HAs are shown in Table 1 [14–17]. Among the aminophenyl-β-carboline derivatives studied, APNH showed the strongest mutagenicity with a value comparable to those for 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 3-amino-1-methyl-5*H*-pyrido [4, 3-*b*]indole (Trp-P-2) and about 500 times higher than that of benzo[*a*]pyrene [17]. The mutagenic activities of other derivatives including APH and AMPH, were 100–200 times lower than those of APNH. In absence of S9 mix, aminophenyl-β-carboline derivatives were not mutagenic in either strain.

Table 1
Mutagenicities of aminophenyl-β-carboline derivatives and HAs in *Salmonella* strains in presence of S9 mix [14–17]

	Mutagenicity (revertants/μg)		
	TA98	TA100	YG1024
APNH[a]	187000	1230	1783000
2′-AMPNH[a]	140	16	3000
3′-AMPNH[a]	41000	2	698000
MeIQx	145000	14000	3500000
Trp-P-2	104200	1800	77000
Glu-P-1	49000	3200	2500000

[a] S9 concentration was used for 5 μl per plate.

In subsequent experiments the DNA adduct formation by norharman in presence of aniline or toluidine isomers (*o*-, *m*- and *p*-toluidines) was analyzed. After incubation of *S. typhimurium* TA98 with norharman plus aniline, or each of the three kinds of toluidine isomers in the presence of S9 mix, DNA adducts were analyzed by the ^{32}P-postlabeling method under modified adduct intensification conditions. Formation of DNA adducts was clearly observed with mixtures of norharman plus aniline or *o*- or *m*-toluidine but not in the case of *p*-toluidine. Three adduct spots (two major and one minor) were found in the reaction mixture of norharman plus aniline, furthermore two major and one minor spots for norharman plus *o*-toluidine, and a single spot for norharman plus *m*-toluidine were detected [18]. In contrast, no adduct spots were produced by incubation of any of the aromatic amines or by norharman alone. The mutagenicities of norharman (100 μg per plate) in presence of aromatic amines (200 μg per plate), such as aniline or toluidine isomers, towards *S. typhimurium* TA98 with S9 mix, as well as the DNA adduct levels are shown in Table 2. Mixtures of norharman with aniline and *o*- or *m*-toluidine, caused mutagenicity, whereas no mutagenic activity was observed with the combination of norharman and *p*-toluidine, or with any of the compounds alone. Thus, the DNA adduct formation by norharman with aromatic amines correlated well with the co-mutagenic action in

Table 2
Relationship between the co-mutagenic action and DNA adduct formation in *S. typhimurium* TA98 seen after incubation of norharman with aromatic amines and S9 mix [18]

	Mutagenicities[a] (revertants per plate)	Adduct levels[b] (adducts/10^8 nucleotides)
Norharman + aniline	6610 ± 1210	10.8 ± 2.27
Norharman + *o*-toluidine	6990 ± 1043	3.74 ± 1.71
Norharman + *m*-toluidine	62 ± 6	0.04 ± 0.01
Norharman + *p*-toluidine	0	N.D.[c]
Norharman	0	N.D.[c]
Aniline	0	N.D.[c]
o-Toluidine	0	N.D.[c]
m-Toluidine	0	N.D.[c]
p-Toluidine	0	N.D.[c]

Numbers of revertants and RALs in *S. typhimurium* TA98 are mean±S.D. values.

[a] Mutagenicity of norharman in combination with aromatic amines examined in *S. typhimurium* TA98 with S9 mix. The doses of norharman and aromatic amines were 200 and 100 μg per plate, respectively.

[b] DNA adduct levels were analyzed by ^{32}P-postlabeling method under modified adduct intensification conditions.

[c] N.D.: not detectable levels (<$1/10^{10}$ nucleotides) of aminophenyl–β-carboline–DNA adducts.

S. typhimurium TA98 [18]. Moreover, the chemical structure of the major APNH–DNA adduct was concluded to be $N^{4'}$-(2′-deoxyguanosin-8-yl)-9-(4′-aminophenyl)-9*H*-pyrido-[3,4-*b*]indole (dG-C8-APNH) using the ^{32}P-postlabeling method and various spectrometry techniques [19]. APNH–DNA adduct formation was observed in various organs of F344 rats fed 40 ppm of APNH for 4 weeks, the highest levels were detected in the liver and colon (1.31 ± 0.26 and 1.32 ± 0.11 adducts/10^7 nucleotides, respectively) [19].

