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Effect of Indomethacin in Endotoxin Shock in the Dog.* (32239)

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Previous publications from these laboratories and elsewhere(1,2,3) indicated that acetylsalicylic acid and phenylbutazone can block the hemodynamic effects of endotoxin in dog. Since the pharmacological activities of these agents overlap with another anti-inflammatory drug, indomethacin, (1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid)(4,5) we became interested to determine whether indomethacin can interfere with the typical cardiovascular response to endotoxin in the dog.

Materials and methods. The early responses of animals that received endotoxin were studied. Experiments were performed on 42 adult mongrel dogs, anesthetized with sodium pentobarbital, 30 mg/kg i.v. The femoral artery and vein were cannulated in all experiments, portal vein pressure was recorded in 10 animals. Arterial pressure was monitored by means of a catheter introduced through the femoral artery. Pressure in the portal vein was assayed by means of a catheter advanced from the splenic vein. Pressures were recorded with Statham pressure transducers connected to a Sanborn direct writing recorder, or to a Grass polygraph. Hematocrit and pH determinations were carried out with blood drawn through a catheter inserted in the femoral vein. Rectal temperature was measured with a thermometer.

Drugs were injected into the femoral vein. Indomethacin was dissolved in a 0.1 M phosphate buffer of pH 8 and was given i.v. in a volume of 5 ml/kg within 2 minutes. Dogs received 20 mg/kg indomethacin 20-30 minutes prior to the i.v. administration of 0.4 mg/kg endotoxin (*E. coli,* Difco Labs). This dose of endotoxin represented the LD_{80}, which was established in separate experiments done with 45 dogs by one of us (Hinshaw). In control studies, dogs received endotoxin only or phosphate buffer and endotoxin. Four dogs were pretreated with 100 mg/kg phenylbutazone HCl instead of indomethacin.

The animals used in the experiments were divided into 5 groups. Group I: 10 animals received 0.4 mg/kg endotoxin only. Group II: 14 dogs were given indomethacin as pretreatment 20-30 minutes before administration of endotoxin; the hematocrit, blood pH and rectal temperature were recorded in 10 animals. Group III: 4 dogs were pretreated with phosphate buffer only. Group IV: In 5 dogs the abdominal wall was opened and portal venous pressure was measured simultaneously with mean systemic arterial pressure. The animals in this group received phosphate buffer only as pretreatment. Group V: 5 dogs were treated similarly to group IV, but indomethacin was given 20 minutes before endotoxin. Group VI: 4 dogs were pretreated with phenylbutazone.

The hydrolysis of benzoyl-L-arginine ethyl ester (BAEe) by highly purified hog pancreatic kallikrein was assayed in a Radiometer pH-Stat. The concentration of BAEe was 3×10^{-3} M in 0.15 M NaCl. The kallikrein used was purified by Dr. E. Werle; it contained 1,000 FU/mg. The reactions were followed at room temperature and the pH was kept constant at 8 by the addition of 0.001 M NaOH. The initial steady rates of hydrolysis were registered.

Results. In the first series of experiments, 10 dogs in Group I were given the LD_{80} dose of endotoxin, while the 14 dogs in

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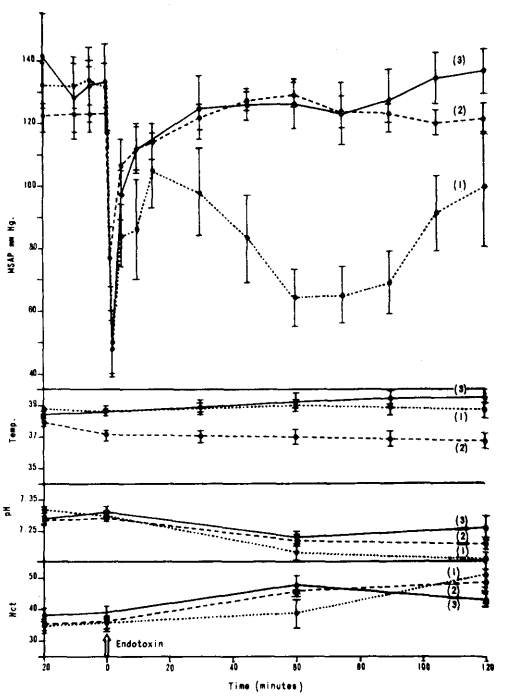

FIG. 1. Effects of indomethacin or phenylbutazone pretreatment in endotoxin shock in dogs. LD_{50} dose of endotoxin was injected i.v. Mean systemic arterial blood pressure (MSAP), rectal temperature (Temp), blood pH (pH) and hematocrit values (Hct) were registered. 1. Control experiments, Group I. 2. Group II, dogs were pretreated with 20 mg/kg indomethacin. 3. Group VI, dogs were pretreated with 100 mg/kg phenylbutazone. The points show the mean values, the vertical bars indicate ± 1 S. E.

