

Complement-1 Inhibitor Attenuates Myocardial Ischemia Reperfusion Injury in a Guinea Pig Model

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Abstract: Complement-1 esterase Inhibitor (C1-INH), an endogenously derived compound, is a key mediator providing regulation of the complement system. In this study, the protective role of C1-INH was investigated in the setting of myocardial ischemia reperfusion injury. Guinea pig hearts (n = 20) were studied in control (n = 10) and experimental (n = 10) groups, using a modified Langendorff perfusion apparatus. Control hearts were perfused with Krebs Henseleit solution during pre-ischemia and reperfusion periods while C1-INH was added to the perfusates of experimental hearts during the reperfusion period. Heart rate (pulse/minute), contractility (mm) and aortic pressure (mmHg) values were recorded at the end of pre-ischemia, post-ischemia and reperfusion periods. Perfusate and tissue analysis for glutathione and malondialdehyde levels and perfusate analysis for nitric oxide levels were obtained at the end of each experimental period. Both increased aortic pressure and cardiac contractility as well as elevated levels of tissue glutathione and MDA were observed in the experimental group during reperfusion. Perfusate levels of glutathione and MDA remained unchanged. As a result, it was concluded that C1 esterase inhibitor preserved cardiac contractility and protected against ischemia reperfusion injury.

Key words: Complement inhibitor, ischemia, reperfusion injury pig model, MDA

INTRODUCTION

A greater understanding of the dynamics behind Cardiopulmonary Bypass (CPB) has led to a decrease in peri-operative mortality and morbidity. Nonetheless, post-operative complications following CPB still occur, mainly due to the immune response that produces inflammatory reactions during ischemia/reperfusion of myocardial tissue. In addition to ischemia/reperfusion, CPB can also initiate an inflammatory cascade that results in the presence of reactive oxygen compounds, cytokines and other cytotoxic substances (Grace, 1994; Granger and Korthius, 1995; Homeister and Lucchesi, 1994). The complement system consists of the biochemical pathways that initiates and produces the immune response of body tissue leading to this inflammation.

Complement activation is one of the protective mechanisms of tissue when it encounters a foreign

surface or a foreign agent and it leads to programmed cell death via numerous enzymes and regulatory proteins. During CPB, when blood contacts surfaces other than endothelium, the complement system is activated (Hess, 2005). In addition, ischemia/reperfusion injuries that can occur during the coronary revascularization process, is caused by an exaggerated activation of the complement system immune response (Chenoweth *et al.*, 1982; Crawford *et al.*, 1998).

There are several regulatory mechanisms present in tissue to compensate for the over-activation of the immune system. Complement 1 esterase Inhibitor (C1-INH) is one enzyme used as an immune regulatory mechanism specific to the first step of the complement system. This enzyme, however, is diluted, along with other blood proteins, during CPB (Buerke and Toyooki, 1995; Bauernschmit *et al.*, 2000). The resultant decrease in complement system regulation leads to insufficient suppression of the immune system activation

in reperfusion injury following CPB and contributes to significant post-operative morbidity and mortality (Sellke and Boodhwani, 2006).

The use of C1-INH as a therapeutic agent during ischemia/reperfusion injury has been previously studied in humans and in animal models. C1-INH was shown to diminish the necrotic area developed secondary to ischemia and to provide protection against myocardial dysfunction following tissue reperfusion (Caliezia *et al.*, 2000; Lehmann *et al.*, 2000). This study investigates the protective role of C1-INH, added to the perfusate during CPB, against myocardial ischemia reperfusion injury in guinea pig hearts.

MATERIALS AND METHODS

Twenty guinea pigs weighing 300-400 g and fed with standard chow were used for this study. The guinea pigs were divided into two groups of ten: A control group and an experimental group. In preparation for heart isolation, the guinea pigs were administered heparin 300 u kg⁻¹ intraperitoneally. Ten minutes after anticoagulation treatment the guinea pigs were anesthetized with 25% urethane 0.7 mL 100 g⁻¹ administered intraperitoneally.

Following isolation of the heart and aorta and cannulation of the ascending aorta, the guinea pig hearts were excised, weighed and attached to a modified Langerdorff apparatus perfusion system. Hearts from both groups were immediately stabilized with normothermic perfusion with a constant flow (7 mL min⁻¹) of a modified Krebs solution for 20 min delivered by a micro-tubing pump (MP3) (Sinci *et al.*, 1994). A 3.0 silk was passed from the cardiac apex through a fixed pulley and attached to isometric tension transducers of a GRASS polygraph (Model 7G), used to record heart rate and contractility levels.

After stabilization, all hearts were subjected to normothermic ischemia for 20 min and the hearts were then reperfused for 20 min. Control group hearts were reperfused with the modified Krebs solution, while experimental group hearts were reperfused with C1-INH 100 U/kg/min (Sigma-Aldrich Company), added to the modified Krebs solution.

