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13. ABSTRACT

Injection of live E. coli organisms to dogs iv causes a lethal shock. Administrations of anti-inflammatory drugs (indomethacin, aminopyrine, flufenamic acid, and phenylbutazone) block partially the effects of shock on the portal vein pressure, and on the hematocrit values. The changes that follow the injection of E. coli were significantly different in the treated animals from those in the control group. Some of the other agents tested were ineffective. Thus only drugs which have an antiphlogistic effect protected against shock.

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# EFFECTS OF ANTI-INFLAMMATORY DRUGS IN SHOCK CAUSED BY INJECTION OF LIVING E. COLI CELLS

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MEDICAL CENTER RESEARCH AND DEVELOPMENT OFFICE
OF THE UNIVERSITY OF OKLAHOMA FOUNDATION, INC.

## Introduction

It was shown previously that the administration of nonsteroid anti-flammatory drugs, such as aspirin (1-3), phenylbutazone (2, 4) or indomethacin (4), blocks the cardiovascular effects of endotoxin in dogs. Injection of live Escherichia coli organisms to dogs iv also induces a lethal shock, although the cardiovascular parameters are somewhat different from those that follow the iv injection of endotoxin extracted from E. coli (5, 6). Because the shock caused by live organisms may resemble clinical conditions closer than that brought about by endotoxin, we tested the effectiveness of some nonsteroidal anti-inflammatory agents in this type of shock.

## Materials and Methods

Experiments were performed on 58 mongrel dogs anesthetized with sodium pentobarbital, 30 mg/kg iv. The femoral artery, vein and portal vein were cannulated. The details of the experimental procedures were described previously (3, 4). The changes in mean systemic arterial blood pressure (SAP), portal venous pressure (PVP), blood pH, and hematocrit (Hct) were recorded for 4 hr. The organism used was an enteropathogenic Dunwald strain of E. coli (6). The solution injected contained approximately $2 \times 10^9$ organisms/ml.

At zero time, all the animals received an iv injection of 2 ml/kg of viable organisms to cause shock. This dose represented an $LD_{100}$. Eighteen animals served as control; 40 dogs were given iv injection of an anti-inflammatory agent.

### Group I

Nine dogs were treated with indomethacin. The drug was dissolved in 0.2 M Tris buffer of pH 7.4. Twenty mg/kg was given 15 min prior to the E. coli injection and 10 mg/kg at 120 min.

Group II

Six dogs received aminopyrine in saline in three doses. The first dose was 50 mg/kg injected 15 min before E. coli was given, then 10 mg/kg was given at 60 min and another 10 mg/kg at 120 min.

Group III

Ten dogs received flufenamic acid dissolved in 0.17 N NaOH and neutralized. Twenty mg/kg was given as pretreatment, 10 mg/kg at 60 min and another 10 mg/kg injection at 120 min.

Group IV

Six dogs were given phenylbutazone sodium salt (100 mg/kg) in saline at 15 min before E. coli injection.

Acetaminophen (20 mg/kg) and salicylamide (50 mg/kg) were administered in a single dose to 2 animals, each 15 min before zero time. Acetaminophen was dissolved in saline with the help of added NaOH, and salicylamide was dissolved in 95% ethanol. Sulfinpyrazone was dissolved in the Tris buffer used, 50 mg/kg were given . 3 dogs at 0 minus 15 min, 10 mg/kg after 60 min, and finally 10 mg/kg after 120 min. Salicylaldoxime was dissolved in 30% ethanol; 50 mg/kg were administered as pretreatment to 2 dogs, 10 mg/kg after 60 and 10 mg/kg after 120 min.

Results

Injection of live E. coli organisms caused a slow decline in the SAP that reached its lowest level at 45% of the original value after 1 hr and remained at this level for 2 hr past injection time (Fig. 1). By the termination of the study (4 hr), the SAP averaged 66% of the initial value in the eight dogs surviving 4 hr.

The mean PVP increased 5 (±0.09 SEM) mm Hg in the first 5 min after injection of organisms and remained elevated above the initial mean value for the entire period of study (Fig. 2).

