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E. coli ENDOTOXIN SHOCK IN THE DOG; TREATMENT WITH LIDOCAINE OR INDOMETHACIN

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1 Dogs treated with lidocaine (1 mg kg^{-1} h^{-1}) or indomethacin (1.5 mg/kg) before and after an LD_{60} dose (1 mg/kg) of *E. coli* endotoxin survived for at least 72 h.

2 Although all dogs in both treated groups survived, only those treated with indomethacin were significantly protected against the fall in the arterial blood pressure 1 to 2 min following endotoxin administration.

3 Endotoxin increased the plasma prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) concentration in the control and lidocaine-treated groups, however, no increase was observed with indomethacin treatment.

4 Neither lidocaine nor indomethacin alone had any significant effect on the parameters measured in this model.

5 Following the administration of endotoxin, lidocaine-treated animals had significantly decreased plasma fibrinogen concentrations when compared to the other groups.

6 This study suggests that lidocaine, a local anaesthetic and a drug widely used for cardiac arrhythmias, might offer protection in endotoxin shock.

Introduction

Previous work in this laboratory (Fletcher, Herman & Ramwell, 1976a; Fletcher, Ramwell & Herman, 1976b; Fletcher & Ramwell, 1977) has documented increased plasma prostaglandin concentrations following the administration of endotoxin intravenously in dogs and primates. The increases in prostglandins were related in time to changes in circulatory function. Therapeutic doses of analgesic-antipyretic drugs given before and after administration of endotoxin diminished the early haemodynamic changes and prevented the increase in the prostaglandins, but failed to improve the survival in the lethal (LD_{100}) baboon model. In contrast, in the LD_{50} dog model, these drugs did improve the survival and the early circulatory function while inhibiting the synthesis of the prostaglandins. Although the prostaglandins were elevated as early as 1 to 2 min after the endotoxin administration, their relationship to the pathophysiology of endotoxin shock is unclear.

The analgesic-antipyretic drugs do improve circulatory function and survival in canine endotoxin shock but the exact mechanism by which they exert a beneficial effect is unknown. We demonstrated (Fletcher & Ramwell, 1977) that while indomethacin or aspirin prevented the increase in plasma prostaglandins following the administration of endotoxin, only indomethacin clearly improved the haemodynamic events. This observation suggested that some effect of indomethacin other than the inhibition of the prostaglandins should be considered. One possible explanation of the improved circulatory function with indomethacin in canine endotoxin shock is that indomethacin may potentiate vascular smooth muscle responses to catecholamines (Zimmerman, Ryan, Gomer & Kraft, 1973; Kadowitz, Joiner & Hyman, 1975). The increased catecholamines present in endotoxin shock (Hall & Hodge, 1971) may improve circulatory function in the presence of indomethacin by improving venomotor tone and thereby increasing venous return to the heart. A decline in venous return in canine endotoxin shock significantly contributes to the impairment of circulatory function (Weil, MacLean, Vischer & Spink, 1956).

Lidocaine, a widely used clinical drug, may also increase vascular smooth muscle sensitivity to catecholamines (Altura, 1967; Somylo & Somylo, 1970). In addition, lidocaine stabilizes cell membranes (Ritchie & Greengard, 1966; Seeman, 1972) and decreases membrane permeability (Scherphof, Scarpa & van Toorenburgen, 1972). Interestingly, lidocaine has been

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used successfully, as a local anaesthetic, on the coeliac ganglion to prevent mesenteric vasoconstriction during endotoxin shock in dogs (Hauman, 1968; Wangensteen, Geissinger, Lovett, Glen & Lefer, 1971). Thus, lidocaine might provide protection during endotoxin shock.

This study was designed to (i) compare the circulatory function in untreated controls given an LD_{60} dose of endotoxin with those treated with indomethacin or lidocaine, (ii) evaluate the effects of pre- and post-treatment with indomethacin or lidocaine on prostaglandin release and on survival, (iii) determine the effects of lidocaine or indomethacin on the white blood cell, platelet, and coagulation changes seen in canine endotoxin shock.