Recently, Ohe et al. reported that APNH, 3′-AMPNH and APH induced sister chromatid exchange (SCE) in Chinese hamster lung (CHL) cells [20]. The cells were incubated with the individual compounds in presence of S9 mix, and SCEs were induced in a dose-dependent manner at concentrations between 0.00125 and 0.01 μg/ml for APNH, and between 0.3125 and 5 μg/ml for 3′-AMPNH and APH. The approximate doses leading to three-fold increase over the control level were 0.05 for APNH, 0.51 for 3′-AMPNH and 1.7 μg/ml for APH. In addition, APNH induced chromosome aberrations in CHL cells at concentrations between 0.00125 and 0.04 μg/ml, the potency of SCE induction and clastogenic activity was much stronger than that seen with potent model clastogens, such as actinomycin D, mitomycin C or 1,8-dinitropyrene [20].

4. The mechanisms of APNH formation

In a number of experiments, the enzymes in rat liver involved in the in vitro formation of APNH from norharman and aniline were investigated. The microsomal fraction was clearly active, whereas no APNH formation was observed with cytosol fraction. Addition of a P450 inhibitor (SKF-525A) to the reaction mixture of norharman, aniline and the microsomal fraction resulted in a decrease to approximately 40% of the APNH level in the reaction mixture without the inhibitor. These findings indicated that P450s mediated the formation of APNH from norharman and aniline [21]. In addition, APNH was also detected in presence of the microsomal fraction derived from human liver. The mechanisms of the reaction are not fully known yet, but, Guengerich suggested the possibility that the two amines are brought together in a P450 complex so an *ipso* attack on the aniline can occur [22]. On the other hand, it has been reported that a mixture of norharman and phenylhydroxylamine (PHA) which is a *N*-hydroxy-derivative of aniline, in the presence of S9 mix, causes three times higher mutagenicity than the mixture of norharman and aniline [3]. Moreover, we confirmed that APNH was formed in the reaction mixture of norharman and PHA with S9 mix. Thus, an other possible mechanism of formation of APNH can be postulated: first, aniline is oxidized to form PHA by P450(s), which might be converted to a phenyl nitrenium cation by further enzymatic reactions. A nitrenium carbo-cation at the C-4 position derived from nitreniumion, might be produced, and could bind to norharman to form APNH (unpublished data). It remains to be clarified which P450 enzyme(s) is (are) involved in the formation of APNH. On the other hand, it is also possible that other types of enzymes are involved in the reaction of norharman and aniline.

The in vivo formation of APNH was studied by detection of APNH in the urine samples using gas chromatography with nitrogen-phosphorous selective detector, a selective and sensitive method for the detection of HAs. A mixture of norharman and aniline was administered by gavage to F344 rats pretreated with phenobarbital and β-naphthoflavone, then the amount of APNH in 24 h urine was analyzed. In urine samples from rats administered 45 mg/kg or 90 mg/kg each of norharman and aniline, 11.5 ± 8.6 ng was detected in the 24 h urine and 19.6 ± 16.9 ng in the 24 h urine [21]. In contrast, APNH was not detected in urine samples from rats exposed to norharman or aniline, alone. Moreover, the amount of APNH was about 2–3-fold increased in urine samples treated with 1N hydrochloric acid at 60 °C for 5 h for hydrolysis of APNH conjugates. As mentioned above, both, norharman and aniline are present in our environment, therefore, it is likely that APNH is similarly produced in humans.

5. Metabolism of APNH

Since APNH shows higher mutagenic activity in *S. typhimurium* YG1024 with high acetyltransferase than in TA98 in the presence of S9 mix, the compound may be metabolized by cytochrome P-450 enzymes with conversion of an exocyclic amino group to a hydroxyamino group, then further activated to form the *N*-acetoxy derivative by the action of acetyltransferase, as is the case for HAs [13,17].

