Original Articles

Induction of Hepatic Drug Metabolizing Enzymes and Mutagen-activation Capacity in Several Animal Species

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To improve Ames' chemical carcinogen screening test system, several species of animals including rats, mice, guinea pigs, hamsters and rabbits were pre-treated with polychlorinated biphenyl (PCB), 3-methylcholanthrene (MC) and phenobarbital (PB), and hepatic S-9 fraction obtained therefrom was tested as to the effects on the increase of revertant colonies of *S. typhimurium* TA98 and TA100. Among 12 strains of the 5 mammalian species, S-9 fraction from PCB-treated Hartley guinea pig proved to be more effective than that from PCB-treated SD rat in detecting three different types of mutagens, Benzo(a)pyrene, 2-acetylaminofluorene and dimethylnitrosamine. The methylcholanthrene-pretreated guinea pig (Hartley) liver S-9 was equally effective to the rat preparation and PCB-treated hamster S-9 fractions were effective to DMN mutagenesis, whereas Wistar and Fisher rats, ICR and dd mice and rabbits were generally no better than SD rat.

INTRODUCTION

One of the detection methods for mutagenicity and carcinogenicity of environmental chemicals including pesticides has been developed by Ames *et al.*^{1,2)} which utilizes several strains of *Salmonella typhimurium* combined with *in vitro* drug metabolizing enzyme systems (S-9) from rat liver. Although Sprague Dawley rats have been often used as the metabolizing enzyme source^{1,2)} in such trials, S-9 fractions of other mammalian species might be better, since metabolic pathways of a compound as well as its carcinogenic potential are varied with mammalian species. The mouse and other species were used in some cases,^{3,4)} but any attempts have hardly been made so far to compare the capacity of hepatic preparations obtained from various mammalian species including rabbits, guinea pigs and hamsters. Therefore, studies were initiated to select the most pertinent mammalian species for preparation of S-9 fraction among rats, mice, rabbits, guinea pigs and hamsters, after

pre-treatment with 3 different types of inducers of drug metabolizing enzymes, polychlorinated biphenyl (PCB), phenobarbital (PB) and 3-methylcholanthrene (MC).

MATERIALS AND METHODS

1. Special Chemicals

Benzo(a)pyrene (BaP), 2-acetylaminofluorene (AAF), dimethylnitrosamine (DMN) and 3-methylcholanthrene (MC) were obtained from Wako Pure Chemical (Osaka). Sodium phenobarbital (PB) and NADPH were purchased from Fuzinaga Chemical (Tokyo) and Oriental Yeast (Osaka), respectively. Polychlorinated biphenyl (PCB, Kanechlor 400) was kindly supplied by Prof. N. Ito of Nagoya City University Medical school.

2. Animals

The following animals species were used in the present experiment; 5 week old Sprague Dawley (SD) (male and female), Fisher (male) and Wistar (male) rats weighing 160-200 g; 6-week-old male ICR and dd mice, 20-30 g;

5-week-old male Golden (80–100 g) and Chinese (20–30 g) hamsters; 6-week-old male Hartley and hybrid guinea pigs, 350–400 g; 10-week-old male New Zealand White rabbits and Japanese native strain rabbits, 1.9–2.3 kg. Rats and mice were obtained from Nihon Clea (Osaka), and other animal species, from Nihon Dobutsu (Osaka).

3. Bacteria

Salmonella typhimurium TA98 and TA100²⁾ were supplied by Dr. B. N. Ames, University of California.

4. Pre-treatment of the Animals with PCB, MC and PB

PCB and MC were dissolved in corn oil (10%) and administered once to the animals intraperitoneally at 0-day. The administered level of PCB was 500 mg/kg for all animals, and MC was given at the rate of 80 mg/kg to rats, mice, hamsters and guinea pigs, and 50 mg/kg to rabbits. PB was dissolved in physiological saline (10%) and given intraperitoneally once a day for five days. The dose levels were 80 mg/kg/day for rats, mice, and hamsters, 50 mg/kg/day for rabbits, and 40 mg/kg/day for guinea pigs.

