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Strain-dependent differences in mutagenicity and genotoxicity of cyclophosphamide in mice

Anna Ejchart and Jadwiga Koziorowska

Drug Institute, Chełmska 30/34, 00-725 Warsaw, Poland

The C57B/6N (Ah^b) and DBA/2N (Ah^d) strains of mice differ in the inducibility of aryl hydrocarbon hydroxylase by 3-methylcholanthrene [1]. This difference is reflected by differences in mutagenic activity of the promutagens/procarcinogens which require metabolic activation [2, 3]. In our previous work we have found differences in the mutagenic activity of benzo[a]pyrene and 2-acetylaminofluorene when the liver S9 fractions from phenobarbital (PB[1])-pretreated B10.A (Ah^b) and DBA/2 (Ah^d) mice were used [4].

The present investigation was undertaken to compare the metabolizing activity of the liver S9 fractions derived from PB-pretreated B10.A and DBA/2 mice towards cyclophosphamide (CP) in the *Salmonella* test, and to examine whether the PB-pretreatment affects the incidence of micronuclei in bone marrow of these mice. CP is a commonly used chemotherapeutic drug but also is a well-known promutagen [5] and a micronucleus inducing agent [6 - 9].

In parallel, cytochrome P-450 contents in liver tissue were determined [10].

The mutagenicity assays of CP, performed according to Maron & Ames [11] in the presence of S9 fractions from the control and PB-pretreated B10.A and DBA/2 mice, showed dose effect-curves (Fig.1). The increasing doses of CP raised the revertant number only in the presence of S9 fractions derived from PB-pretreated DBA/2 mice. Table 1 summarizes the results of micronuclei analysis in bone marrow of both strains of mice exposed to 50 mg/kg of CP or to the same dose of CP plus 100 mg/kg

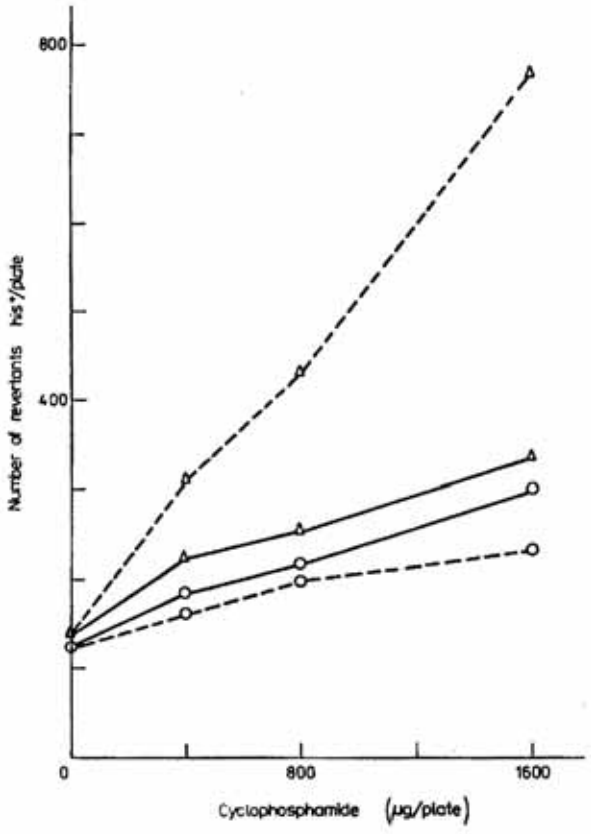

Fig. 1. Mutagenic activity of CP in the presence of S9 fractions of PB-pretreated (Δ) and untreated (○) B10.A (—) or DBA/2 (---) mice

of PB. The frequency of micronuclei in polychromatic erythrocytes was higher in bone marrow of DBA/2 mice after CP injection and PB-pretreatment but was not altered in the bone marrow of B10.A mice. The cytochrome P-450 content in the livers of both strains of

[1]Abbreviations: CP, cyclophosphamide; PB, phenobarbital

Table 1

Incidence of micronucleated polychromatic erythrocytes (PCE) in the marrow of B10.A and DBA/2 mice treated either with cyclophosphamide (CP) or with CP after phenobarbital (PB)-pretreatment

Mice	Treatment (mg/kg)	Number		Micronucleated PCE % (±SE)
		PCE	Micronucleated PCE	
B10.A	Control	9860	30	0.3 (± 0.02)
	CP 50	14994	523	3.5 (± 0.13)
	PB 100 + CP 50	8200	289	3.5 (± 0.10)
DBA/2	Control	8100	21	0.2 (± 0.10)
	CP 50	11300	476	4.2 (± 0.40)
	PB 100 + CP 50	10700	684	6.3 (± 0.70)*

*Significant at $P < 0.001$ as referred to the CP treated mice

Table 2

The influence of cyclophosphamide (CP) and phenobarbital (PB) on the total cytochrome P-450 content in the S9 liver fractions from B10.A and DBA/2 mice (nm/g wet liver ±S.E.)

Treatment	B10.A	DBA/2
Control	17.0 (± 1.6)	19.7 (± 1.2)
CP (50 mg/kg)	21.9 (± 1.6)	19.2 (± 1.2)
PB (100 mg/kg)	23.7 (± 1.0)*	24.7 (± 0.9)*
PB + CP	18.9 (± 1.3)	26.2 (± 0.9)*

*Difference significant at $P < 0.002$ as referred to the controls

mice (Table 2) was similar in control animals and was increased after PB-pretreatment. CP did not change significantly the level of cytochrome P-450 in the S9 fractions from livers of either strain. However, an interstrain difference was observed when CP was given after PB-pretreatment ($P < 0.002$).

The results of our experiments demonstrated strain dependent differences both in the degree of bone marrow chromosome damage and mutagenicity but only when mice were pretreated with PB or when S9 fractions from PB-pretreated mice were used. This suggests that the enzyme system activating CP to a clastogen or to a bacterial mutagen is not inducible in the livers of B10.A mice.

CP is metabolically activated by the PB-inducible forms of cytochrome P-450 [12]. It has been found [13] that mouse P-450 isoenzymes show large variation in their inducibility and that individual PB-inducible P-450 isoenzymes are regulated in different ways. The results shown in this report indicate, however, that the level of cytochrome P-450 after PB-pretreatment increased in a similar way in both strains of mice but after CP treatment in the PB-pretreated animals it decreased, compared to the sole PB administration, exclusively in B10.A mice. This effect could be caused by inactivation of cytochrome P-450 by CP in this strain. Inactivation of cytochrome P-450 by CP in rats has been described by other authors [14]. However, it is not excluded that the elevated cytochrome P-450 content persisting in PB-pretreated and CP-treated DBA/2 mice could depend on some inherent properties of this mouse strain.

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