Group II, received indomethacin prior to injection of endotoxin. The hemodynamic response to endotoxin was different in the two groups (Fig. 1). During the first phase of shock, 2 minutes after injection of endotoxin, the transient drop in the mean systemic arterial blood pressure was 46 mm Hg in Group II and 84 mm in Group I. The difference was significant at the $P<0.02$ level. Five minutes after administration of endotoxin, the blood pressure started to return to normal in both groups. In Group I, 2 dogs died 15 minutes post-endotoxin and a third after 105 minutes.

In the second phase of the shock, the blood pressure started its characteristic slow decline in Group I, 15 minutes post-endotoxin, while it stayed at a normal level in Group II. The difference between the two groups was significant at the $P<0.01$ level from 45 minutes to 105 minutes, at 105 minutes the P was <0.02. The rectal temperature (Fig. 1) decreased in Group II by 1.3°C. The difference between the values recorded at the time of injection of indomethacin and at the end of the experiment was statistically significant ($P<0.05$). The blood pH and hematocrit changed in a parallel manner in both groups (Fig. 1). The heart rate decreased from an average 160 beats/minute to 111 in five minutes post-endotoxin in Group I. It returned to the pre-shock level in 90 minutes. The corresponding values in Group II were 150 and 129 beats/minute in Group II. The return to pre-shock level took 45 minutes in Group II, ($P>0.1$).

Four dogs in Group III received phosphate buffer only as pretreatment. The response of this group was very similar to Group I. From 2 minutes post-endotoxin until the end of the experiments, systemic arterial pressure in Group III was not different from Group I ($P>0.1$).

Administration of indomethacin lowered the portal venous pressure in Group V (Fig. 2). Injection of endotoxin to dogs in Groups IV and V elicited a rise of 7 respectively 10 mm Hg in portal venous pressure concomitant with the initial drop of the systemic arterial pressure. This elevated portal pressure disappeared in Group V after 10 minutes. The difference in portal venous pressure between Group IV and V was significant ($P<0.05$) from 10 to 45 minutes post-endotoxin. It should be mentioned, however, that the portal venous pressure was slowly rising in Group IV and falling in Group V even before endotoxin was given. The mean systemic arterial blood pressure in the dogs pretreated with indomethacin (Group V) was significantly higher ($P<0.01$) than in Group IV during both phases of the shock.

The mean systemic arterial blood pressure of the shocked animals pretreated with phenylbutazone (Group VI) was similar to that of dogs treated with indomethacin, (Group II) (Fig. 1).

Kallikrein inhibition. Indomethacin did not inhibit the hydrolysis of BAEe by highly

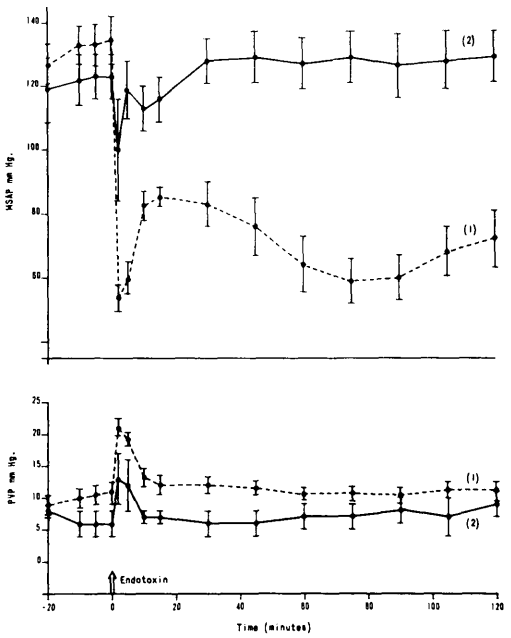

FIG. 2. Effect of endotoxin on portal venous pressure (PVP) and mean systemic arterial blood pressure (MSAP) in dogs pretreated with indomethacin and in control experiments. 1. Control experiments, Group IV. 2. After pretreatment with indomethacin, Group V. The points show the mean values, the vertical bars indicate ± 1 S. E.

purified hog pancreatic kallikrein. Indomethacin in a concentration of 1×10^{-3} M was pre-incubated with the enzyme for 30 minutes. The mean rate of hydrolysis of BAEe was 83 μmole/min/mg in presence or absence of indomethacin.