The Hct increased from an initial 42 (±1.6) to 57 (±2.6) in 2 hr (Fig. 3).

The mean pH of the blood dropped from 7.31 (±0.01) to its lowest point of 7.08 (±0.03) in 2 hrs and then increased toward its original value, reaching 7.21 (±0.04) at termination of the experiments.

Group I

This group received indomethacin; the SAP was maintained at a significantly higher and the mean PVP at a lower level than in the control group (Figs. 1A, 2A). At the termination of the study (4 hr), the SAP was 86% of the initial value in the 9 out of 10 animals that survived.

The Hct rose rapidly during the first hour from an initial value of 41, then it remained elevated at 52. Mean Hct values in Group I were significantly lower than in the control at 3 hr ($p < .05$) (Fig. 3A).

The pH changed from 7.33 (±0.01) to 7.21 (±0.04) in 2 hr. At other times, the pH was not significantly different from control.

Group II

Treatment with aminopyrine maintained the SAP at a significantly higher level than it was in the untreated animals (Fig. 1B). None of the 6 animals studied died.

The mean PVP was fairly constant and lower than in the control (Fig. 2B). The Hct increased from 40 (±3.33) to 46 (±3.21) in 1 hr and afterwards it did not change (Fig. 3B). The blood pH declined to its lowest point from an initial value of 7.30 (±0.21) to 7.18 (±0.03) in 1 hr.

Group III

Flufenamic acid was given in Group III. Here also the SAP and the mean PVP were significantly better than in the untreated animals (Figs. 1B, 2B). At the end of 4 hr 6 animals were alive in this group. The Hct increased but less than in the control (Fig. 3B). The pH remained stable for the first 30 min, then decreased from 7.30 (±0.02) to 7.18 (±0.05).

Group IV

Six animals were pretreated with phenylbutazone. The SAP decreased in this group only 5% after 1 hr (Fig. 1A). The mortality was much greater in the control group because in Group IV only 1 out of 6 animals died. The mean PVP remained fairly constant (Fig. 2A) and the Hct increased significantly less than in the control (Fig. 3A).

The pH decreased from 7.28 (±0.02) to 7.24 (±0.02) during the first hour, but it dropped only 0.08 during the entire study.

Negative results were obtained in exploratory investigations; that is, no protection against E. coli was seen when dogs were treated with the following drugs: acetaminophen (n = 2), sulfinpyrazone (n = 3), salicylaldoxime (n = 2), and salicylamide (n = 2).

Discussion

The dog reacts differently to injection of live E. coli organisms than to endotoxin. The immediate precipitous drop in SAP, followed by a rapid rise caused by injection of endotoxin, was not seen in animals shocked by the administration of live organisms as previously reported (5,6). PVP rises in both groups (6) but ordinarily more in endotoxin-treated dogs. There was no correlation between the decrease in SAP and the rise in PVP in dogs treated with E. coli as also described in an earlier report (6).

The present study showed that treatment of dogs with nonsteroidal anti-inflammatory agents abolished some of the symptoms of E. coli shock. Indomethacin, aminopyrine, flufenamic acid, and phenylbutazone blocked or ameliorated the effects of E. coli organisms on SAP, PVP, Hct, and pH values. On the average, more animals survived until the termination of the experiments in treated groups than in untreated controls. Four other drugs tested, acetaminophen, sulfinpyrazone, salicylaldoxime, and salicylamide, afforded no protection against shock in a small number of exploratory studies. It is improbable that the drugs used have a single mode of action. They may have protected the animals against vasoactive agents released during shock, although it is unlikely that bradykinin is involved (7, 8). Other explanations for the beneficial effects of the drugs tested in shock may include prevention of disseminated intravascular coagulation (9), stabilizing lysosomal membranes (10), blocking the aggregation of platelets (11) or interfering with the reactions of the complement system (12).

Surveying the various properties of the drugs used revealed that the agents which protected against shock have strong anti-inflammatory action (aminopyrine, flufenamic acid, phenylbutazone, and indomethacin), while those which were ineffective are not anti-inflammatory (13-15). Because the Hct rose in all animals, probably the antiphlogistic activity was not exerted on the capillaries although the animals were not splenectomized. A likely explanation for the effect is that the drugs stabilized some cell membranes and thereby blocked metabolic processes usually brought about by shock.

