Effect of Acetylsalicylic Acid on the Liver Response to Endotoxin* (32684)

LURA A. SOLOMON AND LERNER B. HINSHAW
Veterans Administration Hospital and Department of Physiology, University of Oklahoma Medical Center, Oklahoma City, Oklahoma 73104

General responses of the dog to a lethal intravenous injection of endotoxin have been well defined (1-9). The ensuing developments, known as endotoxin shock, are characterized by an initial marked drop in systemic arterial pressure accompanied by a distinct rise in portal venous pressure and a decrease in cardiac output. The canine liver, previously suggested as the primary site of action of endotoxin (10), elicits significant increases in hepatic artery and portal vein pressures, vascular resistances and organ weights in response to endotoxin. Experiments on intact dogs have shown that acetylsalicylic acid (ASA) pretreatment prevents the fall in mean systemic arterial pressure and associated portal hypertension following injection of endotoxin (11). To evaluate the protective role of salicylates in endotoxin shock, a method of *in situ* liver preparations with controlled liver blood flow was developed.

Materials and Methods. Nineteen mongrel dogs of either sex, weighing between 9 and 14 kg, were intravenously anesthetized with 30 mg sodium pentobarbital/kg and placed in a supine position on a canine surgery table. Following a laparotomy, splenectomy and gastrectomy were performed. The hepatic artery, previously surgically denervated, and portal vein were separately cannulated and perfused with blood obtained from the femoral artery and inferior vena cava, respectively. Constant blood flow to the hepatic artery was delivered via a Sigmamotor pump at physiological pressures (100–140 mm Hg), while constant flow to the portal vein circuit was delivered by another Sigmamotor pump at pressures between 6 and 15 mm Hg. The portal venous inflow catheter tip was placed in the inferior vena cava just downstream from exiting hepatic veins. Celiac, superior mesenteric, and inferior mesenteric arteries were separately ligated following removal of the spleen and stomach and cannulation of hepatic artery. Blood flow to hepatic artery and portal vein was separately interrupted (less than 60 sec) during cannulation procedures, and permitted a continuous flow to the liver through at least one inflow vessel throughout the cannulation period. Fifty mg/kg acetylsalicylic acid dissolved in 90% ethyl alcohol (150 mg/ml) was administered intravenously to 12 animals. The ASA and alcohol (3–4.5 ml volume) infusion, administered via a Harvard infusion pump into the jugular vein, was simultaneously accompanied by a 30- to 40-ml saline flush. Acetylsalicylic acid injection was accomplished in 15 min. Seven of these preparations then received an LD_{80} dose of endotoxin (0.4 mg/kg), and the remaining five animals received no endotoxin and served as controls. Seven other animals were administered alcohol and saline (no ASA) followed by endotoxin. All preparartions were observed for 30 min after the endotoxin injection time.

Pressures were monitored via Statham pressure transducers on a Sanborn direct-writing recorder. Systemic arterial pressure was obtained by advancing a polyethylene catheter from the femoral artery into the aorta. Catheters were placed in the tips of the hepatic artery and portal vein inflow tubing to record pressures at these sites.

Blood flow to the liver was constant throughout each experiment. Table I shows mean liver blood flows and standard errors.

Results. Figure 1 shows mean systemic arterial pressures and portal venous pressures in the three group of animals. Systemic arterial pressure fell significantly ($p<.01$) by 3 min after endotoxin injection and remained significantly depressed for 30 min in the animals not receiving ASA. Control (no endotoxin) preparations and those animals treated with ASA prior to endotoxin administration maintained initial values of systemic arterial pres-

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TABLE I. Liver Blood Flow (Means ± SE).

Group	Blood flow (ml/kg body wt.)
ASA Pre-endotoxin	17.9 ± 1.4
Alcohol blank Pre-endotoxin	20.8 ± 1.5
ASA	19.4 ± 3.3

sure throughout the 30-min observation period.

The typical early rise in portal venous pressure after endotoxin injection in untreated dogs was not observed in ASA-pretreated animals ($p<.01$). Portal venous pressure in untreated preparations was significantly elevated above portal venous values in treated animals at 3 and 15 min after endotoxin injection (p .01, $p<.05$, respectively). Essentially unchanged portal venous pressures were observed in control and ASA-pretreated experiments.