Methods

Adult male mongrel dogs (15 to 23 kg) were anaesthetized with sodium pentobarbitone (30 mg/kg, by intravenous administration), intubated and allowed to breathe spontaneously. Indwelling catheters were inserted into the femoral and pulmonary arteries through a cut-down in the right groin. The position of the pulmonary artery catheter (Swan-Ganz thermodilution) was monitored by pressure tracings and the femoral artery catheter was advanced into the descending aorta. The animals were kept in a supine position. Cardiac output was measured by the thermal dilution method and calculated on a computer programme utilizing a PDP-12 computer (Digital Equipment Corporation). Systemic and pulmonary artery pressures were measured with transducers (Sanborn models 267AC and 267BC) and an 8-channel recorder (Sanborn model 958-100). All parameters were measured just before giving endotoxin and then 1 to 2, 15, 60, 120 and 240 min after the administration of endotoxin.

Blood analysis

Blood samples for prostaglandin analysis were collected from the pulmonary artery, and then immediately centrifuged at 4°C at 2450 rev/min. The plasma was then removed and frozen (−20°C) until analysed. The thawed plasma (2 ml) was extracted with redistilled ethyl acetate (2 × vol × 2) at pH 4.0. The dried ethyl acetate extract was reconstituted in benzene, ethyl acetate, methanol (60:40:10) and separated on a silicic acid column (0.5 g). The prostaglandin F (PGF) fractions were eluted with 5 ml benzene, ethyl acetate, methanol (60:40:30). Recovery of prostaglandin tracers added to plasma was 75 to 85% for $PGF_{2\alpha}$. $PGF_{2\alpha}$ antiserum was obtained from Dr H. Behrman (Yale, New Haven, Conn.). Radioimmunoassay was performed on the PGF fractions at two dilutions. Plasma standards were extracted with each group of unknown samples. The coefficient of variation for between, and within, assay reproducibility was required to be no greater than 10%. Data were calculated by the Rodbard and Hutt computer programme and expressed as pg/ml of plasma.

Mixed venous platelet counts were determined by an electronic particle counter (Coulter, Model ZBI). The one-stage method of Quick (1966) was used to assess the prothrombin time (PT). The activated partial thromboplastin time was performed with Celite-activated phospholipid (Nye & Graham, 1962) and fibrinogen was measured turbidometrically (Parfentjev, Johnson & Clifton, 1953). Quantitation of the fibrin-split products was carried out by the Staphylococcal clumping test (Hawiger, Niewearoski, Gusewich & Thomas, 1970).

Intravenous injections

Endotoxin (*E. coli* 0 55:B5, Difco) was reconstituted in Ringer lactate solution on the day of the experiment. An LD_{60} dose of endotoxin (1 mg/kg) was prepared in sterile Ringer lactate solution on the day of the experiment. Indomethacin (2 mg/ml) was prepared in sterile distilled water by the addition of anhydrous sodium carbonate on the day of the experiment. Lidocaine hydrochloride (10 mg/ml) was diluted in Ringer lactate solution to provide a continuous infusion of 1 mg kg^{-1} h^{-1}. Endotoxin, indomethacin, and lidocaine were given intravenously.

Three groups of animals were studied. Group I (10 dogs) was given only an LD_{60} dose of endotoxin; Group II (10 dogs) was treated with indomethacin (1.5 mg/kg) 45 min before, and 3 h after, an LD_{60} dose of endotoxin; Group III (11 dogs) received a continuous infusion of lidocaine from 45 min before, to 2 h after, an LD_{60} dose of endotoxin.

No attempts were made to resuscitate the animals after endotoxin. All animals received a total of 400 ml of Ringer lactate solution during the study. Upon completion of the experiment, the dogs were placed in a recovery room, observed for 72 h and considered to be survivors if they were alive and well at this time.

Each dog was observed during a control period of 45 min before the administration of the endotoxin. At the end of the control period, baseline values were determined and endotoxin was injected into a peripheral vein. At the time of the first fall in blood pressure, measurements were taken and haemodynamic parameters assessed. In the indomethacin-(Group II) and lidocaine-(Group III) treated animals, there was an additional 45 min time period between giving the drugs and the administration of the endotoxin. Statistical analysis was performed by the paired two-tailed Student's *t* test for the difference between the

