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Protective Effect of indomethacin in experimental gram-negative bacteremia in dogs[x]

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Summary: In this experimental study, central venous pressure, portal venous pressure, hematocrit, leukocyte, platelet levels and arterial blood gases were measured initially and 10,30,60 and 120[th] minutes after gram-negative bacteremia induced with the intravenous injection of live Pseudomonas aeruginosa (10^{11}/ml and 0.5 ml/kg) in dogs. The study was carried out in three groups; Group-1 (Control group-Indomethacin treatment), Group-2 (Study group-Pseudomonas aeruginosa) and Group-3 (Treatment group-Protective indomethacin treatment and Pseudomonas aeruginosa). We observed decrease in central venous pressure, arterial blood pH, leukocyte and platelet levels; increase in portal venous pressure and hematocrit levels after Pseudomonas aeruginosa injection. Acidosis had developed in both study and treatment groups. Gram-negative bacteremia caused pathologic changes in the lung when compared with control group. The protective effect of indomethacin treatment 60 minutes before bacteremia were investigated. Results were compared to those of the literature. It was seen that the indomethacin treatment had some favourable effects on gram-negative bacteremia caused by Pseudomonas aeruginose but it is not significant.

Key words: Sepsis, bacteremia, indomethacin.

Sepsis contineus to be the major cause of death in most surgical departments despite the use of broad spectrum antibiotics and probably the most common cause of adult Respiratory Distress Syndrome (ARDS). Lysosomal enzymes released from white cells sequestered in the lung (3), microembolic blockage of the pulmonary microcirculation (4,7,10,12) and some vasoactive mediators (2) have been incriminated from the lung pathology in sepsis. There is increasing evidence of arachidonic acid metabolites as mediators of lung injury in sepsis.

In this study, we have tested the effect of indomethacin which inhibits arachidonic acid metabolism on live Pseudomonas aeruginosa infusion-induced lung injury in dogs.

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Material and Method

Fifteen adult mongrel dogs, weighing 11-18 kg(mean 14 kg) were used in this study. Dogs were fed with the regular labaratory chow and were fasted 24 hours prior to the experiment. Anesthesia was induced by the intravenous injection of thiopental sodium (25 mg/kg) with 5 mg/kg of supplementary dose as needed. The dogs were intubated endotracheally and allowed to breathe the room air spontaneously. The dogs were kept in supine position during the study.

Femoral vein was cannulated with a polyvinyl catheter and this catheter was used for the central venous pressure measurement, indomethacin and Pseudomonas aeruginosa injections, to get blood samples for hematocrit, leukocyte and platelet counts. This vein was also used for infusion of 0.9 % Na Cl (8 ml/hr/kg) during the experiment and for the anesthetic injections.

The femoral artery was also exposed and was used to obtain blood samples for measuring of blood gases.

A sterile midline laparotomy was performed and the portal vein was cannulated via the splenic vein. The portal venous pressure was measured via this catheter.

Indomethacin (100 mg) was prepared in a sterile 0.9 % na Cl solution by adding anhydrous sodium carbonate (37.5 mg) on the day of experiment.

Indomethacin was infused before 60 minute of basal measurement at 10 mg/kg dose.

All dogs received 0.9 % Na Cl 8 ml/hr/kg, during the experiment. Pseudomonas aeruginosa suspension (10^{11}/ml and 0.5 ml/kg) was given intravenously in a bolus injection.

The study was carried out in three groups, 5 dogs each . Dogs were randomly assigned. We measured the blood parameters just before the experiment and at 10,20,60,90 and 120^{th} minutes. The lung biopsies were taken at 60 and 120^{th} minutes from the contralateral lung.

Group-1 (n=5) (Control group) : Anesthesia, laparotomy and indomethacin
Group-2 (n=5) (Study group) : Anesthesia, laparotomy and pseudomonas aeruginosa
Group-3 (n=5) (Treatment group) : Anesthesia, laparotomy indomethacin and pseudomonas aeruginosa.

The lung biopsies were fixed with 10 % formalin for later histologic examinations. Sections were stained with the Hematoxylin-Eosin stain and examined with light microscope. Each sections were examined for the criterion of atelectasis, vascular congestion, polymorphonuclear infiltration, hemorrhage and edema. Each pathological change recorded and graded between 0 and 3 (0= Normal and 3= maximal pathological change). The average Pathologic Index (A.P.I.) were calculated as the mean value of 5 dogs in each group. Every A.P.I. were compared with each other. Student's t test was used for the statistical analysis.

Results

A- Hemodynamic parameters: The injection of live Pseudomonas aeruginosa rapidly produced a significant reduction on platelet and leukocyte counts and central venous pressure in both group-2 and 3. After the injection of live Pseudomonas aeruginosa, the portal venous pressure and hematocrit levels were increased. There was no change in arterial blood pH in group-1, but pH decreased directly proportional with the time in both group-2 and 3, and acidosis developed.

B- Pulmonary histopathology: The A.P.I. was 0.28 ± 0.04 at the 60^{th} minute and 0.36 ± 0.04 at the 120^{th} minute in Group-1 (Table I). The increase in A.P.I. was observed in Group-2 after Pseudomonas aeruginosa injection at the 60^{th} and 120^{th} minutes (respectively $P<0.05$ and $p<0.05$). There was also significant increase in A.P.I. (Table II)in Group-3 when compared with the control group at the 60^{th} and 120^{th} minutes ($P<0.05$ and $p<0.05$ respectively)(Table III). But there was no significant difference in A.P.I. between Group-2 and 3 at the same period of observation ($P>0.05$).