5. S-9

The hepatic S-9 fraction of each animal species was prepared by the method described by Ames *et al.*²⁾

6. Mutation Test

The mutagenic potentials of BaP, AAF and DMN were tested on *S. typhimurium* TA98 and TA100 in the presence of S-9 fraction, according to the procedures reported elsewhere,⁶⁾ which were essentially the same as Ames' method.^{1,2)} The number of spontaneous revertant colonies was 15–40/plate in TA98 and 80–160/plate in TA100 after 48 hr incubation at 37°C.

7. Measurement of Cytochrome P-450 Content

The contents of cytochrome P-450 (P-450) in S-9 fraction were measured according to the methods described by Ohmura and Sato.⁷⁾

Fig. 1 Cytochrome P-450 contents in hepatic S-9 from several animal species after administration of PCB, PB or MC.

○ rat, ● mouse, □ guinea pig, ■ rabbit, △ hamster.

RESULTS AND DISCUSSIONS

The drug metabolizing capacities of S-9 fraction are known to depend to some degrees on the quantity of P-450, so, the levels of hepatic P-450 in S-9 fraction were measured in SD rats, ICR mice, Golden hamsters, Hartley guinea pigs and New Zealand White rabbits after intraperitoneal application of the three inducers. Figure 1 presents the results. In PCB-treated animals, P-450 contents increased in all species and the maximum levels were observed on 5th day after PCB treatment, 1.8–2.9 times higher than the controls. By consecutive treatment with PB, P-450 increased

in all animals during the administration, which, however, tended to decrease on cessation of PB treatment. The maximum levels were 2–3 times higher than the controls. MC treatment did not affect P-450 levels rats and mice during the subsequent 7 days, while in hamsters, guinea pigs and rabbits P-450 levels increased and reached maximum on 5th day after administration.

To confirm the correlation of P-450 levels with mutation-inducing capacities, S-9 fraction prepared from SD rats was used for mutation test with BaP, AAF or DMN. Although the data are not presented here, the number of mutants caused by BaP, AAF and DMN was increased by PCB-treatment and the maximum was observed with S-9 prepared at 3 to 5 days after treatment. By PB-treated S-9, only AAF increased mutation, and the fraction obtained at 5 days post-treatment was most effective.

On the 3rd to 5th day after MC-treatment the S-9 fraction increased the mutation by BaP and AAF appreciably, in contrast to the unaltered content of P-450 in rats and mice. DMN-induced mutation was not affected.

Thus, roughly, the higher P-450 contents reflected the increased number of the mutants. Based on the above experiments, effects of the inducers on mutagen-activating capacities of S-9 fraction were tested by sacrificing the animals at 5 days after the treatments. The animals pre-treated with MC were also sacrificed after 24 hr, as is usually the case.¹⁾ The effects on reverse mutations of S-9 fraction obtained from the liver of various animal species pre-treated with PCB, PB or MC are collectively summarized in Table 1. All the examinations were carried out at three or more concentrations of the mutagens, replicated at least three times, as typical examples are given in Fig. 2. However, to facilitate the comparison, the data with one concentration are presented in Table 1, namely BaP 10 $\mu\text{g}/\text{plate}$, AAF 80 $\mu\text{g}/\text{plate}$ and DMN 3,000 $\mu\text{g}/\text{plate}$. As DMN did not substantially induce reverse mutation in the TA98 strain in the presence of S-9 of any mammalian species, the results are not included in the Table.

As evident in Table 1, generally two strains of mice, Fisher, Wister rats were no better

Fig. 2 Increase of *S. typhimurium* reverse mutants in the presence of S-9 fractions obtained from several animal species pretreated with inducers.

- A) Mutants of TA98 strain by BaP in the presence of PCB-treated SD rat and PCB-treated Hartley guinea pig S-9.
 B) Mutants of TA98 strain by AAF in the presence of PB-treated guinea pig and PCB-treated SD rat S-9.
 C) Mutants of TA100 strain by DMN in the presence of PCB-treated Golden hamster and PCB-treated SD rat S-9.