Figure 2 shows that hepatic artery pressure rose significantly by 3 min after endotoxin administration in untreated animals, yet fell to starting levels by 15 min after endotoxin

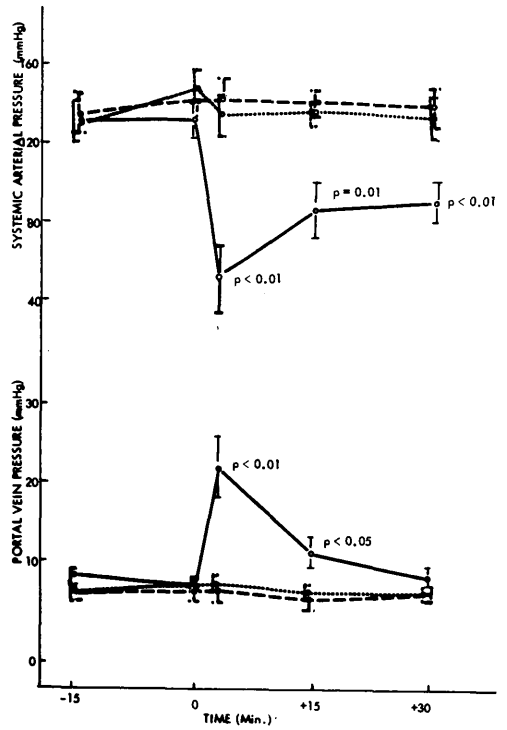

FIG. 1. (– – –) Controls; (- - -) ASA pre-endotoxin; (——) endotoxin + alcohol blank.

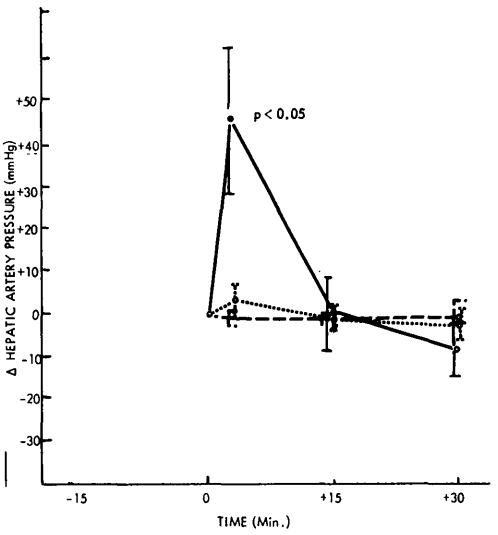

FIG. 2. (– – –) Controls; (- - -) ASA pre-endotoxin; (——) endotoxin + alcohol blank.

was given. Pretreatment with ASA obliterated the sharp rise in hepatic artery pressure seen in untreated animals given endotoxin ($p<$ .05). Treated and control groups demonstrated almost unchanged hepatic artery pressures throughout the experimental period. Table II shows absolute hepatic artery pressures (mean ± SE) in the three groups of animals.

Discussion. The results show that ASA treatment prior to insult with endotoxin prevents constriction characteristically seen in both the hepatic artery and hepatic vein of the dog.

Previous work (10) has shown that the rise of hepatic artery pressure with endotoxin is due to a myogenic response triggered by hepatic venous constriction (12). Portal vein pressure elevation is a passive response to hepatic vein constriction which results in extensive liver pooling within 1–5 min after endotoxin administration (1, 4, 8, 10). The site of action of ASA in blocking the liver response to endotoxin therefore appears to be hepatic veins.

Recent work (11) has shown that ACA pretreatment prevents the decrease in systemic arterial pressure due to endotoxin administration. The present study confirmed these findings in a different experimental preparation with the exception of one animal in which

TABLE II. Hepatic Artery Pressures (mm Hg; means ± SE).

