Original article

Effect of nitric oxide scavengers, carboxy-PTIO on endotoxin induced shock in sheep

Andualem Mossie Ayana[1], Hassen Taha Sherief[2], Stefan Eriksson[3], Legesse Zeriehun[2]

Abstract: Physiological changes associated with septic shock are due to an interplay of a number of inflammatory mediators which increase capillary permeability and vasodilation leading to circulatory disturbance. Research evidence shows that sepsis-associated vascular relaxation is mediated by nitric oxide. Nitric oxide formation is stimulated by endotoxin, cytokines such as Tumor necrosis factor, and Interleukines. The stimulation is due to the activation of an inducible nitric oxide synthase, which transforms an amino acid L-arginine into nitric oxide in endothelial cells and macrophages. In the present study, administration of endotoxin (LPS, E. coli extract, 70 μg/kg, iv) in six unanesthesized ewes resulted in a decrease in the mean arterial blood pressure (MAP) by 38%, $P<0.01$ and an increase in cardiac index of $P<0.05$. Injection of 50 mg/kg of carboxy-PTIO, as an antidote, after an hour of endotoxin dose, reestablished the normal baseline values of the cardiovascular parameters considered in this study. This indicates that carboxy-PTIO is an efficient nitric oxide scavenger chemical of trapping nitric oxide immediately after its synthesis. Therefore, based on the current result, carboxy-PTIO can be used as one possible treatment agent against septic shock. [*Ethiop. J. Health Dev.* 2000;14(1):85-89]

Introduction

Circulatory shock is a clinical syndrome characterized by a decrease in blood pressure, an increase in heart rate, an alteration in tissue perfusion, an alteration in mentation, a decrease in urine output and cutaneous vasoconstriction (1). It reflects an acute impairment of cellular oxygen availability, which leads to anaerobic metabolism, and an increase in the production of lactic acid. Hence, an increase in blood lactate level is considered to be a hallmark of circulatory shock (2).

Septic shock is one of the leading causes of death with a mortality rate of 30% (3). This high mortality rate has stimulated an intense research to elucidate the pathophysiology of septic shock and to search for novel therapies (4,5). Septic shock usually results from infection by Gram-negative bacteria (30-80% of the cases) and Gram-positive bacteria (6-24%), the rest being caused by fungi and viruses (6, 7). The development of Gram-negative septic shock is initiated by the cell wall component of the bacteria, a lipopolysaccharide known as endotoxin (3, 8). Endotoxin has an antigenic effect capable of stimulating macrophages, neutrophils, platelets and endothelial cells to produce cytokines (particularly tumor necrosis factor (TNF-α)) and interleukines (IL_1, IL_2, IL_4, IL_8). Interleukines and TNF-α are known to be the mediators of septic shock (9, 10, 11). These mediators cause many of the signs and symptoms of septic shock either by their direct effect or by their stimulatory effect on the release of other cytokines, such as prostaglandins, platelet activating factor, bradykinin, and histamine (12, 13).

Macrophages and endothelial cells synthesize an inducible nitric oxide synthase enzyme in response to endotoxin and cytokine stimulation. This enzyme causes an over-production of nitric oxide. Excessive production of nitric oxide leads to vasodilation

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and hypotension (14). It also leads to cardiac depression and generalized cytotoxin effect (18). Nitric oxide is now a days conventionally accepted as the ultimate mediator in septic shock (15, 16).

Therapeutic agents have so far failed to show a significant improvement in the mortality rate due to septic shock. Recent research on septic shock therapy is focused on the possible role of excessive production of nitric oxide and suggests that inhibition of either the synthesis or the action of nitric oxide could have an effective therapeutic implication in septic shock (3, 14, 17).

There are different types of nitric oxide blockers. Some of them inhibit the enzyme called nitric oxide synthase, while others trap nitric oxide before it reaches its target tissue (19). Free nitric oxide can be inactivated by scavenger chemicals such as methylene blue, fuscidic acid, and carboxy-PTIO (3,20). Carboxy-2-phenyl-4,4,5,5-tetramethy-limidozolene-1-oxyl-3-oxide (Carboxy-PTIO) is a stable, water soluble nitric oxide scavenger molecule. The inhibitory effect of Carboxy-PTIO on NO is found to be two-fold stronger than those of nitric oxide synthase inhibitors (20).

