Bull. Environ. Contam. Toxicol. (1987) 38:565–570

Bulletin of Environmental Contamination and Toxicology

Comparison of the Promutagenic Activity of Liver Homogenates from Fish and Rat in the Ames Test

Mitsuko Yamashita,[1] Naohide Kinae,[1] Isao Tomita,[1] and Ikuo Kimura[2]

[1]Laboratory of Health Science, Shizuoka College of Pharmaceutical Sciences, Shizuoka, 422 Japan, and [2]Laboratory of Viral Oncology, Aichi Cancer Center Research Institute, Nagoya, 464 Japan

Several mutagenic assay systems using bacteria have been developed as pre-screening methods for the detection of carcinogens from the industrial and the environmental materials (Kada 1975; Green and Muriel 1975; Beardmore 1980; Suter and Jaeger 1982). A liver homogenate, so-called S9 (supernatant at 9000 x g), prepared from the livers of rat, mouse or golden hamster, which were administered a certain chemical such as polychlorinated biphenyl (PCB), phenobarbital, 3-methylcholanthrene and flavone is used for the metabolic activation in these systems. Recently, it was demonstrated that the activity of microsomal mixed function oxidase and the content of cytochrome P-450 in fish was increased by exposure to these chemicals (Bend et al. 1977; Usui and Fukami 1978; Elcombe and Lech 1979; Kachole et al. 1979; Stegeman 1980; Gooch and Matsumura 1983) and environmental pollutants (Payne 1977; Stegeman 1978; Kurelec et al. 1979) as observed in mammals.

Several mutagenic and carcinogenic substances were isolated from samples of sediment, sea water and marine fish from the estuary area in which a high incidence of croaker nibe (Nibea mitsukurii) skin tumors were observed (Kinae et al. 1981a; Kinae et al. 1981b; Kimura et al. 1984; Kinae et al. 1986). But, the incidences of other fish diseases in the coastal area were not significant from those of the control areas. These results suggest that nibe has a high metabolic potency against the xenobiotics and are induced the tumor by these materials.

In this paper, we report the comparison of liver microsomal enzymic activities of nibe and carp (Cyprinus carpio), freshwater fish, as well as that of rat with or without PCB administration. We also describe an application of liver homogenates to the bacterial mutagenicity test developed by Ames et al.(1975).

MATERIALS AND METHODS

4-Nitroquinoline-1-oxide (4NQO) and benzo(a)pyrene (BaP) used as

Send reprint requests to N. Kinae at the above address.

reference compounds were purchased from Aldrich Co. 3-Hydroxybenzo(a)pyrene (3-OH-BaP) was kindly supplied by Dr. Hiroo Kinoshita, Kyushy University, Japan.

A rat liver S9 and the S9 mix were prepared from Sprague-Dawley rat (200 g body wt.) according to the method of Ames after the administration of PCB, Kanechlor 500 (10% olive oil soln. 500 mg/kg).

Each five individuals of nibe (80-120 g body wt.) from the non-polluted water or carp (200-250 g body wt.) from the hachery were intraperitoneally administered Kanechlor 500 (10% olive oil soln., 250 mg/kg) 2 times on the first and the third days. During experimental period, the animals were fed freely. After 3 days of the second administration each animal was sacrified and dissected the liver of nibe or the hepatopancreas of carp. The preparation of S9 and S9 mix was done using the same method as that of the rat samples. In the preparation of microsome, an S9 isolated above was centrifuged at 105,000 x g for 60 min. The residue was rinsed with 1.15% KCl and then suspended into 100 mM phosphate buffer solution (pH 7.4).

The AHH activity of the liver homogenates, S9 and microsome, was measured according to the method of Nebert and Gelboin (1968). An aliquot of the sample was mixed with Tris-HCl buffer solution (pH 7.5) containing BaP, NADPH and $MgCl_2$. After the incubation of the reaction mixture at 37°C (rat) or 28°C (fish) for 30 min, the reaction was stopped by the addition of acetone and then treated with n-hexane and 1N NaOH solution. For the determination of the content of 3-OH-BaP formed in each sample, the fluorescent intensity was measured at the wavelengths of the excitation at 396 nm and the emission at 522 nm.

