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Effects of Endotoxin on the Pharmacology of Antineoplastic Agents

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Summary. *Patients with cancer often develop serious gram-negative bacterial infections. Since bacterial endotoxins have been shown to affect the in vitro hepatic metabolism of antineoplastic agents, significant infection may adversely affect drug pharmacokinetics and metabolism in these patients. To evaluate the clinical significance of these effects, bacterial endotoxin (0.5 mg/kg, IV) was administered to male beagle dogs 1 or 24 h prior to the administration of radiolabeled 5-fluorouracil (5-FU), methotrexate (MTX), arabinosylcytosine (Ara-C), or vinblastine (VLB) as an IV bolus. Drug levels in plasma and urine were measured at various times after administration and standard pharmacokinetic parameters were calculated. The pharmacokinetics of all four agents were found to be significantly altered by the administration of bacterial endotoxin. However, there were no detectable patterns to these changes so that no predictions could be made. In studies on rats, chronic, nonlethal endotoxin administration (0.8 mg · kg^{-1} · day^{-1} for 10 days) resulted in a dramatic decrease in the distribution of [^{14}C]methylglyoxal bis(guanylhydrazone) (MGBG) in liver, kidney, intestine, heart and lung tissue. This suggests that bacterial endotoxin may also affect drug pharmacokinetics by altering drug penetration into various organs. In studies on hepatic microsomes isolated from rats, bacterial endotoxin incubation affected aniline hydroxylase activity only at concentrations greater than 0.4 mg/ml, at least tenfold higher than the LD_{50} of endotoxin in rats. It therefore seems likely that the endotoxin may require in vivo metabolism to affect changes in drug metabolism and disposition.*

Introduction

Gram-negative bacterial infections are a serious threat to patients with cancer. These patients are often immunocompromised as a result of their disease [5, 9], radiation [28], or chemotherapy [11]. Opportunistic overgrowth of gram-negative bacteria frequently causes chronic life-threatening infections in these patients. Bodey [4] has shown that 70% of patients with leukemia or lymphoma develop septicemia caused by gram-negative organisms.

Patients with infections in general respond less favorably to antineoplastic therapy and show a greater rate of toxic side-effects than those without infections. Because tolerance of chemotherapy is poor in the presence of sepsis, most oncologists delay therapy until recognized sepsis has been controlled. This is usually possible in solid tumor patients; it may not be possible in leukemics in relapse, where chronic sepsis is a fact of life. Freireich et al. [6] have suggested that infection may be prognostic of response to chemotherapeutic agents. Factors which may contribute to the poor responsiveness of these patients include: altered tumor sensitivity caused by bacterial endotoxins [3], general debilitation [25], nutritional imbalance [25], and alterations in the metabolism and disposition of antineoplastic agents [21].

Alterations in hepatic function are central to the compensatory adjustments that occur in response to severe infection [17]. Bacterial endotoxins have been found to affect significantly hepatic microsomal enzyme activity [7], decrease mitochondrial membrane permeability [22], and reduce hepatic clearance of indocyanine green dye [16]. In rat hepatocytes, *Escherichia coli* endotoxin decreased the bilirubin uridine diphosphoglucuronic acid transferase activity, aniline hydroxylase activity, and cytochrome P-450 content [8]. Renton [26] has shown that cytochrome P-450- and cytochrome P-450-dependent mixed-function oxygenase activities were depressed in hepatic microsomes of rats treated with *E. coli* endotoxin and *Bordetella pertussi* vaccine. Cancer chemotherapeutic agents such as 6-mercaptopurine, MTX, and VLB

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have been reported to potentiate the toxicity of bacterial endotoxins in mice [18]. On the other hand, bacterial endotoxins significantly affected the metabolism and cytoxicity of phenobarbital and cancer chemotherapeutic agents [19, 20, 27]. Rose et al. [27] have demonstrated that bacterial endotoxins enhanced phenobarbital-induced sedation in mice, suggesting a decrease in microsomal mixed-function oxygenase activity. In addition, a sublethal dose of *Salmonella typhosa* endotoxin (2 mg/kg) decreased the LD_{50} of cyclophosphamide (CTX) 4.7-fold, procarbazine (MIH) 13.1-fold, MTX 10.8-fold, and vincristine 1076-fold [27]. These phenomena are apparently not mediated through immunological processes since 'lipid A', a constituent of endotoxin which, though toxic and mutagenic was not immunogenic, also increased 5-FU lethality [20].