The main aim of this study is to evaluate changes in cardiovascular parameters such as the mean arterial pressure, systemic vascular resistance, heart rate and cardiac index, before and after the administration of endotoxin alone and endotoxin with carboxy-PTIO.

Methods

Six adult Texel cross-breed ewes (body weight 65±5 kg) were used in all experiments. Acetpromazine (10mg/kg), sodiumthiopental (25 mg/kg), and a muscle relaxant celocurine (50 mg/kg) were used as anesthetic medications during surgical operations of each sheep. Striptocillin, 4ml daily for five days, intramuscular, was given as a prophylactic antibiotic after the surgery.

During carotid loop surgical operation, anesthetized ewes were kept in supine position on the operation table. The cervical region was shaved and cleaned with antiseptics. Two midline incisions were made on the skin. The underlying tissue was dissected carefully to separate the vagus nerve and to grasp the carotid arteries. The carotid arteries, once isolated, were wrapped by the overlying skin and suturing was done thereafter.

After two weeks of healing, the ewes were ready for experimentation. One of the exteriorized carotid artery was catheterized with arterial cannula. Measurement of he arterial blood pressure and the heart rate was taken from the recorder (ABB SE 120 Polygraph) connected with the arterial cannula.

A flow directed thermodilution Swan Gantz catheter was introduced into the pulmonary artery via the right jugular vein with a small incision made under local anesthesia. The catheter was positioned with the help of continuous pressure monitoring and the final position of the catheter was in the pulmonary arterial branch, usually 50-60 cm away from the jugular entrance. The central venous pressure was measured via a proximal part of the Swan Gantz catheter, while the pulmonary arterial pressure was recorded at the distal end of the catheter. Intravascular pressure was recorded by a transducer and displayed on a Grass Polygraph after an initial repeated calibration of the baseline values (Figure 1A).

The systemic vascular resistance was calculated by dividing the systemic pressure gradient by the cardiac out put SVR = (MAP - CVP)/CO. The systemic pressure is the mean arterial pressure minus the central venous pressure (MAP - CVP).

Cardiac output was approximated as described by Rubini, et al, (1995). It is measured by means of a thermodilution technique (rapid iv injection of 10 ml of cold 3-4°C isotonic saline). Signals from the catheter were processed on an Edwards Lab cardiac output computer (Model 95 10-A). The temperature dilution curve was graphically displayed and the cardiac output values were read. The cardiac index was calculated by dividing the cardiac output by the body weight in order to minimize variations due to body size.

All the parameters considered in this study

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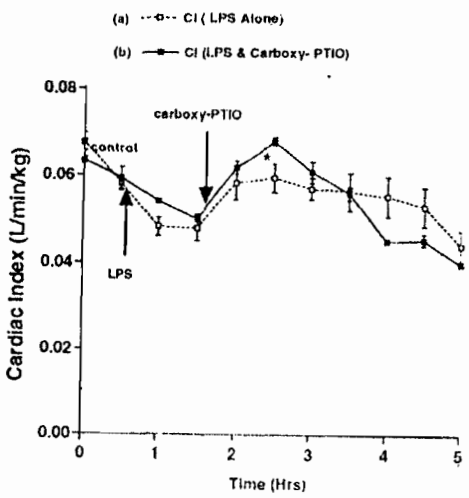

Figure 3: Cardiac Index (CI): The time course change in CI following intravenous administration of endotoxin (LPS, 70 μg/kg) alone and with carboxy-PTIO (50 mg/kg) after LPS injection tested on six awake sheep. Control values were recorded at the beginning of each experiment before endotoxin injection. Results are plotted on the graph as mean plus or minus SEM and computed as (a) changes in CI after LPS injection were compared with control values and were found to be statistically significant *($p<0.005$). (b) changes in CI after carboxy-PTIO were compared with values recorded with LPS alone and were found to be statistically not significant ($P>0.05$).