The content of cytochrome P-450 was measured according to the method of Matsubara et al.(1976) and was calculated by the following equation.
P-450(nmole/g tissue) = $(OD_{450}-OD_{490}) \times 1000/104\ mM^{-1}cm^{-1} \times A$.
A is protein content of the sample determined by the Lowry method.

Salmonella typhimurium strains TA98 and TA100 were kindly provided by Dr. B.N.Ames of University of California, U.S.A. The mutagenic assay of each chemical was carried out according to the method of Ames et al.(1975) with some modification (Yahagi et al. 1977). BaP was dissolved in dimethylsulfoxide (DMSO) at an appropriate concentration, and then subjected to the test with and without metabolic activation using S9 or microsomal preparation. The number of His^+ revertant colonies exhibited the mean values of three plates.

RESULTS AND DISCUSSION

Simply state that the fluorescence and the excitation spectra of BaP was changed by the action of S9 liver fractions prepared from PCB-administered rats and nibes as shown in Fig. 1a and b. These

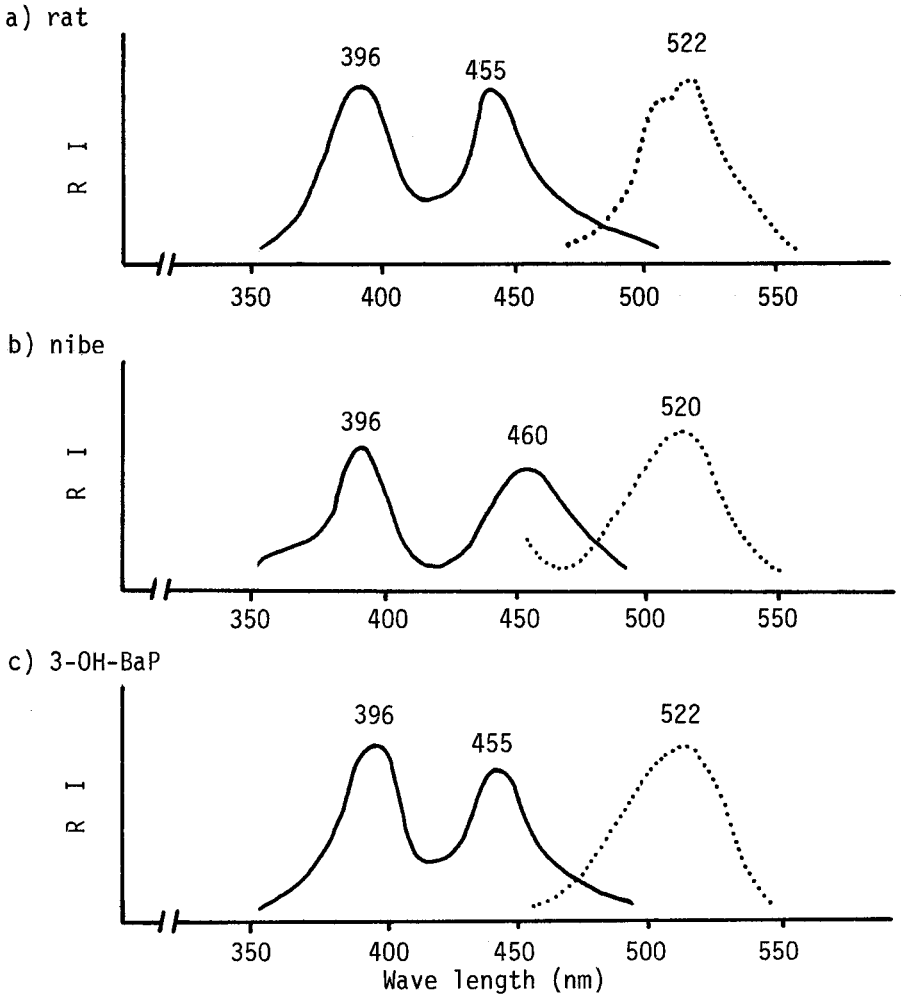

Figure 1. Fluorescent spectra of benzo(a)pyrene after treatment with rat and nibe S9. ——— : fluorescent spectra kept at 522 nm. ····· : fluorescent spectra excited at 396 nm.(sensitivity 11 x 10) RI : relative intensity

spectra were very similar with that of 3-OH-BaP shown in Fig 1c and they were not present before treatment with the liver S9.