It is clear that bacterial endotoxins can dramatically alter in vivo drug metabolism and disposition. This is especially critical with antitumor agents, since their effectiveness depends very much on their pharmacologic disposition. Attention thus far has been focused on the interactions of bacterial endotoxins with the metabolism of anticancer agents, and the only agent studied in some detail was 6-mercaptopurine [1, 10, 19]. We have therefore investigated the effects of bacterial endotoxin on the metabolism and pharmacokinetics of five clinically useful antineoplastic agents: 5-FU, MTX, arabinosylcytosine (ara-C), MGBG, and VLB, with a view to evaluating the clinical significance of these effects.

Materials and Methods

Methotrexate-3′,5′,9′, (n) [^{3}H] sodium salt (MTX) 25 Ci/mmole and [G-^{3}H] vinblastine sulfate (VLB) 4.5 Ci/mmole were purchased from Amersham; [2-^{14}C]5-fluorouracil (5-FU) 13 mCi/mmole, and cytosine-2-[^{14}C]-arabinoside (ara-C) 51 mCi/mmole were provided by Stanford Research Institute. [^{14}C] Methylglyoxal bis(guanylhydrazone) (MGBG) 1.9 μCi/μmole was synthesized by a published procedure [23]. Bacterial endotoxin (*Escherichia,* B: 0626) was acquired from Difco Laboratories, Detroit, MI, USA. All chemicals and reagents purchased from regular commercial sources were reagent grade or higher. Glass-distilled solvents for chromatography were from Burdick and Jackson Labs., Muskegon, MI, USA.

Dog Studies. Beagle dogs of either sex (8–12 kg) were lightly anesthetized with sodium pentabarbital. Bacterial endotoxin (0.5 mg/kg) was administered IV (femoral vein) over 5–10 min; 30 min or 24 h later, various antitumor agents were administered by IV bolus. At various intervals, blood samples (10 ml) were collected from the opposite femoral vein in a glass tube containing heparin as an anticoagulant. They were immediately centrifuged at 12,000 *g* for 10 min at 25° C. The plasma was separated from the cells and frozen until analysis. Prior to HPLC analysis, plasma samples were deproteinated by 20% sulfosalicylic acid (1 : 10). Urine was collected with an indwelling Foley catheter. After each urine collection the bladder was flushed with normal saline and the washings were combined.

[^{14}C]CO_2 was collected by passing expired air through a solution of phenethylamine and methanol (1 : 1, v/v). Radioactivity of the solution was determined with a Packard model 2650 liquid scintillation spectrometer. At the end of the experiment, the animals were killed with an overdose of the anesthetic.

Tissue Distribution Studies of MGBG in Rats. Male Sprague-Dawley rats (250–300 g) received bacterial endotoxin (0.8 mg/kg in 0.5 ml saline) or saline alone (IV tail vein) for 10 days. [^{14}C]MGBG (0.5 μCi) in 0.5 ml saline was administered IV and 24 h later the animals were anesthetized with ether. Blood samples were obtained by cardiac puncture and the animals were killed by decapitation. Representative tissue samples were removed and frozen until analysis. Whole blood was added to 10-ml plastic centrifuge tubes containing sodium heparin and centrifuged at 10,000 *g* for 15 min. The plasma was decanted and frozen until analyis.

Hepatic Microsomal Studies. These were performed according to standard procedures [13]. Male Sprague-Dawley rats (200–300 g) were killed by decapitation and their livers removed. A 25% liver homogenate in cold 150 m*M* Tris buffer (pH 7.7) was prepared and centrifuged at 8,000 *g* for 20 min to remove large particulates. The supernatant was centrifuged at 105,000 *g* for 60 min. The pellet was again resuspended in cold Tris buffer containing 10 m*M* EDTA and resedimented at 105,000 *g* for 30 min. The final microsomal pellet was reconstituted to the original liver volume in 50 m*M* Tris buffer containing 150 m*M* KCl and 10 m*M* $MgCl_2$. The microsomal enzyme mixture (1 ml) was added to 4 ml Tris buffer containing 2 μmoles NADP, 25 μmoles glucose-6-phosphate, 3 U of glucose-6-phosphate dehydrogenases, various amounts of bacterial endotoxin, and 1 ml aniline hydrochloride (0.648 mg/ml H_2O). Aniline hydroxylase activity in the mixture was measured as previously described [14].