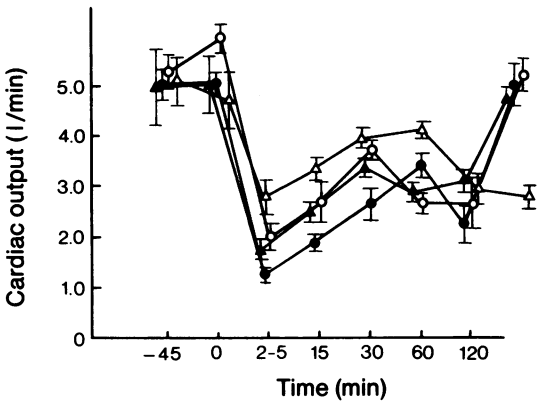

Figure 1 Mean cardiac outputs during endotoxin shock. Group I animals that lived (○) and died (●) consisted of 10 animals given endotoxin alone. Group II (△) consisted of 10 dogs treated with indomethacin (1.5 mg/kg) 45 min before, and 3 h after, an LD_{60} dose of endotoxin. Group III (▲) consisted of 11 animals which received a continuous infusion of lidocaine (1 mg kg^{-1} h^{-1}) from 45 min before, to 2 h after, an LD_{60} dose of endotoxin. The times indicate the following: 45 min (baseline measurements for all animals); 0 min (measurements in all groups immediately before endotoxin; in Groups II and III the values indicate the direct effects of indomethacin or lidocaine). The values represent mean results from all animals in each group; vertical lines show s.e. means.

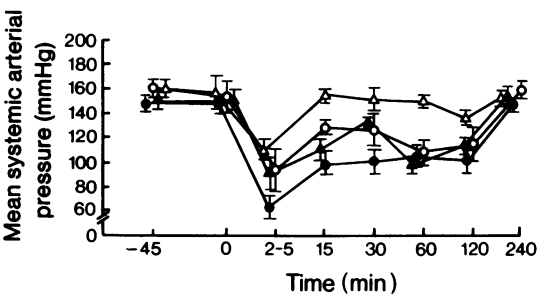

Figure 2 Mean systemic arterial blood pressures (mmHg) in dogs given only endotoxin (lived, ○; died, ●) or given endotoxin and indomethacin (△) or lidocaine (▲). Endotoxin was given to each group at 0 min. Mean values are shown; vertical lines indicate s.e. means.

baseline values and the experimental values in the same animals and the unpaired two-tailed *t* test for the difference between the groups. Chi square analysis was used to compare the survival data of the groups.

Results

Survival

All animals that received indomethacin (Group II) or lidocaine (Group III) survived, compared with only 40% in the endotoxin alone group. The survival rates were significantly ($P < 0.05$) different between the controls and the treated groups (II and III).

Haemodynamic parameters

Cardiac outputs for the three groups are shown in Figure 1. Neither lidocaine nor indomethacin had any significant effect on cardiac output. After endotoxin administration, cardiac outputs for the controls and the lidocaine-treated dogs were significantly ($P < 0.01$) less than the baseline values for the first 2 h. Interestingly, the cardiac outputs were greater during the first 30 min in those control animals that lived when compared to those that died. Lidocaine-treated animals had cardiac output values that were not different from control dogs that lived. Indomethacin treatment significantly ($P < 0.05$) improved the cardiac output during the first hour after the administration of endotoxin when compared to the control group.

Systemic mean arterial pressures (MAP) for all groups are presented in Figure 2. Neither lidocaine nor indomethacin alone had any significant effect on MAP. The animals that died in Group I had significantly ($P < 0.02$) decreased MAP only for the first 30 min of the study when compared to the control dogs that lived. Lidocaine-treated dogs had similar MAP values to those control dogs that lived. In contrast, indomethacin-treated animals had increased MAP values from 15 min to 2 h after the administration of endotoxin when compared to Groups I and III. The greatest fall in MAP (62 ± 10 mmHg; mean ± s.e.) was observed within 2 to 5 min after endotoxin administration in control dogs that died. Surprisingly, MAP values in all groups were not different from the baseline values by 4 h.

Pulmonary arterial pressures (PAP) are shown in Figure 3. Neither lidocaine nor indomethacin alone had any significant effect on PAP. *Only* at 2 to 5 min after endotoxin administration in the control (I) and lidocaine (III) groups were the PAP values greater than the baseline values ($P < 0.05$). Indomethacin-treated dogs (II) had PAP values that were significantly ($P < 0.05$) less than the endotoxin alone and the lidocaine-treated animals at that time.