Heart rate (pulse min⁻¹), aortic pressure (mmHg) and contractility (mm) were recorded at three time points (pre-ischemia, post-ischemia and post-reperfusion) to assess the level of cardiac function. Perfusate samples were taken during the same three time points and the samples were then frozen and stored at -70°C for subsequent Malondialdehyde (MDA), glutathione and Nitric Oxide (NO) level analyses. Tissue samples were

obtained following the 20 min reperfusion period in order to measure MDA and glutathione tissue levels. Spectrophotometric analysis of plasma and tissue levels of MDA (412 nm), glutathione (532 nm) and NO (544 nm) were performed with the Spectronic 21 device (Bausch and Lomb). MDA, glutathione and NO were used to evaluate the efficacy of C1-INH in preventing ischemia/reperfusion injury.

Data were analyzed using SPSS version 13.0 (Chicago, IL). Independent group means medians were compared with Kruskal Wallis and Mann Whitney U tests, post Hoc tests were used for detected significances. Dependent groups were compared according to control and experimental values by the Wilcoxon paired sample test. $p < 0.05$ was considered significant.

RESULTS

There were no statistically significant differences in mean heart rate between the experimental and control

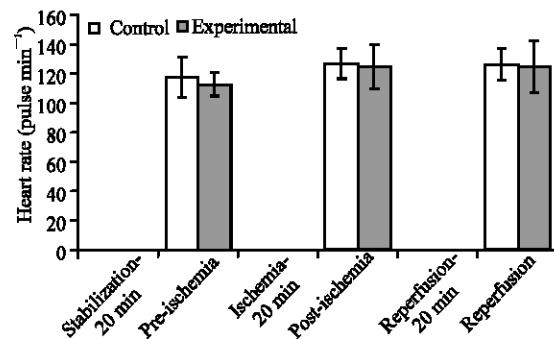


Fig. 1: Heart rate (pulse min⁻¹) values of control and experimental groups (X ± SD)

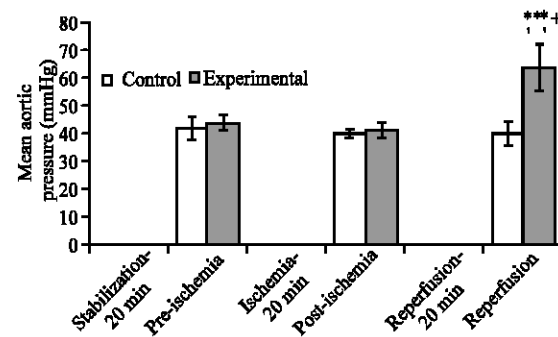


Fig. 2: Mean aortic pressure (mmHg) values of control and experimental groups (X ± SD), * $p < 0.05$: Compared to control groups, ** $p < 0.05$: Compared to pre-ischemic data, + $p < 0.05$: Compared to post-ischemic data

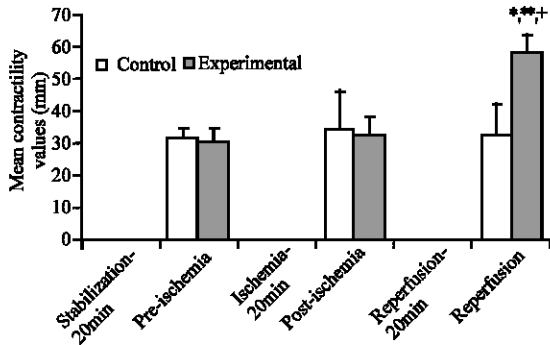


Fig. 3: Mean contractility (mm) values of control and experimental groups ($X \pm SD$), * $p < 0.05$: Compared to control groups, ** $p < 0.05$: Compared to pre-ischemic data, + $p < 0.05$: Compared to post-ischemic data

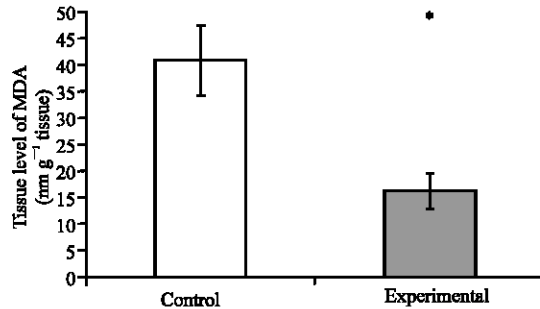


Fig. 4: Tissue levels of MDA (nm mL^{-1}) in control and experimental groups ($X \pm SD$), * $p < 0.05$: Compared to control groups

groups and between each time point of each group (Fig. 1). The mean aortic pressure and mean contractility measurements did not show any significant differences at the pre-ischemia and post-ischemia time points; however, the mean aortic pressure and mean contractility of the experimental group at the post-reperfusion time point was significantly higher ($p < 0.05$) than the control group's post-reperfusion measurement and the experimental group measurements observed at pre-ischemia and post-ischemia (Fig. 2 and 3).