Radiochemical Techniques. Radioactivity was determined with a Packard Tri-carb liquid scintillation spectrometer (model 2650); quenching was corrected by the external standard channel ratio method and all counts were corrected to DPM. Aliquots (0.2 ml) of plasma, urine, or [$^{14}CO_2$] trapped in phenylethylamine/methanol solution were counted in 11 ml PCS scintillant (Amersham Corp., Arlington Hts., IL, USA). Radioactivity in tissue samples was determined after combustion of 0.5 g tissue samples in a Packard Sample Oxidizer model 306 B.

Chromatography. Analyses of 5-FU, MTX, and VLB were carried out with a Waters Associates Model 204 liquid chromatograph equipped with the following accessories from Varian Associates: A Vari-chrom variable wavelength detector, a CDS 111 integrator and a Model 9176 recorder. A Waters μ Bondapack C_{18} reverse phase column (30 cm × 4.0 mm ID) was used for separation. These methods have been described elsewhere [2; J.A. Benvenuto et al., unpublished work].

MTX. A deproteinated plasma or urine sample was injected into the column eluted with 0.005 *M* ammonium formate buffer in 20% methanol (pH 3.5) at a flow rate of 2 ml/min. The absorbance of the eluent was monitored at 254 nm. MTX had a retention time of 8.7 min. The effluent was collected for 2 min per tube and the radioactivity was determined by liquid scintillation.

5-FU. Deproteinated plasma or urine samples were injected into the column and eluted with double-glass-distilled water at a flow rate of 2 ml/min under 2,000 psi with the absorbance of the eluent monitored at 270 nm. 5-FU had a retention time of 2.8 min.

VLB. Deproteinated plasma or urine samples were injected into the column and eluted with 0.001 *M* phosphate buffer, pH 7.5, in 50% acetonitrile at a flow rate of 2.5 m/min. The wavelength of the monitor was set at 254 nm. Unlabeled VLB was added to all samples to facilitate the UV monitoring of the absorbance of the effluent, since the concentration of VLB was not sufficiently high to produce significant absorbance. The eluate was collected at 1-min intervals, and whenever significant absorbance was detected on the chromatogram the entire elution volume of the peak was combined as a single fraction and its radioactivity determined. The efficiency of the method was over 87% [15].

Ara-C. All samples were kept in heparinized tubes containing 1×10^{-4} *M* tetrahydrouridine (THU) and were deproteinated and separated by paper chromatography (Whatman #1) [12]. The solvent system consisted of isopropanol-water-ethyl acetate (22.5 : 125 : 65 v/v/v). In descending flow for 7 h, the Rf value of ara-C was 0.12. The chromatogram was cut into 2 × 2 cm squares and put in a counting vial containing 11 ml PCS. Radioactivity was determined as described above.

Results and Discussion

Bacterial endotoxin significantly affected the pharmacokinetics of all antitumor agents, as revealed in this study. As an example, endotoxin administration decreased the terminal plasma half-life of vinblastine (VLB) as well as the area under the curve (Fig. 1). This may be accounted for, in part, by the increased urinary excretion of VLB shown in Fig. 2.

A summary of changes in the pharmacokinetics of anticancer agents induced by bacterial endotoxin is shown in Table 1. The pharmacokinetics of all agents appeared to be affected, although to varying extents. Changes in 5-FU pharmacokinetics were observed 24 h after endotoxin administration only; simultaneous administration of the endotoxin produced no effect. In control dogs after [^{14}C]5-FU administration, 5-h cumulative [$^{14}CO_2$] excretion was 51% ± 6% of the total radiolabel administered. However, 24 h after endotoxin administration, the 5-h cumulative [$^{14}CO_2$] excretion was 17% ± 4% of the dose, suggesting a decrease in the metabolism of 5-FU to CO_2. On the other hand, endotoxin may affect the pulmonary endothelium and may compromise CO_2 exchange through an unknown mechanism.

To examine the effects of endotoxin on various organs, male Sprague-Dawley rats received [^{14}C]-MGBG by injection after endotoxin treatment for 10 days. Figure 3 shows that bacterial endotoxin treatment reduced the disposition of the agent into various tissues except the spleen. Since MGBG is not metabolized [24], this may be a direct effect of endotoxin on the organs.