We determined the AHH activity and the content of cytochrome P-450 in the liver homogenates from fishes as well as those from rat (Table 1). The AHH activity of the liver S9 from nibe and carp increased 1.2 - 5 times by the PCB treatment. The activity of each microsome were 3 - 5 times higher than that of S9. But the values of the AHH activity and the P-450 content of the liver samples from carp and nibe were very low compared with those of rat. These results suggest that aquatic animals such as nibe and carp can metabolize the xenobiotics, but the metabolization potency was one third to one tenth of that of rat.

Table 1 AHH activity and cytochrome P-450 content in the liver samples from fish and rat

Samples	PCB-treatment	AHH activity[a] (nmole/mg protein)	Cytochrome P-450 (nmole/g)
Nibe S9[b]	-	0.50	1.71
	+	2.53	7.12
Nibe M[c]	-	1.38	2.53
	+	3.91	16.9
Carp S9	-	1.21	0.99
	+	1.60	2.70
Carp M	-	3.55	2.70
	+	9.17	12.2
Rat S9	+	9.87	11.6
Rat M	+	1060	1260

a) 3-hydroxybenzo(a)pyrene formed b) supernatant at 9000 x g
c) microsome fraction

Table 2 Mutagenic activity of benzo(a)pyrene after incubation with liver S9 from PCB-administered animals

Strains	S9 content (μl)	Mutagenicity (Net His^+ revs.)	Specific activity (Revs./mg protein)
TA98	Nibe(50)	99	85
	" (100)	299	256
	Carp(50)	62	62
	" (100)	90	90
	Rat (50)	463	691
	" (100)	750	1119
TA100	Nibe(50)	216	185
	" (100)	820	703
	Carp(50)	89	89
	" (100)	95	95
	Rat (50)	889	1327
	" (100)	825	1179

Five μg of BaP was used per plate. The control values (TA98, 42; TA100, 100) were subtracted.

We tried to use liver S9 prepared from PCB-administered fish as a tool of the metabolic activation in the Ames test. The mutagenic activity of BaP with S9 from rat and fish was shown in Table 2. In both strains, His^+ revertant colonies of BaP were induced as the amount of S9 increased in three animals. At the dose of 5 μg of BaP, 820 revertants were induced in the S.typhimurium TA100 by using 100 μl of nibe S9. The induction effect of mutagenic activity of each liver S9 was proportional to their AHH activity and the P-450 content. From these experiments, it was found that the metabolization potency of the nibe liver S9 against BaP was not

superior to that of rat, but it was 2 - 2.5 fold higher than that of carp.

Recently, an application of fish liver S9 to the mutagenicity test was discussed(Guobaitis et al. 1986; Milling and Maddock 1986). We are trying to examine the mutagenic activity of several environmental contaminants by using these fish liver S9. The data will be presented in the next paper.

REFERENCES

Ames BN, McCann D, Yamasaki E (1975) Methods for detection carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat Res 31:347-364

Beardmore JA (1980) Genetic considerations in monitoring effects of pollution. Rapp P-V Réum Cons Int Explor Mer 179:258-266

Bend JR, James MO, Dansette PM (1977) In vitro metabolism of xenobiotics in some marine animals. Ann New York Acad Sci 298:505-521

Elcombe CR, Lech JJ (1979) Induction and characterization of hemoprotein(s) P-450 and monooxygenation in rainbow trout(Salmo gairdneri). Toxicol Appl Pharmacol 49:437-450

Gooch JW, Matsumura F (1983) Characteristics of the hepatic monooxygenase system of the goldfish (Carassius auratus) and its induction with β-naphthoflavone. Toxicol Appl Pharm 68:380-391