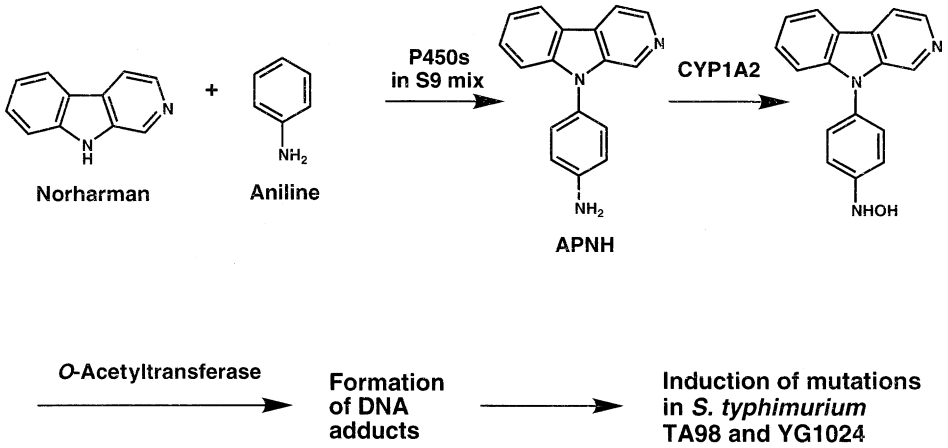

Fig. 2. Schematic illustration of the reaction of norharman with aniline in the presence of S9 mix leading to appearance of mutagenicity.

For most HAs, except for Trp-P-1, the principal cytochrome P-450 isozyme responsible for *N*-hydroxylation is known to be CYP1A2 [23]. Using genetically engineered *S. typhimurium* OY 1002 which contains one of the various forms of human cytochrome P-450 and NADPH–P-450 reductase, APNH was found to be mainly *N*-hydroxylated by CYP1A2, also CYP2C9 elicited weak activity, but other isozymes, including CYP1A1, 1B1, 2D6, 2E1 and 3A4, showed no ability to *N*-hydroxylate APNH [24]. In addition, APNH yielded identical DNA adducts in *S. typhimurium* YG1024 as those observed with N-OH-APNH and also the mixture of norharman and aniline [14]. Moreover, it was reported that 3′-AMPNH produces the same DNA adducts in *S. typhimurium* YG1024 as those observed with the mixture of norharman and *o*-toluidine [16]. From these observations, possible mechanisms of the co-mutagenic action of norharman with aniline are suggested, as follows (Fig. 2): (i) the coupled mutagenic compound, APNH, is formed by an enzymatic reaction, presumably with P450(s); (ii) subsequently, the exocyclic amino group is metabolically activated by CYP1A2 and the *N*-hydroxyamino derivative further metabolized to the *N*-acetoxy form by the action of acetyltransferase. The ultimate metabolite produces DNA adducts which induce mutations in *Salmonella* strains. This might also be the case for compounds generated by norharman or harman with other aromatic amines.

6. In vivo toxicity of APNH in F344 rats

Since humans may be continuously exposed to both norharman and aniline in their daily life, the possible toxic and carcinogenic effects of APNH in mammals should be studied. To assess this question, a short-term experiment was conducted in F344 rats. Ten-week-old males were treated with a single intragastric injection of APNH at doses of 45 mg/kg or 90 mg/kg body weight and sacrificed 1, 3 or 6 days thereafter. With the highest dose, the food intake was suppressed and body and testis weights were decreased by day 6. Histopathological examinations clearly demonstrated that APNH induced severe testicular damage, such as vacuolation of Sertoli cells, appearance of multinucleated giant cells and loss of round spermatids and spermatogonia in the seminiferous tubules (Fig. 3) [25]. However, Leydig cells

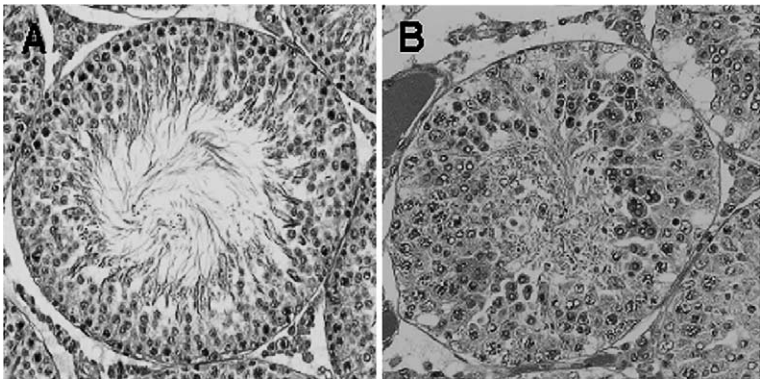

Fig. 3. Histopathological appearance of seminiferous tubules in rats 6 days after administration of water (A) or 90 mg/kg of APNH (B) (H&E, original magnification 20×).