Shock caused by septicemia is a grave and frequently lethal clinical condition (16). Because of the strikingly beneficial effects of nonsteroidal anti-inflammatory agents in both endotoxin (2-4) and E. coli shock, their use to improve this condition in man may merit consideration.

Summary

Injection of live E. coli organisms to dogs iv causes a lethal shock. Administrations of anti-inflammatory drugs (indomethacin, aminopyrine, flufenamic acid, and phenylbutazone) block partially the effect of shock on the portal vein pressure, and on the hematocrit values. The changes that follow the injection of E. coli were significantly different in the treated animals from those in the control group. Some of the other agents tested were ineffective. Thus only drugs which have an antiphlogistic effect protected against shock.

References


1. Blair, E., A. Wise, and A. G. Mackay. J. Amer. Med. Asso. 207; 333, 1969.

2. DiPalma, J. R., ed. Drill's pharmacology in medicine, 3rd ed. McGraw-Hill, New York, 1965.

3. Emerson, T. E., Jr., and F. C. Kelly. J. Appl. Physiol. 23: 609, 1967.

4. Erdös, E. G. Biochem. Pharmacol. Suppl. 283, 1968.

5. Erdös, E. G., L. B. Hinshaw, and C. C. Gill. Proc. Soc. Exp. Biol. Med. 125: 916, 1967.

6. Erdös, E. G., and H. Y. T. Yang. Bradykinin, Kallidin and Kallikrein Handbook of Experimental Pharmacology (E. G. Erdös, ed.), p. 289, 1970.

7. Goodman, L. S., and A. Gilman. Eds., The pharmacological basis of therapeutics, 4th ed. Macmillan Co., New York, 1970.