Table I. Pathologic changes group-I dogs(0: No pathology, 3: Maximal pathology, and A.P.I.: Average Pathologic Index).

60. min Dog Number	Athelectasia	Vas.Conges.	P.N.L. infilt.	Hemorrhage	Edema	Pathologic index
1	1	1	0	0	0	0.4
2	1	1	0	0	0	0.4
3	1	0	0	0	0	0.2
4	1	0	0	0	0	0.2
5	0	0	0	1	0	0.2
A.P.I.						0.28±0.04
120.min						
1	1	1	0	0	0	0.4
2	2	0	0	0	0	0.4
3	1	0	0	0	0	0.2
4	1	1	0	0	0	0.4
5	1	0	0	1	0	0.4
A.P.I.						0.36±0.04

Table II Pathologic changes Group-2 dogs. (0:No pathology, 3: Maximal pathology, and A.P.I.: Average Pathologic Index).

60.min. Dog Number	Athelectasia	Vas.Conges.	P.N.L. Infilt.	Hemorrhage	Edema	Pathologic Index
1	2	1	1	0	0	0.8
2	1	1	1	1	1	1.0
3	1	2	1	0	0	0.8
4	1	2	1	1	1	1.2
5	1	1	1	1	0	0.8
A.P.I.						0.92±0.08
120.min.						
1	2	1	1	0	1	1.0
2	1	2	0	2	1	1.2
3	2	1	2	1	2	1.6
4	1	2	1	0	0	0.8
5	1	1	0	1	2	1.0
A.P.I.						1.12±0.13

Table III.Pathologic changes Group-3 dogs.(0:No pathology, 3: Maximal pathology, and A.P.I. : Average Pathologic Index).

60.min. Dog Number	Athelectasia	Vas.Conges.	P.N.L. Infilt.	Hemorrhage	Edema	Pathologic Index
1	1	1	0	0	1	0.6
2	1	2	1	2	1	1.4
3	1	1	1	0	0	0.6
4	1	1	2	0	2	1.2
5	1	0	1	1	1	0.8
A.P.I.						0.92±0.16
120.min.						
1	1	1	1	0	1	0.8
2	2	1	0	1	2	1.2
3	1	2	0	1	1	1.0
4	1	1	2	0	1	1.0
5	1	2	0	0	1	0.8
A.P.I						0.96±0.07

Discussion

The Sepsis is an acute infection and characterized by episodic bacteremia and associated with the systemic changes. Although there are many cause of ARDS, the sepsis probably is the most common cause. Despite the strong antimicrobial agents, the mortality rate in ARDS is still high. One of the models used to produce sepsis is the intravenous infusion of live organisms like Pseudomonas aeruginosa (16). İntravenous infusion of Pseudomonas aeruginosa produce a fulminant ARDS smiliar to that seen with the severe sepsis in man (8,9). Because of the increasing importance of Pseudomonas aeruginosa in gram-negative sepsis, this bacteria was chosen for the present study.

Lysosomal enzymes released from white cells, sequestered in the lung (3), microembolic blockage of the pulmonary microcirculation (4,7,10,12) and some vasoactive mediators (2) (such as prostaglandins) have been incriminated from the lung pathology in sepsis. If this agents have an important role, then inhibition oftheir synthesis might be of benefit in sepsis-induced lung pathology.

Demling et al (3), reported that lysosomal enzymes are released into the lung after endotoxin administration, probably from sequestered leukocytes, the degree of release corresponding to the degree of vascular injury. Indicator of the cell injury is the increase in plasma lysosomal enzymes. It was found that lysosomal enzymes increased in plasma after endotoxin-induced lung injury. These enzymes alter the microvascular membrane resulting in the increased vascular permeability. Non-steroidal anti-inflamatory agents stabilize lysosomes, that means they might prevent the rise of lysosomal enzymes in plasma and might prevent some of the harmfull effect of sepsis-induced lung injury.

Arachidonic acid metabolites may act as a central factor in initiating septic-induced respiratory failure (14). Prostaglandins (2), thromboxane (5,8,9,13) are involved in the pathophysiology of lung injury. Indomethacin, cyclooxygenase inhibitor, blockes the increased plasma thromboxane B_2, the stabile metabolite of thromboxane A_2, and inhibits prostaglandin synthesis (17). Inhibition of the effects of thromboxane A_2 or blocking thromboxane A_2 production has improved survival in rats following endotoxin administration (1,18). Pseudomonas aeruginosa infusion produces respiratory dysfunction (8,9) and a cyclooxygenase inhibitor which prevents the production of prostaglandins including thromboxane, alleviates some but not all of the problems observed in ARDS.

Thromboxane causes both platelet aggregation (6) and white blood adhesion (15). It is also suggested that the beneficial effect of indomethacin pretreatment results from inhibition of platelet aggregation (11).

Theoretically, indomethacin might be of benefit in sepsis-induced lung injury by decreasing the levels of lysosomal enzymes, by inhibiting the prostaglandin synthesis and thromboxane or by inhibition of the platelet aggregation. It is also reported that indomethacin is beneficial in endotoxin shock hemodynamically and histologically in several species in the literature. In this study, we could not demonstrate a significant beneficial effect of indomethacin pretreatment on the lung pathology seen in live Pseudomonas aeruginosa administration. But it is hypothetized that the septic-induced ARDS is probably due to the release of multiple inflammatory mediators in addition to thromboxane, therefore the treatment will most likely require polypharmacy (8). In summary our results suggest that the indomethacin pretreatment may have a protective effect in live Pseudomonas aeruginosa-induced lung injury but it is not significant.

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