Systemic vascular resistance (Figure 4) was not significantly affected by either indomethacin or lidocaine alone. In control animals that died, the vascular resistance was significantly ($P < 0.05$) greater 30 and

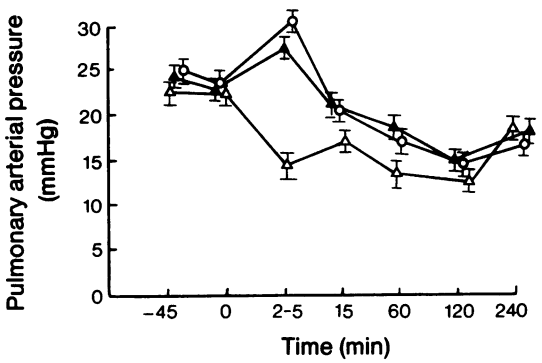

Figure 3 Mean pulmonary arterial pressures (mmHg) in dogs given only endotoxin (lived, ○; died, ●) or given endotoxin and indomethacin (△) or lidocaine (▲). Endotoxin was given at time 0. Mean values are shown; vertical lines indicate s.e. means.

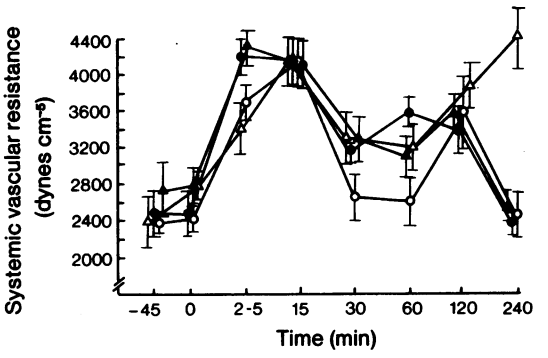

Figure 4 Mean systemic vascular resistance (dynes cm^{-5}) in dogs given only endotoxin (lived, ○; died, ●) or given endotoxin and indomethacin (△) or lidocaine (▲). Endotoxin was given at time 0. Mean values are shown; vertical lines indicate s.e. means.

60 min after the administration of endotoxin when compared with those animals that lived. The vascular resistance was significantly ($P < 0.01$) increased at 4 h with endotoxin and indomethacin when compared with the other groups.

Heart rates were not affected by lidocaine or indomethacin alone. After the administration of endotoxin, heart rates decreased in a similar fashion in all the groups, then returned to baseline values by the end of the study. There were no significant differences between the groups.

White blood cells, platelets, and coagulation. Neither lidocaine nor indomethacin alone altered the white blood cell or platelet counts; no effect on the coagulation tests were observed. There were no significant differences in the white blood cell counts between the groups. Interestingly, a severe leukopaenia was present within 2 to 5 min after the administration of endotoxin in all groups (1500 ± 300/μl; mean ± s.e.) when compared to the baseline value (11,706 ± 470/μl). Similarly, a severe thrombocytopaenia occurred in all groups within 2 to 5 min after the administration of endotoxin (14.1 ± 0.80 × 10^3/μl) when compared to the baseline value (210 ± 65 × 10^3/μl). However, there were no consistent differences between the groups. Surprisingly, only in the control animals (both those that lived and those that died) did the platelet counts return to normal by 4 h. In contrast, lidocaine- or indomethacin-treated animals had 4 h platelet counts (53.5 ± 5.0 × 10^3/μl) that were significantly ($P < 0.01$) less than those in the control group (205 ± 50 × 10^3/μl). The platelet counts were not different in control animals that lived when compared with those that died.

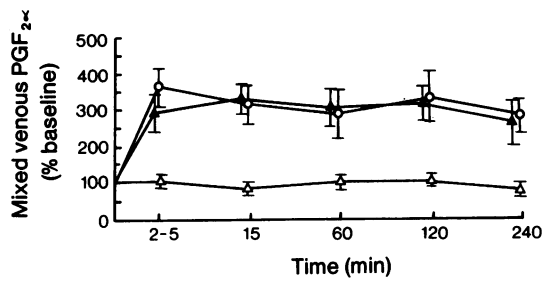

Figure 5 Prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) levels following the administration of endotoxin alone (○) or endotoxin with indomethacin (△) or lidocaine (▲). For details of treatment see text. Treatment with indomethacin completely prevented the increase in circulating $PGF_{2\alpha}$ that resulted from endotoxin administration. Baseline = 236 ± 31 pg/ml. Mean values are shown; vertical lines indicate s.e. means.