Green MHL, Muriel WJ (1976) Mutagen testing using Trp^+ reversion in Escherichia coli. Mutat Res 38:3-32

Guobaitis RJ, Ellingham TJ, Maddock MB (1986) The effects of pretreatment with cytochrome P-450 inducers and preincubation with a cytochrome P-450 effector on the mutagenicity of genotoxic carcinogens mediated by hepatic and renal S9 from two species of marine fish. Mutat Res 164:59-70

Kachole MS, Pawar SS, Mahajan AG (1979) Effect of phenobarbital and hexobarbital treatment on Lepidocephalichthys thermalis, a fresh water fish. Bull Environ Contam Toxicol 21:488-491

Kada T (1975) Mutagenicity and carcinogenicity screening of food additives by the Rec-assay and reversion procedures, in "Screening tests in chemical carcinogenesis" In: Montesano R, Bartsch H, Tomatis L (ed) IARC Scientific Publication No.12:105-115

Kimura I, Taniguchi N, Kumai I, Tomita I, Kinae N, Yoshizaki K, Ito M, Ishikawa T (1984) Correlation of epizootiological observations with experimental data: Chemical induction of chromatophoromas in the croaker Nibea mitsukurii. National Cancer Inst Monogr 65 Use of small fish species in carcinogenicity testing 139-154

Kinae N, Hashizume T, Makita T, Tomita I, Kimura I (1981a) Studies on the toxicity of pulp and paper mill effluents(I). Mutagenicity of sediment samples derived from kraft pulp mills. Water Res 15: 17-24

Kinae N, Hashizume T, Makita T, Tomita I, Kimura I (1981b) Studies on the toxicity of pulp and paper mill effluents(II). Mutagenicity of the extracts of the liver from spotted sea trout (Nibea mitsukurii). Water Res 15:25-30

Kinae N, Yamashita M, Takebuchi K, Ichinose N, Tomita I, Kimura I (1986) Detection of mutagenic compounds in areas inhabited by

Nibea mitsukurii and their tumor-inducing effects in fish. J Hygienic Chem (in Japan) 32:p-10
Kurelec B, Matijasevic Z, Rijavec M, Alacevic M, Britvic S, Müller WEG, Zahn RK (1979) Induction of benzo(a)pyrene monooxygenase in fish and the Salmonella test as a tool for detecting mutagenic/carcinogenic xenobiotics in the aquatic environment. Bull Environ Contam Toxicol 21:799-807
Matsubara T, Koike M, Touchi A, Tochino Y, Sugeno K (1976) Quantitative determination of cytochrome P-450 in rat liver homogenate. Anal Biochem 75:596-603
Milling DM, Maddock MB (1986) Activation and detoxication of promutagens by toadfish (Opsanus tau) hepatic postmitochondrial fractions in the Salmonella assay. Mutat Res 164:81-89
Nebert D W, Gelboin HV (1968) Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. J Biol Chem 243:6242-6249
Payne JF (1977) Mixed function oxidases in marine organisms in relation to petroleum hydrocarbon metabolism and detection. Mar Poll Bull 8:112-116
Stegeman JJ(1978) Influence of environmental contamination on cytochrome P-450 mixed-function oxygenases in fish: Implications for recovery in the wild harbor marsh. J Fish Res Board Can 35: 668-674
Stegeman JJ (1980) Mixed function oxygenase studies in monitoring for effects of organic pollution. Rapp P-V Réum Cons Int Explor Mer 179:33-38
Suter W, Jaeger I (1982) Comparative evaluation of different pairs of DNA repair-deficient and DNA repair-proficient bacterial tester strains for rapid detection of chemical mutagens and carcinogens. Mutat Res 97:1-18
Usui T, Fukami J (1978) Comparative study of microsomal mixed-function oxidases in several animals, with special references to contents cytochrome P-450 and oxidase activity. J Pest Sci (in Japan) 3:149-154
Yahagi T, Nagao M, Seino Y, Matsushima T, Sugimura T, Okada M(1977) Mutagenicities of N-nitrosamines on Salmonella. Mutat Res 48:121-130

Received September 18, 1986; accepted November 24, 1986