Discussion Endotoxin shock in dog is characterized by an immediate fall in systemic arterial blood pressure and a simultaneous rise in portal venous pressure. After this early, transient phase, the arterial pressure rises again followed by a slower progressive decline. The rise in portal pressure and the initial sudden hypotension is usually attributed to pooling of blood in the liver. Our findings on the effect of endotoxin are in agreement with those of numerous previous observers(6,7).

The experiments shown here, however, demonstrated that treatment with indomethacin alleviates the hemodynamic effects of endotoxin shock in dogs. In the pretreated animals the initial fall in systemic blood pressure was smaller than in the control group. The most impressive consequence of the administration of indomethacin was that after the transient fall of the mean systemic arterial blood pressure during the first phase of shock, the blood pressure returned to normal level in the treated animals and stayed there for the duration of the experiments, for 2 hours (Fig. 1, 2). Treatment with the phosphate buffer solvent of indomethacin only was not effective.

The effect of endotoxin has been attributed to the release of variety of vasoactive agents(7,8). Lately the liberation of hypotensive peptides, collectively called plasma kinins, has been implicated in shock(9,10). The evidence is still fragmentary, but the administration of an inhibitor of proteolysis, Trasylol, prevented the depletion of the bradykinin precursor (kininogen) in blood (11) and improved the rate of survival of shocked animals(1).

The present studies indicate that the beneficial effects of indomethacin in shock are not due to a direct inhibition of a kinin releasing enzyme. Indomethacin did not inhibit glandular kallikrein, one of the enzymes that can liberate a kinin.

Another anti-inflammatory drug, acetylsalicylic acid, also blocked the hemodynamic effects of endotoxin in dogs(1,2,3) but there were differences between the action of acetylsalicylic acid and indomethacin. Acetylsalicylic acid blocked the effects of endotoxin both in the first phase and the second phase of shock(2). That is after pretreatment with acetylsalicylic acid, the initial fall in arterial pressure was much smaller, the concomitant rise in portal pressure and the secondary decrease in arterial pressure, caused by injection of endotoxin to control dogs, were not seen. In contrast, indomethacin did not block the rise in portal venous pressure. Although acetylsalicylic acid was given in 2-5 times higher dose than indomethacin, the difference in the effects of 2 drugs cannot be attributed to the difference in dosage. Pretreatment with another anti-inflammatory drug, with 100 mg/kg of phenylbutazone did not protect the animals against the initial fall of the systemic arterial blood pressure

(Fig. 1), but maintained normal pressure during the second phase of shock. The effect of phenylbutazone in this respect was similar to that of 20 mg/kg indomethacin.

The use of nonsteroidal anti-inflammatory drugs has a beneficial effect in various forms of experimental shock(1,2,12), but their mode of action in the area of the splanchnic circulation may be different.

Summary. Pretreatment of dogs with 20 mg/kg of indomethacin blocked some of the hemodynamic effects of endotoxin. In the treated animals i.v. injection of the LD_{80} dose of endotoxin (*E. coli*) caused a significantly smaller transient fall in the first phase of shock than in the control group. After the initial brief fall, the arterial blood pressure stayed at normal level for the duration of the experiment in dogs that received indomethacin. This was in contrast to the characteristic decline of arterial pressure in the untreated dogs in the second phase of shock. Administration of indomethacin lowered the rectal temperature and portal venous pressure of dogs. The results were discussed and compared to those obtained with other anti-inflammatory agents.

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Methyl 5 (or 4)-(3,3-Dimethyl-1-Triazeno)imidazole-4 (or 5)-Carboxylate (NSC 87982): Mouse Tissue Concentrations as Determined by Microbiological Assay.* (32240)

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Several triazenoimidazoles have been shown to have antitumor activity in experimental neoplasms(1,2). Although lacking noteworthy antitumor activity, a new member of this group of compounds, methyl 5 (or 4)-(3,3-dimethyl-1-triazeno)imidazole-4 (or 5)-carboxylate (designated NSC 87982 by the Cancer Chemotherapy National Service Center, National Cancer Institute), has been reported to have broad spectrum antimicrobial activity *in vitro*, and therapeutic activity against experimental infections of *Staphylococcus aureus* in mice has been demonstrated(3,4). The knowledge that this compound is being considered for clinical trial prompted the development of an assay procedure applicable to the determination of concentrations

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