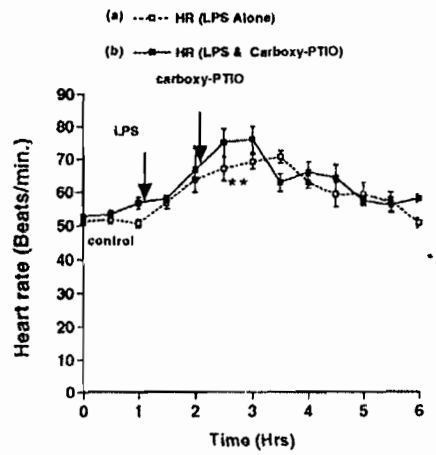

Figure 4: Heart rate (HR): The time course change in heart rate following intravenous injection of endotoxin (LPS, 70 μg/kg) alone and with carboxy-PTIO (50 mg/kg) after LPS injection. Control values were recorded initially at the start of the experiment. Results are plotted on the graph as mean plus or minus SEM and computed as follow (a) the change in HR after LPS injection were compared with control values and found to be statistically significant **($p<0.001$). (b) changes in HR after administration of carboxy-PTIO were found to be statistically not significant ($P>0.05$) compared to values recorded with LPS alone.

stabilization to the baseline value. Carboxy-PTIO had no significant effect on reversing endotoxin-induced tachycardia.

Discussion

The main findings of the present study show that (a) administration of 70 μg/kg of endotoxin to sheep produces marked hypotension, (b) administration of 50 mg/kg of carboxy-PTIO reverse endotoxin induced cardiovascular changes.

Endotoxin decreases the mean arterial pressure and systemic vascular resistance due to the effect of endotoxin triggering the release of septic mediator cytokines such as TNF-∝, from endothelial cells and macrophages (9). These cells synthesize an inducible nitric oxide synthase in response to endotoxin and cytokine stimulation. This stimulation results in the production of nitric oxide that leads to vasodilation and hypotension.

Intravascular administration of 50 mg/kg of carboxy-PTIO reversed endotoxin induced hypotension. Carboxy-PTIO traps nitric oxide released in response to endotoxin and cytokines stimulation. The reversing action of this chemical returned the mean arterial pressure and systemic vascular resistance to the baseline values.

Related work done by Kilbourn et al (1990) indicated that administration of 40 μg/kg of endotoxin to anesthetized dogs caused a reduction in peripheral vascular resistance (% = 33) and a fall in the mean arterial pressure (%Δ = 54) within an hour. Both the vascular resistance and the arterial pressure returned to normal after administration of N-methyl-L-arginine (20 mg/kg), a potent and selective inhibitor of Nitric oxide synthase. L-arginine, precursor of nitric oxide, reversed the effect of this drug and restored the hypotension.

The heart rate increased after endotoxin injection. This is probably due to sympatho-adrenal stimulation in response to endotoxin induced hypotension. Carboxy-PTIO has no significant effect on reversing endotoxin induced changes in heart rate.

Endotoxin produced a biphasic alteration of cardiac index with an initial slight fall followed

by immediate increase towards the baseline values. The initial fall might be caused by the myocardial depressant effect of endotoxin, TNF-α, and nitric oxide. It could also be due to coronary hypoperfusion. The subsequent increase in cardiac index might be due to sympathoadrenal stimulation. This result is supported by the work of Piepoli et al, (1995). Carboxy-PTIO was shown to have no effect on reversing endotoxin induced changes of cardiac index.

In conclusion this study, administration of carboxy-PTIO on endotoxin shocked ewes, reversed the mean arterial pressure, and systemic vascular resistance significantly. Based on this finding, we can conclude that carboxy-PTIO can be used as a therapeutic agent for septic shock. However, further investigation is needed on this compound to find out the dose-response relation, its adverse effects and its pharmacokinetics.

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