Group	Time (min)				
	Preinfusion	0	+3	+15	+30
Controls	65 ± 6	64 ± 7	63 ± 6	61 ± 7	62 ± 6
EtOH + endotoxin	80 ± 6	74 ± 8	119 ± 22	82 ± 17	66 ± 7
EtOH + endotoxin + ASA	82 ± 13	79 ± 13	81 ± 15	77 ± 12	76 ± 11

portal vein pressure increased and systemic arterial pressure fell after endotoxin injection. This experiment was excluded from the current series but signified again the inverse relationship between portal vein and systemic artery pressure changes in nonprotected animals. The failure of ASA to prevent portal hypertension in the previously mentioned experiment was believed due to an insufficient dosage of ASA.

Results of the present study demonstrate that ASA prevents the fall in systemic arterial pressure after endotoxin administration by obliterating the decrease in venous return due to hepatic venous constriction and subsequent liver pooling. The mechanism of action of ASA is presently unknown. It may occupy receptor sites on the liver venules and thus block the action of endotoxin or released humoral substances. There is evidence that ASA blocks the vascular actions of certain vasoactive agents including histamine, serotonin, and bradykinin (13–24), which may be released after endotoxin. The intimate mechanism of its protective action is a subject for further investigation.

Summary. General responses of the dog to a lethal intravenous injection of endotoxin have been previously reported. The present investigation was devised to study the mechanism of the canine liver response to endotoxin. Experiments were carried out on an *in situ* liver preparation with controlled hepatic arterial and portal venous blood flows. Fifty mg acetylsalicylic acid/kg administered intravenously prior to endotoxin injection prevented the decrease in mean systemic arterial pressure and obliterated the constriction observed in both hepatic artery and hepatic vein in animals given endotoxin alone. The mechanism of action of acetylsalicylic acid is unknown.

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An Autoclavable Powdered Culture Medium for Mammalian Cells (32685)

ISAO YAMANE, YUTAKA MATSUYA, AND KATSUHIKO JIMBO
(Introduced by R. H. Kahn)
The Microbiology Division, Research Institute for Tuberculosis, Leprosy, and Cancer, Tohoku University, Sendai, Japan

Recently, the successful preparation of premixed powdered tissue culture media and their favorable application to virological studies were reported (1,2), and since then some of these media have become commercially available. However, these powdered culture media still require sterilization by filtration before use. The procedure for filter sterilization of these media is time-consuming and laborious, compared with that of the usual bacteriological media. We have prepared a thermostable powdered culture medium that lacks only one component, glutamine. The components of this medium are somewhat modified from that of Eagle's MEM (3), so as to provide a favorable dehydrated state and to permit autoclaving. The present report describes the medium components, the autoclaving procedure, and the cellular growth with this medium.

Table I shows the medium components. Phosphate, calcium, and magnesium salts in the medium were utilized in their anhydrous forms as indicated in the table. The most massive component, sodium chloride, was dehydrated as completely as possible before mixing. Furthermore, choline bitartrate was used instead of the usual choline chloride to avoid the latter's hygroscopic property. The amount of the pH indicator, phenol red, was decreased to 6 mg/liter medium, differing from the original MEM formula, and heat-stable kanamycin was added in the amount of 60 mg/liter medium as an antibiotic before mixing. The special additives in the formulation of the present medium are succinic

TABLE I. Main Components of the Autoclavable Powdered Medium.

Components	mg/liter	gm/100 liters
Arginine	105	10.5
Cystine	24	2.4
Histidine	31	3.1
Isoleucine	52	5.2
Leucine	52	5.2
Lysine	58	5.8
Methionine	15	1.5
Phenylalanine	32	3.2
Threonine	48	4.8
Tryptophan	10	1.0
Tyrosine	36	3.6
Valine	46	4.6
Glucose	1000	100.0
NaCl	6800	680.0
KCl	400	40.0
$CaCl_2$	200	20.0
$MgSO_4$	93.5	9.35
NaH_2PO_4	115	11.5
Kanamycin[a]	*60*	*6.0*
Phenol red	*6*	*0.6*
Sodium succinate	*100*	*10.0*
Succinic acid	*75*	*7.5*
Thiamine	1	0.1
Riboflavin	0.1	0.01
Pyridoxal	1	0.1
Nicotinamide	1	0.1
Pantothenate	1	0.1
Folic acid	1	0.1
Inositol	2	0.2
Choline bitartrate	*1.80*	*0.180*

[a] Italicized components are the ones different from the amounts of original MEM formula or lacking in it.