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Decreased Liver and Lung Drug-metabolizing Activity in Mice Treated with *Corynebacterium parvum*[1]

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SUMMARY

Injections of killed suspensions of *Corynebacterium parvum* (i.p.) in young male mice were followed by time- and dose-dependent decreases in the drug-metabolizing activity of liver microsomes and lung homogenates. *In vitro* assays with model substrates [aminopyrine, aniline, *p*-nitroanisole, and benzo(*a*)pyrene] were used to quantitate drug-metabolizing activity. It is likely that such decreases in mixed-function oxidase activity will act to significantly alter the pharmacokinetics of concurrently or subsequently administered drugs. The results provide a possible mechanism to explain several previously reported immunochemotherapeutic interactions.

INTRODUCTION

Killed suspensions of CP[2] have been tested clinically as immunotherapeutic agents for patients with cancer. A recent symposium (7) and a review (11) have summarized much of the experimental and clinical data.

It has been suggested that at least part of the utility of such treatment is that patients tolerate larger doses of chemotherapy (2). Furthermore, it was reported that CP increased the toxicity of short-acting barbiturate anesthetics but not that of diethyl ether (9). Castro (1) commented on the abnormal sensitivity to Nembutal of mice treated i.v. with CP, and ascribed this to parenchymal damage. Foster (5) found that pretreatment with CP increased the hematopoietic toxicity of a cell cycle-specific chemotherapeutic agent. We therefore investigated the possibility that CP might alter drug metabolism by means of *in vitro* assays with model substrates for the mixed-function oxidases of hepatic microsomes and lung homogenates.

MATERIALS AND METHODS

Animals. Male C57BL/6J mice from The Jackson Laboratory, Bar Harbor, Maine, were maintained under standard conditions. The animals were 44 to 58 days of age at the beginning of the experiments.

Treatment. CP was obtained from Burroughs-Wellcome (Lot 997-0), Research Triangle Park, N. C., as a suspension of washed, formalin-killed organisms in physiological pyrogen-free 0.9% NaCl solution containing 0.01% thiomersal. A preparation, 7 mg dry weight per ml, was injected i.p.

Drug Metabolism Assays. These are described in detail elsewhere (13). Briefly, microsomes were prepared and the production of colored or fluorescent metabolites from aminopyrine, aniline, *p*-nitroanisole, and benzo(*a*)pyrene was measured after incubation at 37° in air in the presence of a NADPH-generating system. Reaction rates were linear over the short incubation periods (3 to 30 min). Assays were selected as representative of type I (aminopyrine) and type II (aniline) substrate reactions, and *p*-nitroanisole and benzo(*a*)pyrene were selected as representative of those reactions dependent on cytochrome P_1-450. The latter is commonly referred to as AHH activity. Only AHH activity was studied with lung homogenates.

One-g aliquots of liver were homogenized in 6 ml of isotonic sucrose-EDTA-Tris buffer (pH 7.8) at 4° and centrifuged at 10,000 × *g* for 20 min. The supernatant was then centrifuged at 100,000 × *g* for 60 min, and the resultant pellet was resuspended in 4.5 ml of the buffer. Each sample was assayed in duplicate.

Formaldehyde production from aminopyrine was trapped with Nash's reagent, and the increase in absorbance compared to that from a nonincubated control from the same mouse was determined at 412 nm in a spectrophotometer (Gilford Instrument Co., Oberlin, Ohio).

p-Aminophenol produced by hydroxylation of aniline was coupled with phenol reagent and determined at 630 nm.

p-Nitrophenol resulting from *O*-demethylation of *p*-nitroanisole was determined in alkali at 400 nm.

AHH activity was determined by the method of Nebert and Gelboin (10), except that the substrate was dissolved in acetone and NADH was added, as well as NADPH. The fluorescence of the alkaline-extractable metabolites was determined in an Aminco-Bowman spectrofluorophotometer (Aminco-Bowman Co., Silver Springs, Md.), excitation 396 nm, emission 522 nm. A quinine sulfate solution was used for daily standardization of the instrument.

Substrates were prepared daily. Reagents were obtained from Sigma Chemical Co., St. Louis, Mo.