Prothrombin times and partial thromboplastin times were prolonged in all groups after the administration of endotoxin; however, there were no significant differences between the groups. In contrast, fibrin-split product formation during the first hour was significantly ($P < 0.01$) greater in the control animals that died when compared to those that lived. Indomethacin-treated dogs exhibited significantly ($P < 0.05$) decreased fibrin-split product formation for the first 2 h after the administration of endotoxin when compared with the controls and Group III.

Fibrinogen values were significantly less in control animals that lived (303 ± 47 mg %) at 2 to 5 min after the administration of endotoxin when compared with control dogs that died (480 ± 20 mg %). The lowest fibrinogen values after endotoxin were present

in the lidocaine-(III) treated dogs at 2 h (177 ± 27 mg %). The fibrinogen values during lidocaine treatment were significantly less ($P < 0.01$) at all sampling times when compared with values in controls and in Group II. In all control as well as all indomethacin-treated dogs (III), fibrinogen levels had returned to normal by 4 h.

Prostaglandins The mixed venous $PGF_{2\alpha}$ concentrations are shown in Figure 5. Before the administration of lidocaine or indomethacin they were 243 ± 38 pg/ml. Following lidocaine or indomethacin the mixed venous $PGF_{2\alpha}$ levels were 224 ± 41 pg/ml.

The $PGF_{2\alpha}$ concentrations were significantly increased ($P < 0.02$, at all sampling times) in the control and the lidocaine-treated groups after endotoxin. Indomethacin treatment prevented the increase in the prostaglandin concentrations after the administration of endotoxin when compared with the control and lidocaine-treated groups. The greatest increase in the $PGF_{2\alpha}$ concentration was within 2 to 5 min after the administration of endotoxin in the control group.

Discussion

The important findings in this study are: (i) intravenous lidocaine in therapeutic doses was as effective as therapeutic doses of indomethacin in improving the survival during canine endotoxin shock; (ii) indomethacin and lidocaine afforded significantly different degrees of attenuation of the haemodynamic events evoked by endotoxin; (iii) indomethacin inhibited $PGF_{2\alpha}$ release after endotoxin, whereas lidocaine had no apparent effect; (iv) neither lidocaine nor indomethacin alone altered any of the parameters measured in this model; (v) the exact mechanism by which lidocaine affords protection in this model is undetermined.

The use of intravenous lidocaine for the treatment of canine endotoxin shock has not been reported previously. The facts that both lidocaine and indomethacin have been found to increase vascular smooth muscle sensitivity to circulatory catecholamines and that improved circulatory function is observed with indomethacin-treated canine endotoxin shock, suggested that lidocaine might be a logical drug to evaluate in this model. In addition to its reported effects on vascular smooth muscle, lidocaine has many other effects. It antagonizes the binding of calcium and other ions to phospholipids (Feinstein, 1964; Blaustein & Goldman, 1966a). Local anaesthetics displace calcium from red blood cell membranes (Kwant & Seeman, 1969) and are competitive inhibitors of the binding of mono- and divalent cations to submitochondrial particles (Scarpa & Azzi, 1968). A decrease in membrane permeability by local anaesthetics appears to be related to membrane phospholipase activity (Scherphof *et al.*, 1972). Inhibition of the increase in Na^+ and K^+ conduction in axon membranes occurs with local anaesthetics (Blaustein & Goldman, 1966b). A lidocaine concentration of 50 μM inhibits a major prostaglandin degrading enzyme, 15-hydroxy prostaglandin dehydrogenase (Tai & Hollander, 1976). Platelet aggregation and adhesion are inhibited by local anaesthetics which prevent the release of the internal pool of Ca^{2+} (Feinstein, Fiekers & Fraser, 1976) and compete for the calcium binding sites on the platelet surface (O'Brien, 1962). Which of these effects of lidocaine, if any, are beneficial in the treatment of canine endotoxin shock is uncertain. The findings in this study do suggest that lidocaine does have some observable effect on the mean arterial pressure and the cardiac output following endotoxin. However, our hypothesis that lidocaine and indomethacin may have similar effects on vascular smooth muscle sensitivity to circulating catecholamines in canine endotoxin shock cannot be strongly supported.