Several studies have shown that bacterial endotoxin administration in the intact animal inhibits the metabolism of certain anticancer agents by hepatic

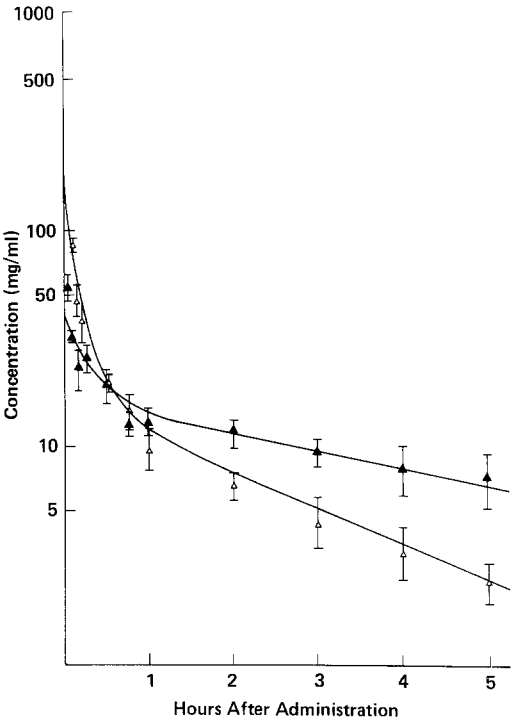

Fig. 1. Disappearance of vinblastine from the plasma of beagle dogs from 30 min after saline (▲) or endotoxin (△) administration. Each *point* represents the mean ± SEM of duplicate determinations of three separate animals

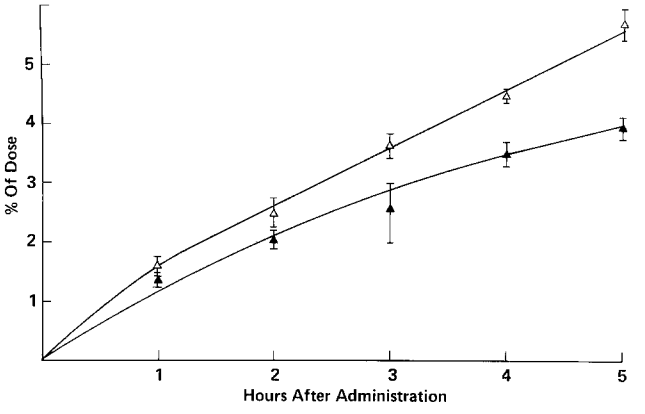

Fig. 2. Urinary excretion of vinblastine in beagle dogs from 30 min after administration of saline (▲) or bacterial endotoxin (△). *Points* and *bars* represent the mean ± SEM, respectively, of duplicate determinations of three separate animals in each case

microsomal enzymes [18, 27]. It is not known whether endotoxin itself or an activated endotoxin intermediate is responsible for these effects. Equally obscure is the mechanism by which the endotoxin exerts its effects. We therefore incubated rat hepatic microsomes with *E. coli* endotoxin at various concentrations. Aniline hydroxylase activity was measured as a

Table 1. Changes in the pharmacokinetics of antineoplastic agents in beagle dogs after endotoxin administration

Drug (dose; mg/kg)	Endotoxin	Plasma $t_{1/2}$		Vd (ml/kg)	C × t (mg/l × min)	Total clearance (ml/kg/min)	5-h excretion in urine (% of dose)
		Initial	Terminal				
MTX	–	2.9 ± 0.3	1.6 ± 0.2	254 ± 17	80 ± 10.6	6.5 ± 0.2	41.5 ± 5.3
(0.5)	+	12.6 ± 0.9	5.2 ± 0.02	171 ± 7.8	344 ± 39.5	1.2 ± 0.3	26.5 ± 4.1
% Change		+ 334	+ 225	− 67	+ 330	− 82	− 36
FU[a]	–	2 ± 0.2	0.66 ± 0.01	409 ± 34.1	151 ± 8.7	7.2 ± 0.5	19 ± 1.0
(65)	+	3 ± 0.23	0.42 ± 0.18	292 ± 65.8	264 ± 10.8	4.1 ± 0.9	5 ± 0.6
% Change		+ 50	− 36	− 29[b]	+ 75	− 43	− 74
Ara-C	–	6.5 ± 0.03	5.2 ± 0.4	247 ± 3	4,662 ± 46	2.1 ± 0.1	25 ± 5
(10)	+	11.0 ± 0.4	2.5 ± 0.2	391 ± 76	2,838 ± 43	3.8 ± 0.9	17 ± 0.7
% Change		+ 69	− 52	+ 58	− 39	+ 81	− 32[b]
VLB	–	8.5 ± 0.3	5.3 ± 0.2	3,037 ± 22	7.5 ± 0.9	28 ± 1.1	3.9 ± 0.4
(0.15)	+	7.4 ± 0.9	1.9 ± 0.6	952 ± 71	3.8 ± 0.4	40.0 ± 0.8	5.7 ± 0.8
% Change		− 13[b]	− 64	− 69	− 49	+ 43[b]	+ 46