Microsomal protein concentration was determined by the method of Lowry *et al.* (8), with bovine serum albumin as the standard.

Experimental Design. Two studies were performed, one a time-course experiment in which serial measurements were made in 6 groups of mice, ranging from 1 hr to 21 days following CP. In the 1st study, all CP injections were in amounts of 350 μg. The 2nd was a dose-response study,

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[2] The abbreviations used are: CP, *Corynebacterium parvum*; AHH, aryl hydrocarbon hydroxylase.

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using varied amounts (22, 88, and 175 μg).

Statistical Analysis. Data of the time-course experiments were compared by Student's 1-tailed t test, whereas those of the dose-response experiment were analyzed by analysis of variance and Newman-Keuls tests.

RESULTS

Time-Course Experiment

Organ and Body Weights. The most marked effect was an increased weight of the spleen in animals injected with 350 μg CP i.p. (Table 1). Three days after treatment, spleen weights were approximately double those of controls and, by 7 days, spleens had tripled in weight. Splenomegaly slowly declined, but remained significantly elevated for the full 3 weeks of the study. Liver weight increased in the treated animals to a lesser degree and duration than spleen weight. Hepatomegaly was no longer present at the end of 3 weeks. Lung and body weights of treated animals did not differ from those of controls.

Drug Metabolism. Yields of microsomal protein were decreased 3 days after CP, returned to the control level by 7 days, and then exceeded those of controls at 14 days, before returning to the base line (Table 2).

The specific activities for aminopyrine *N*-demethylase, aniline hydroxylase, and *p*-nitroanisole *O*-demethylase were decreased from 1 to 14 days following CP treatment. AHH activity in liver microsomes decreased more slowly and was significantly lower than controls only at 14 days ($p < 0.01$). In contrast, AHH activity in lung homogenates decreased to a significantly lower level by Day 1. Lung AHH activity remained depressed throughout the 3 weeks of the experiment; however, because of the low level of activity in lung and considerable variability, the depression did not reach statistical significance at 14 and 21 days.

Table 1

Effect of CP on body and organ weights

Time after injection	Wt (g) in Control	Wt (g) in CP-treated mice	CP/control × 100
	Body		
1 day	23.0 ± 0.9[a]	23.9 ± 1.1	104
3 days	20.9 ± 0.6	21.0 ± 0.3	100
7 days	22.6 ± 0.9	21.8 ± 0.5	96
14 days	23.5 ± 1.0	22.0 ± 0.5	94
21 days	24.2 ± 0.9	22.8 ± 0.7	94
	Liver		
1 hr	1.12 ± 0.05	1.34 ± 0.04	119[b]
1 day	1.05 ± 0.03	1.20 ± 0.09	114
3 days	1.24 ± 0.05	1.54 ± 0.04	124[b]
7 days	1.38 ± 0.10	1.78 ± 0.09	128
14 days	1.30 ± 0.06	2.00 ± 0.04	154[b]
21 days	1.40 ± 0.08	1.40 ± 0.11	100
	Spleen		
1 hr	0.055 ± 0.002	0.057 ± 0.003	104
1 day		Not done	
3 days	0.047 ± 0.003	0.095 ± 0.006	202[b]
7 days	0.056 ± 0.003	0.180 ± 0.007	321[b]
14 days	0.062 ± 0.002	0.150 ± 0.02	242[b]
21 days	0.052 ± 0.004	0.090 - 0.009	173[b]

[a] Mean ± S.E.; $N = 5$.
[b] Significant at $p < 0.01$; 1-tailed t test.

Table 2

Effect of CP on hepatic microsomal protein, enzyme activity, and lung homogenate AHH activity