□ PCB-Hartley guinea pig, ○ PCB-SD rat, △ PCB-Golden hamster.

than SD rats, and male SD rats were better than the females. Some of the non-treated (control) animal species, especially guinea pigs, rabbits and hamsters gave the same levels of mutants as, or in some cases higher than, PCB-treated SD rat. Among the three inducers, PCB was generally most effective in increasing

Table 1 Number of mutants of *S. typhimurium* TA 98 and TA100 caused by BaP, AAF, or DMN combined with S-9 fractions obtained from inducer-treated animals species.

Animal species	Strains	Inducers	Increasing number of revertants/plate				
			TA98		TA100		
			BaP	AAF	BaP	AAF	DMN
Rat	SD (male)	control	24	361	112	120	183
		PCB	125	728	518	427	616
		PB	43	595	123	247	185
		MC (1)*	37	407	219	255	158
		MC (5)*	42	401	362	269	129
	SD (female)	control	18	109	64	74	77
		PCB	32	163	283	342	1347
		PB	22	79	130	131	143
		MC (1)*	23	201	187	175	159
		MC (5)*	21	166	206	134	87
	Fischer	control	25	98	85	79	111
		PCB	30	350	260	167	727
		PB	13	80	64	61	87
		MC (1)*	22	124	112	84	83
		MC (5)*	26	130	193	191	225
	Wistar	control	40	332	122	149	115
		PCB	56	563	486	375	269
		PB	21	313	140	133	251
		MC (1)*	57**	437**	272	177	167
		MC (5)*	345**	730**	378	308	231
Mouse	dd	control	27	245	108	164	245
		PCB	18	1335	198	586	846
		PB	14	462	128	207	182
		MC (1)*	24	920	518	609	311
		MC (5)*	15	434	264	344	322
	ICR	control	28	39	86	93	291
		PCB	24	334	147	263	523
		PB	21	140	91	115	323
		MC (1)*	14	21	75	78	140
		MC (5)*	27	78	108	97	138
Guinea pig	Hartley	control	132	1723	558	95	260
		PCB	413	1773	751	720	753
		PB	109	1997	360	111	400
		MC (1)*	475	1555	523**	271**	359**
		MC (5)*	597	1718	544	542	692
	hybrid	control	32	1483	166	602	138
		PCB	85	1894	352	266	1029
Rabbit	New Zealand White	control	31	879	231	515	176
		PCB	84	1642	648	335	273
		PB	17	1603	150	691	782
		MC (1)*	38	763	244	350	150
		MC (5)*	108	1646	733	404	284
	Japanese native	control	21	77	135	113	136
		PCB	42	1769	268	342	493

Table 1. (continued)

Animal species	Strains	Inducers	Increasing number of revertants/plate				
			TA98		TA100		
			BaP	AAF	Bap	AAF	DMN
Hamster	Golden	control	19	1327	195	904	929
		PCB	71	1365	301	319	1327
		PB	31	1608	177	710	1157
		MC (1)*	104	1598	838**	282**	984**
		MC (5)*	54	1775	361**	491**	1925**
	Chinese	control	42	2000	274	587	613
		PCB	35	2022	166	127	1168

* [MC (1)] and [MC (5)] indicate 1 and 5 day samples after MC treatment, respectively.

** These samples gave high background reversion levels.

Dosage levels of mutagens were 10 µg/plate for BaP, 80 µg/plate for AAF and 3 000 µg/plate for DMN.

reverse mutation in TA98 and TA100, followed by MC and PB. PB-treatment was effective only on a limited part of AAF-induced mutagenesis. MC-treatment was effective on BaP- and AAF-mutagenesis, but in some cases it gave high background level of mutants, disturbing to accurately count the actual reversion by the mutagens, probably because of contaminated mutagen(s) which might be derived from pre-treated MC. The 5th day S-9 fractions [MC(5)] were more effective than those of the first day [MC(1)] in all species except dd strain mouse.