E. coli, endotoxin (0.5 mg/kg) was administered IV to anesthetized beagle dogs. After a 30-min interval, MTX, Ara-C or VLB was administered IV. For each agent, three animals were studied. Unless noted, the statistical significance of observed differences is $P < 0.05$

[a] 5-FU administered 24 h after endotoxin

[b] $P < 0.1$

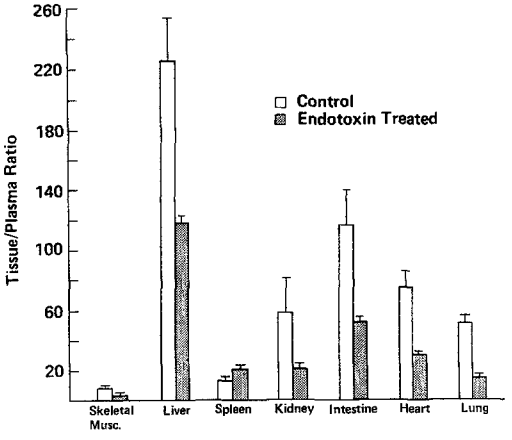

Fig. 3. Disposition of [^{14}C]MGBG in male Sprague-Dawley rats (200–250 g) after administration of bacterial endotoxins (IV, 0.8 mg/kg) daily for 10 days (*shaded columns*) or saline (*open columns*). Radiolabeled MGBG was then injected, and the animals killed 24 h after injection. Values shown are the mean ± SEM of duplicate determinations of six separate animals in each case

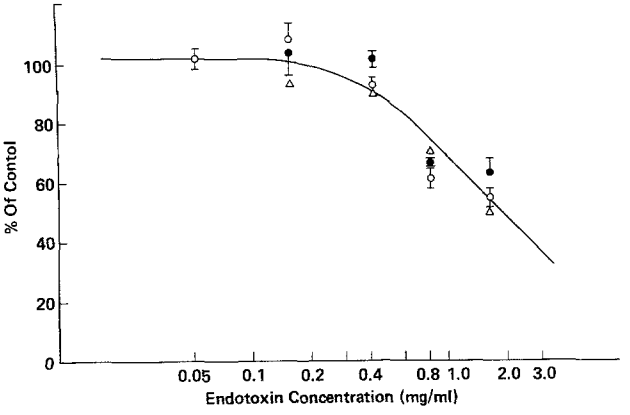

Fig. 4. Effect of bacterial endotoxin on hepatic microsomal aniline hydroxylase activity. Microsomes isolated from normal rat liver were incubated with aniline in the presence of saline containing various amounts of bacterial endotoxin. Aniline hydroxylase activity was then determined as discussed in *Materials and Methods* and expressed as a percentage of control. Each *symbol* and *bar* represents the mean ± SEM of triplicate determinations on microsomes isolated from the livers of three rats

function of the endotoxin concentration. Figure 4 shows that an endotoxin concentration of 1.5 mg/ml is required for 50% inhibition of enzyme activity. This dose is three orders of magnitude higher than the approximate LD_{50} for rats (1.5 mg/kg). This suggests that in the rat, the effect of endotoxin on hepatic metabolism may occur after in vivo metabolism or activation of endotoxin itself. Alternatively, endotoxin effects may occur via a second messenger.

In summary, we have shown that bacterial endotoxin administration in the dog causes significant changes in the pharmacokinetics of anticancer agents, which may persist for at least 24 h after endotoxin administration. As previous studies have shown, endotoxin administration may significantly affect not only the metabolism, but also the disposition of antineoplastic agents. The mechanism by which these effects are exerted remain unknown; however, we

have shown that bacterial endotoxin itself does not affect metabolism of standard substrates of microsomal enzymes in vitro at sublethal concentrations.

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