Time after injection	Control	CP	CP/control × 100
	Microsomal protein (mg/g wet wt)		
1 hr	21.3 ± 1.04[a]	18.9 ± 0.7	89
1 day	21.2 ± 0.5	20.5 ± 0.9	97
3 days	18.5 ± 0.8	16.4 ± 0.6	89
7 days	19.5 ± 1.6	18.4 ± 0.7	94
14 days	18.1 ± 0.5	21.0 ± 0.5	116[b]
21 days	19.7 ± 1.0	20.1 ± 0.5	102
	Aminopyrine demethylase (Δ A/mg/3 min)		
1 hr	0.139 ± 0.020	0.120 ± 0.004	86
1 day	0.072 ± 0.006	0.067 ± 0.010	83[b]
3 days	0.134 ± 0.004	0.104 ± 0.005	78[b]
7 days	0.126 ± 0.008	0.088 ± 0.002	70[b]
14 days	0.130 ± 0.001	0.106 ± 0.010	82[b]
21 days	0.114 ± 0.004	0.108 ± 0.005	95
	Aniline hydroxylase (Δ A/mg/10 min)		
1 hr	0.116 ± 0.009	0.116 ± 0.003	100
1 day	0.120 ± 0.005	0.072 ± 0.006	60[b]
3 days	0.088 ± 0.002	0.064 ± 0.001	73[b]
7 days	0.083 ± 0.004	0.050 ± 0.005	60[b]
14 days	0.069 ± 0.002	0.060 ± 0.005	87
21 days	0.073 ± 0.005	0.068 ± 0.004	93
	p-Nitroanisole demethylase (Δ A/mg/10 min)		
1 hr	0.456 ± 0.080	0.384 ± 0.021	84
1 day	0.504 ± 0.004	0.330 ± 0.027	65[b]
3 days	0.297 ± 0.021	0.234 ± 0.011	79
7 days	0.328 ± 0.020	0.162 ± 0.015	49[b]
14 days	0.410 ± 0.016	0.302 ± 0.011	74[b]
21 days	0.329 ± 0.017	0.298 ± 0.017	91
	AHH (FU[c]/mg/30 min)		
1 hr	62.7 ± 4	71.3 ± 4	113
1 day	76.7 ± 2.6	68.4 ± 3.7	89
3 days	95.3 ± 2	92.7 ± 2	97
7 days	85.5 ± 4	74.4 ± 4	87
14 days	60.9 ± 3	74.1 ± 1.4	81[b]
21 days	69.5 ± 5.5	64.6 ± 2.4	93
	Lung AHH (FU/mg/30 min)		
1 hr	0.85 ± 0.06	0.97 ± 0.03	114
1 day	0.58 ± 0.13	0.47 ± 0.06	81[b]
3 days	1.05 ± 0.14	0.76 ± 0.05	72
7 days	0.72 ± 0.07	0.50 ± 0.02	69[b]
14 days	0.50 ± 0.04	0.57 ± 0.06	88
21 days	0.58 ± 0.01	0.44 ± 0.02	76

[a] Mean ± S.E.; $N = 5$; each performed in duplicate.
[b] Significant at $p < 0.01$.
[c] FU, fluorescence units.

Dose-Response Experiment

Organ and Body Weights. The smallest dose of CP used (22 μg) caused significant splenomegaly at 3 days postinjection (Table 3). Increases in spleen weight were not clearly dose related, although splenomegaly was less with all doses in this study compared with that found with the 350-μg dose in the time-course study. Liver, lung, and body weights were not affected. Microsomal protein yields were not affected by the doses of CP used (Table 3).

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Drug Metabolism. Aminopyrine demethylase activity was diminished by the smallest dose of CP (22 μg) and was significantly lower ($p < 0.01$) than after a dose of 175 μg (Table 4). Aniline hydroxylase activity was significantly lower ($p < 0.05$) than controls after all doses. Effects on *p*-nitroanisole demethylase activity were unusual, in that the 22-μg dose group differed significantly from all other groups. AHH in liver microsomes was not affected by the 175-μg dose, the only group examined in this study. AHH activity in lung was decreased by the 2 higher doses of CP (88 and 175 μg) to an extent comparable to that seen with the 350-μg dose in the time-course study.