Among 51 species-inducer combinations including the control (non-treated), several of the highly efficient S-9 fractions are listed:

1) Bap-induced mutation

TA98;

PCB-, MC(1)- and MC(5)- Hartley guinea pig

TA100

PCB- and MC(5)- New Zealand White rabbit

PCB- and MC(5)- Hartley guinea pig

PCB- and MC(5)- Wister and SD rat

MC(1)- dd mouse

2) AAF-induced mutation

TA98;

PB, PCB, MC(5)- and control- Hartley guinea pig

PCB- hybrid guinea pig

MC(5)- New Zealand White rabbit

MC(5)- Japanese native rabbit

MC(5)- Golden hamster

PCB- and control- Chinese hamster

TA100;

PB- and control- Golden hamster

PCB- and MC(5)- Hartley guinea pig

PB- and control- New Zealand White rabbit

control- hybrid guinea pig

control- Chinese hamster

3) DMN- induced mutation

TA100;

PCB-, PB- and control- Golden hamster

PCB- Chinese hamster

PCB- SD rat (female)

PCB- hybrid and Hartley guinea pig

Thus, depending on the combination of mutagen tested and bacterial strain used, the best S-9 fraction (or more exactly mammal-inducer combination) was different. Moreover, as shown in Fig. 2, the optimal concentration of mutagen giving rise to maximum revertant colonies was variable with S-9 fraction used.

Because of the above reasons, it is not easy to specify one mammalian species-inducer combination as the best S-9 fraction to be applicable to any mutagen to be tested. However, since the PCB-induced Hartley guinea pig S-9 was generally more effective in increasing the revertant colonies of both *S. typhimurium* TA98 and TA100 by 3 different types of mutagens, the S-9 fraction can be proposed to be even better than PCB-induced SD rat which is commonly used. MC-induced Hartley guinea pig liver preparation [MC(5)] can be also used instead of PCB-induced SD rat, as the former gave as many mutant colonies as the latter. Moreover, DMN induced mutants of TA100

were remarkably more increased by S-9 fractions from PCB-treated both hamster strains than by that of Hartley guinea pig.

Guinea pigs have not been considered so far a species suitable for carcinogenicity study, and rats and mice are the preferred experimental animals. So, for S-9 fraction in the Ames test detecting carcinogens as mutagens, the rat preparation might be better for the coherence purpose. Actually, in one of their recent report Ames *et al.*⁸⁾ stated that approximately 90% of various types of chemical carcinogens were detected as mutagens by *S. typhimurium* strains combined with rat S-9 fraction. Moreover, for example, guinea pigs were reportedly not susceptible to carcinogenic potential of AAF due to the lack of AAF N-hydroxylase activity.⁹⁾ However, since rat hepatic S-9 fraction is not intrinsically the best drug metabolizing enzyme source, and that metabolic pathways in a mammalian species are one of the prerequisites for a certain type of chemicals to be carcinogen, S-9 fraction prepared from their species of mammals than rats including guinea pigs might be used to enable Ames and similar carcinogen-screening tests to be more sensitive and more reliable.

要 約

突然変異性発ガン性化合物のスクリーニング方法として一般的に用いられている Ames の方法について、その *in vitro* 代謝系の改良をするため、PCB、フェノールピタールおよび 3-メチルコラントレンのそれぞれを投与し薬物代謝系を誘導したラット、マウス、モルモット、ハムスター、およびウサギよりの肝臓S-9画分を用い、

サルモネラ菌 TA98 株、TA100 株の突然変異体出現数の変化を調べた。

5 動物種 12 系統のなかで、PCB 処理ハートレー系モルモットより得た S-9 画分は、3 種の異なった化合物 (ベンツ (a) ピレン, 2-アセチルアミノフルオレン, ジメチルニトロソアミン) の変異性検出において、通常用いられている PCB 処理 SD ラットよりすぐれていた。また 3-メチルコラントレン処理モルモット (ハートレー系) は、PCB 投与 SD ラットとほぼ同等であることが認められ、ハムスターの S-9 は、DMN の突然変異の検出に有効であった。

一方、ウィスター系およびフィッシャー系ラット、ICR 系、dd 系マウス、ウサギ、などは、SD 系と比較してとくにすぐれているとはみせない。

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