The concentration of lidocaine employed in this study (1 mg kg^{-1} h^{-1}) was selected because it is in the low therapeutic dose range recommended for the treatment of clinical cardiac arrhythmias (Harrison & Collinsworth, 1974). This *in vivo* lidocaine concentration is difficult to compare with the *in vitro* concentrations in the studies mentioned above. However, it is clear that this concentration of lidocaine is sufficient to increase the survival in this model.

The improvement in circulatory function in endotoxin shock with the analgesic-antipyretic drugs was introduced by Northover & Subramanian (1962). Others (Hinshaw, Solomon, Erdos, Reins & Gunter, 1967; Culp, Erdos, Hinshaw & Holmes, 1971; Greenway & Murthy, 1971; Parratt & Sturgess, 1974; 1975a, b & c; 1976) have confirmed the original work and extended it to various species. The concept that has emerged from these studies and others (Anderson, Jubiz, Fralios, Tsagaris & Kuida, 1972; Collier, Herman & Vane, 1973; Fletcher *et al.*, 1976a, b; Fletcher & Ramwell, 1977; Parratt & Sturgess, 1977) is that the arachidonic acid-prostaglandin system participates in the pathophysiology of endotoxin shock. Our previous work (Fletcher *et al.*, 1976a, b; Fletcher & Ramwell, 1977) clearly demonstrated that the prostaglandin concentrations were elevated at times when circulatory function was changing. There is evidence that $PGF_{2\alpha}$ participates in the rise in pulmonary arterial pressure seen following the administration of endotoxin (Anderson, Tsagaris, Jubiz & Kuida, 1975; Parratt & Sturgess, 1975a; 1977; Fletcher *et al.*, 1976; 1977; Fletcher & Ramwell, 1977). The pulmonary arterial hypertension is prevented by treatment with prostaglandin synthetase inhibitors. Indomethacin, like most prostaglandin synthetase inhibitors, can

completely block the cyclo-oxygenase system (Flower, 1974). The measurement of $PGF_{2\alpha}$, as done in this study, is only an indicator of the degree of activity of the arachidonic acid-postaglandin system. However, it is known (Flower, 1974) that at these doses of indomethacin, there is also inhibition of the production of the endoperoxides (PGG_2 and PGH_2), PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2. To state that $PGF_{2\alpha}$ alone is responsible for the pulmonary vasoconstriction is misleading since the effects of the endoperoxides and thromboxanes cannot be excluded. However, of more important interest, is whether or not the prostaglandins participate as a cause in the irreversibility of endotoxin shock. Our previous study (Fletcher & Ramwell, 1977) suggested that some effect of the indomethacin other than prostaglandin inhibition may be important for survival since those untreated animals that *lived* had plasma prostaglandin levels that were not significantly different from those untreated animals that *died*. The results of the present study suggest a similar conclusion; that is, the prostaglandin levels were not significantly different between the lidocaine-treated dogs (of which 100% lived) and the untreated control dogs (of which 40% lived). In consequence, the primary factor in survival in this model may not be the effects of these drugs on prostaglandin synthesis.

We could not demonstrate any effect of lidocaine or indomethacin alone on the white blood cell or platelet counts or on the coagulation parameters. However, following endotoxin there were differences in the thrombocytopaenia, fibrin-split products, and fibrinogen concentrations among the groups. Lidocaine or indomethacin did not alter the changes in the white blood cell counts following endotoxin. Surprisingly, the platelet counts were significantly less in the indomethacin- and lidocaine-treated dogs at 4 h when compared to the untreated controls. Indomethacin (Koscis, Hernandovich, Silver, Smith & Ingerman, 1973) and lidocaine (Feinstein *et al.*, 1976) are known inhibitors of platelet aggregation, therefore, less of a thrombocytopaenia in canine endotoxin shock was expected. Perhaps lidocaine or indomethacin have additional effects *in vivo* that interfere with platelet production. The effect of indomethacin on fibrin-split product formation and the effect of lidocaine on fibrinogen concentrations following the administration of endotoxin are unexplained by this study and require additional detailed studies to determine the significance of these effects.

In summary, this study suggests that in addition to the use of the analgesic-antipyretic drugs, another group of drugs, namely local anaesthetics might offer protection in human endotoxin shock. Lidocaine is administered in coronary care units throughout the world in greater concentrations than employed in this study (Harrison & Collinsworth, 1974). We utilized lidocaine or indomethacin prophylactically for canine endotoxin shock. Additional experiments, in which treatment is instituted after shock has occurred, are indicated.

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