Table 3
CP dose-response study

Dose (μg)	Comparison of organ wt (g, wet wt) in		CP/control × 100
	Control mice	CP-treated mice	
		Body	
22	20.0 ± 0.9[a]	21.2 ± 0.5	106
88	20.0 ± 0.9[a]	20.7 ± 0.9	104
175	20.6 ± 0.7	20.1 ± 0.6	98
		Liver	
22	1.1 ± 0.08	1.3 ± 0.09	118
88	1.1 ± 0.08	1.3 ± 0.05	118
175	1.3 ± 0.07	1.3 ± 0.05	100
		Lung	
22	0.10 ± 0.008	0.104 ± 0.005	104
88	0.10 ± 0.008	0.09 ± 0.007	90
175	0.09 ± 0.006	0.096 ± 0.008	107
		Spleen	
22	0.044 ± 0.002	0.062 ± 0.007	141[b]
88	0.044 ± 0.002	0.06 ± 0.005	136[b]
175	0.052 ± 0.001	0.073 ± 0.003	146[b]
		Microsomal protein (mg/g wet wt)	
22	17.9 ± 0.3	17.1 ± 1.3	96
88	17.9 ± 0.3	17.7 ± 0.5	99
175	18.9 ± 0.3	17.4 ± 0.9	92

[a] Mean ± S.E.
[b] $p < 0.05$.

DISCUSSION

Data from these preliminary experiments strongly suggest that the i.p. injection of mice with CP diminished hepatic drug-metabolizing activity. A clear time-course was seen with decreased activity found from 1 to 14 days postinjection, which paralleled the course of splenohepatomegaly. From the dose-response data, it would appear that aniline hydroxylase activity was the most sensitive to the CP effect. The lowest dose used (a single injection of 22 μg) can be compared to 35 μg injected weekly for 14 weeks for a total dose of 5.25 mg/sq m, estimated by Scott and Warner (12) to be the "human equivalent" dose for mice.

Viewed from the perspective of the overall drug-metabolizing activity of the animal, the decreased specific activities found would be additive to the decreased microsomal protein yield (a measure of the amount of endoplasmic reticulum), leading to a combined decrease in metabolizing capacity. This effect may explain in part the results of Fisher *et al.* (4) that the interval between administration of CP and of cyclophosphamide, a drug requiring activation by microsomal enzymes, was critical in determining its antitumor activity.

Our results are entirely compatible with those of Farquhar *et al.* (3) who found that *Bacillus Calmette-Guérin* administered i.v. or s.c. to rats resulted in decreased aniline hydroxylase activity and microsomal protein yields. Their data, as do those of Castro (1), suggest that parenchymal liver damage may be the underlying basis of these effects.

The lack of a clear dose-dependent relationship is disconcerting, but is consistent with the nonlinear dose-response seen with CP and a variety of immunological measurements (11).

Quite probably there are still other mechanisms by which CP alters sensitivity to chemotherapy. Studies in progress

Table 4
Drug-metabolizing activity in dose-response experiment

Activity	Control	Activity		
		CP, 22 μg	CP, 88 μg	CP, 175 μg
Aminopyrine *N*-demethylase	0.144 ± 0.012[a] (7)[b]	0.114 ± 0.009 (5)	0.134 ± 0.012 (5)	0.164 ± 0.006 (10)
% of control		79[c]	93	113
Aniline Hydroxylase	0.111 ± 0.008 (9)	0.083 ± 0.004 (4)	0.096 ± 0.004 (5)	0.091 ± 0.003 (10)
% of control		75[c]	86[c]	82[c]
p-Nitroanisole *O*-demethylase	0.364 ± 0.044 (10)	0.202 ± 0.025 (5)	0.361 ± 0.026 (5)	0.334 ± 0.020 (10)
% of control		55[c]	99	92
Microsomal AHH	90.4 ± 6 (5)			85.3 ± 3 (5)
% of control				94
Lung AHH	1.40 ± 0.10 (10)	1.22 ± 0.10 (5)	0.90 ± 0.12 (5)	0.96 ± 0.09 (10)
% of control		87	64[c]	68[c]

[a] Mean ± S.E.
[b] Numbers in parentheses, number of animals.
[c] Differs from controls, $p < 0.05$.

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(6) suggest an increase in the rate of proliferation of granulocyte-macrophage progenitor cells increases their sensitivity to cell cycle-specific drugs but not to cycle-nonspecific drugs.

Since many drugs are activated and inactivated by mixed-function oxidase enzymes, the possibility that administration of CP could either increase or decrease the effects of drugs in man demands further investigation.

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