Table 3
Numbers and areas of GST-P positive foci in the livers of F344 rats treated with APNH [26]

Treatment	No. of rats	GST-P-positive foci	
		Number/cm^2	mm^2/cm^2
Control diet	3	0	0
10 ppm APNH	5	0.52 ± 0.03	0.006 ± 0.01
20 ppm APNH	5	1.3 ± 0.9	0.01 ± 0.01
50 ppm APNH	6	21 ± 5.8	2.3 ± 1.3

Values are mean ± S.D. for foci larger than 0.1 mm in diameter.

and serum follicle-stimulating hormone and luteinizing hormone levels were not affected, so that disruption of spermatogenesis might not be related to hypothalamic-pituitary dysfunction. The morphological alterations in rats given APNH were similar to those caused by Sertoli cell toxicants, such as 1,3-dinitrobenzene and 2,5-hexanedione. In addition, erosive changes in urinary bladder, thymic atrophy, and panmyelophthisis were observed.

When 7-week-old male rats were fed diets containing 10 ppm, 20 ppm or 50 ppm of APNH for 4 weeks, clear cell or mixed type foci were observed in the livers. Almost all of these foci stained immunohistochemically positive for glutathione *S*-transferase placental (GST-P) form [26]. In livers of rats fed the control diet, no such GST-P-positive altered foci larger than 0.1 mm in diameter were found. The data for induction of liver GST-P-positive foci in rats by APNH are summarized in Table 3. In organs other than the liver, there is no evidence of toxicity or carcinogenicity in this medium-term assay. MeIQx, one mutagenic and carcinogenic HA, at a dietary dose of 400 ppm long-term induced liver tumors in 100% in male F344 rats [27]. GST-P-positive foci were first observed after 12 weeks, whereas APNH at doses of 10–50 ppm for 4 weeks clearly produced GST-P-positive foci in the liver in a dose-dependent manner, with quantitative values for the dose of 50 ppm after 4 weeks comparable to those with 400 ppm MeIQx for 12 weeks [28]. Moreover, 40 ppm of MeIQx did not induce GST-P-positive foci in the liver of rats [29]. This comparison indicates that APNH might be a much more potent liver carcinogen than other HAs, such as MeIQx. Indeed, APNH was recently demonstrated to induce liver tumors in F344 rats when given at doses of 20 and 40 ppm in diet for 85 weeks (our unpublished data).

7. Discussion

We summarized here that in this survey the mutagenicity of β-carbolines with aromatic amines in the presence of S9 mix is due to the formation of novel mutagenic aminophenyl-β-carboline derivatives. In addition, APNH, the most potent of the coupled compounds formed from β-carboline and aromatic amines, demonstrated severe toxicity in testes and carcinogenicity in the livers of F344 rats. The in vivo toxicity of APNH was much higher than that of other mutagenic HAs, such as MeIQx. It has been reported that norharman also shows co-mutagenicity with 3-aminopyridine, 2-amino-3-methylpyridine, yellow OB and *N*,*N*-diphenylamine, in the presence of S9 mix [1,3]. Although the mechanisms underlying the appearance of mutagenicity by these reactions remain to be clarified, it is likely that similar derivatives to APNH could be formed in presence of S9 mix.

As mentioned above, both β-carbolines and aromatic amines are widely present in food and cigarette smoke and therefore it is likely that humans are simultaneously exposed to both, so that aminophenyl-β-carboline derivatives may be produced in our bodies. *N*-Nitroso compounds are well known as endogenous mutagens and carcinogens, and aminophenyl-β-carboline derivatives may be classified for one of the novel type of endogenous mutagens and carcinogens. Based on the yields of APNH, exposure levels may be lower than with HAs but their health risk in terms of human cancer development might be comparable, because of the stronger genotoxic activity of APNH than HAs. To clarify the effects of APNH and its derivatives on human health, it is important to further elucidate their detailed biological properties.