8. Hardaway, R. M., III. Syndromes of disseminated intravascular coagulation. Thomas, Springfield, Ill., 1966.

9. Hinshaw, L. B., L. A. Solomon, E. G. Erdös, D. A. Reins, and B. J. Gunter. J. Pharmacol. Exp. Ther. 157: 565, 1967.

10. Hinshaw, L. B., L. A. Solomon, D. D. Holmes, and L. J. Greenfield. Surg. Gynecol. Obstet. 127: 981, 1968.

11. Lichtenstein, L. M., H. Gewurz, N. F. Adkinson, Jr., H. S. Shin and S. E. Mergenhagen. Immunology. 16:327, 1969.

12. Massion, W. H., and E. G. Erdös. J. Okla. State Med. Ass. 59:467, 1966.

13. Mustard, J. F., and M. A. Packham. Pharmacol. Rev. 22:97, 1970

14. Northover, B. J. and G. Subramanian. J.Pathol. Bacteriol. 83:463, 1962.

15. Weissman, G. N. Engl. J. Med. 273:1084, 1965.

16. Winder, C. V., J. Wax, B. Serrano, E. M. Jones, and M. L. McPhee. Arthritis Rheum. 6:36, 1963.

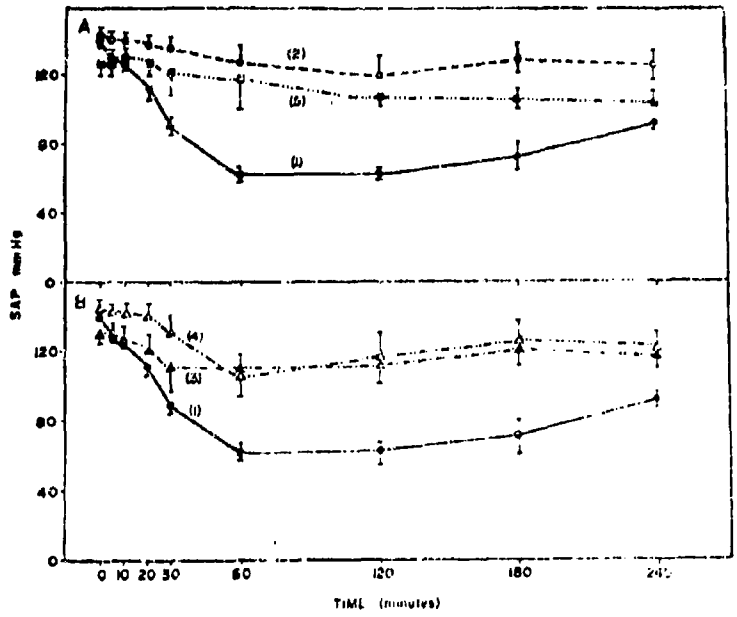


FIG. 1. Effect of anti-inflammatory drugs on shock caused by injection of live *E. coli* organisms to dogs. Mean systemic arterial pressure (SAP) registered. (1) control experiments; (2) indomethacin treated group; (3) aminopyrene-treated group; (4) flufenamic acid-treated group; (5) phenylbutazone-treated group. The difference in SAP between the treated and untreated animals was significant at $P < 0.05$ or lower level throughout the experiments.

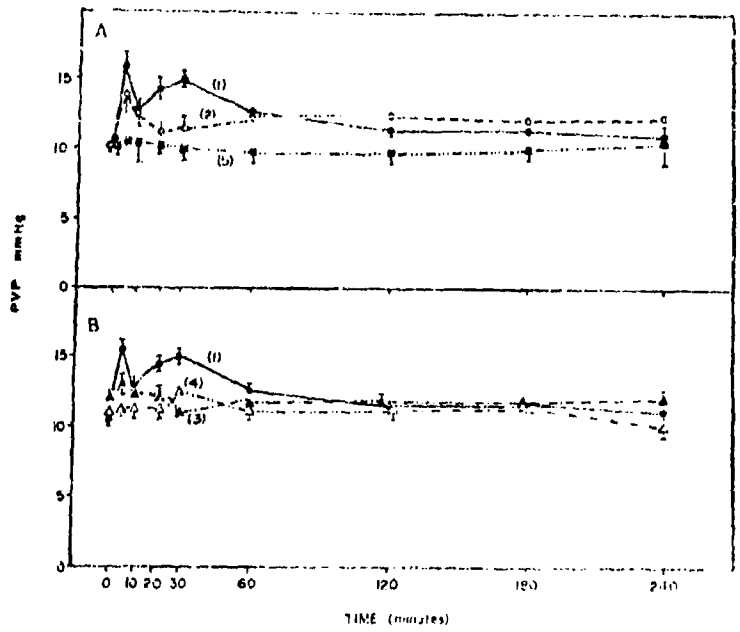


FIG. 2. Effect of treatment with anti-inflammatory drugs on the rise in portal venous pressure (PVP) caused by injection of *E. coli* organisms. Marking of groups as in Fig. 1. The mean PVP was significantly lower ($p<0.01$) in group I after 20 min; in groups II and IV after 5 min ($p<0.05$); and in group III after 10 min ($p<.05$) than in the control groups.

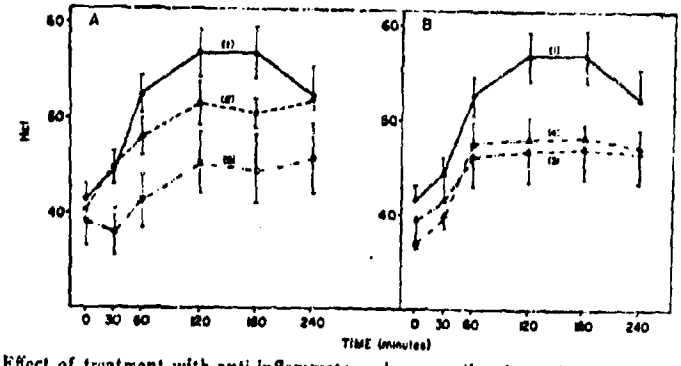


FIG. 3. Effect of treatment with anti-inflammatory drugs on the rise in hematocrit (Hct) values caused by injection of *E. coli* organisms. Marking of groups as in Fig. 1. In general the values were significantly lower in the treated animals (e.g., $p<0.05$ after 3 hr).

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