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References

[1] M. Nagao, T. Yahagi, T. Sugimura, Biochem. Biophys. Res. Comm. 83 (1978) 373.
[2] M. Nagao, T. Yahagi, M. Honda, Y. Seino, T. Matsushima, T. Sugimura, Proc. Jpn. Acad. 53B (1997) 34.
[3] T. Sugimura, M. Nagao, K. Wakabayashi, in: R. Snyder, D.J. Jollow, D.V. Parke, C.G. Gibson, J.J. Kocsis, C.M. Witmer (Eds.), Biological Reactive Intermediates-II, Chemical Mechanisms and Biological Effects (Part B). Plenum Press, NY, 1982, p. 1011.
[4] Y. Totsuka, H. Ushiyama, J. Ishihara, R. Sinha, S. Goto, T. Sugimura, K. Wakabayashi, Cancer Lett. 143 (1999) 139.
[5] H. Ushiyama, A. Oguri, Y. Totsuka, H. Itoh, T. Sugimura, K. Wakabayashi, Proc. Jpn. Acad. 71B (1995) 57.
[6] W. Pfau, K. Skog, J. Chromatogr. B (2003) in press.
[7] F. Luceri, P. Giuseppe, G. Moneti, P. Dolara, Toxicol. Ind. Health 9 (1993) 405.
[8] IARC (International Agency for Research on Cancer), IARC Monographs on the Evaluation of Carcinogenic Risks of Chemicals to Humans, vol. 27, IARC, Lyon, 1982, p. 39.
[9] IARC Monographs on the Evaluation of Carcinogenic Risks of Chemicals to Humans, vol. 27, IARC, Lyon, 1982, p. 155.
[10] M. Riffelmann, G. Muller, W. Schmieding, W. Popp, K. Norpoth, Int. Arch. Occup. Environ. Health 68 (1995) 36.
[11] K.E. Bayoumy, J.M. Donahue, S.S. Hecht, D. Hoffmann, Cancer Res. 46 (1986) 6064.

[12] L.S. DeBruin, J.B. Pawliszyn, P.D. Josephy, Chem. Res. Toxicol. 12 (1999) 78.
[13] R. Kato, Y. Yamazoe, Jpn. J. Cancer Res. (Gann) 78 (1987) 297.
[14] Y. Totsuka, N. Hada, K. Matsumoto, N. Kawahara, Y. Murakami, Y. Yokoyama, T. Sugimura, K. Wakabayashi, Carcinogenesis 19 (1998) 1995.
[15] T. Sugimura, Environ. Health Perspect. 106 (1998) A522.
[16] N. Hada, Y. Totsuka, T. Enya, K. Tsurumaki, M. Nakazawa, Mutat. Res. 493 (2001) 115.
[17] K. Wakabayashi, M. Nagao, H. Esumi, T. Sugimura, Cancer Res. 52 (1992) 2092s.
[18] M. Mori, Y. Totsuka, K. Fukutome, T. Yoshida, T. Sugimura, K. Wakabayashi, Carcinogenesis 17 (1996) 1499.
[19] Y. Totsuka, T. Takamura-Enya, N. Kawahara, R. Nishigaki, T. Sugimura, K. Wakabayashi, Chem. Res. Toxicol. 15 (2002) 1288.
[20] T. Ohe, T. Takata, Y. Maeda, Y. Totsuka, N. Hada, A. Matsuoka, N. Tanaka, K. Wakabayashi, Mutat. Res. 515 (2002) 181.
[21] Y. Totsuka, T. Kataoka, T. Enya-Takamura, T. Sugimura, K. Wakabayashi, Mutat. Res. 506-507 (2002) 49.
[22] F.P. Guengerich, Chem. Res. Toxicol. 14 (2001) 611.
[23] T. Aoyama, F.J. Gonzalez, H.V. Gelboin, Mol. Carcinog. 1 (1989) 253.
[24] Y. Oda, Y. Totsuka, K. Wakabayashi, T. Shimada, Mutat. Res. 483 (Suppl.1) (2001) S132.
[25] Y. Totsuka, T. Kawamori, S. Hisada, K. Mitsumori, J. Ishihara, T. Sugimura, K. Wakabayashi, Toxicol. Appl. Pharmacol. 175 (2001) 169.
[26] T. Kawamori, Y. Totsuka, J. Ishihara, N. Uchiya, T. Sugimura, K. Wakabayashi, Cancer Lett. 163 (2000) 157.
[27] H. Ohgaki, S. Takayama, T. Sugimura, Mutat. Res. 259 (1991) 399.
[28] M. Hirose, K. Wakabayashi, M. Ochiai, H. Kushida, T. Sato, T. Sugimura, M. Nagao, Jpn. J. Cancer Res. 86 (1995) 516.
[29] H. Sone, K. Wakabayashi, H. Kushida, T. Sugimura, M. Nagao, Carcinogenesis 13 (1992) 793.