Tissue MDA levels were significantly lower ($p < 0.05$) in the experimental group as compared to the control group and glutathione levels were found to be significantly higher ($p < 0.05$) in the experimental group as compared to the control group (Fig. 4 and 5). There were no significant differences between perfusate MDA and glutathione levels between the experimental and control groups. In addition, there were no significant differences in perfusate MDA and glutathione levels between each time point with each group (Fig. 6 and 7). Perfusate NO

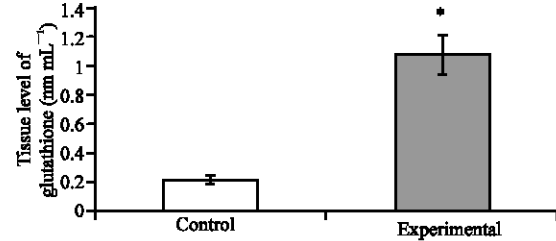


Fig. 5: Tissue levels of Glutathione (nm mL^{-1}) in control and experimental groups ($X \pm SD$), * $p < 0.05$: Compared to control groups

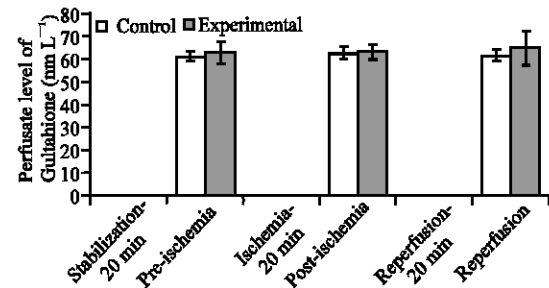


Fig. 6: Perfusate levels of Glutathione (nm mL^{-1}) in control and experimental groups ($X \pm SD$)

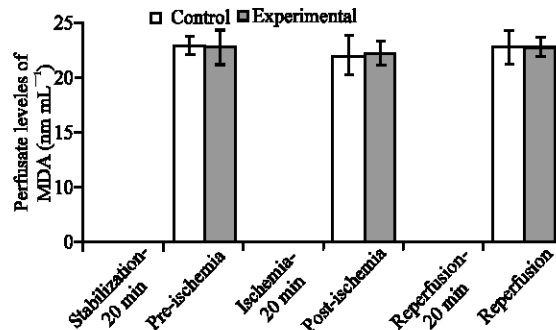


Fig. 7: Perfusate levels of MDA (nm mL^{-1}) in control and experimental groups ($X \pm SD$)

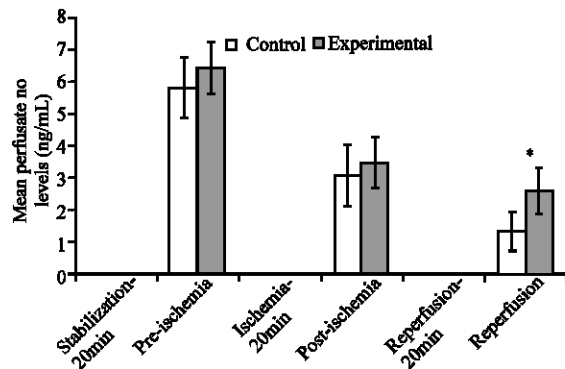


Fig. 8: Perfusate levels of NO (ng mL^{-1}) in control and experimental groups ($X \pm SD$) * $p < 0.05$: Compared to control group

level measurements at the post-reperfusion time point were significantly higher ($p < 0.05$) in the experimental group as compared to the control group (Fig. 8).

DISCUSSION

Prolonged ischemia results in cell death and corresponding tissue damage and reperfusion following ischemia can cause additional tissue injury (Werns *et al.*, 1986; Das *et al.*, 1986; Engler, 1989; Forman *et al.*, 1993). The complement system plays a major role in initiating some of the inflammatory responses that cause tissue injury during ischemia and reperfusion. The classical pathway of the complement system is activated by antibodies, cardiac mitochondrial particles, cardiolipin or by stimulation of the fibrinolytic system and, as such, the complement system may be activated during CPB (Buerke *et al.*, 1998). Furthermore, the hemodilutive effect of CPB reduces the tissue levels of major inhibitors that regulate the complement system.

C1-INH inhibits the first step of the complement system, thereby preventing the complement cascade to occur during tissue ischemia. Previous animal and human studies have shown the actual effectiveness of C1-INH in minimizing ischemia/reperfusion injuries (Buerke *et al.*, 1998; Horstick *et al.*, 1987; Bauernschmitt *et al.*, 1998). Buerke *et al.* (1998) demonstrated that C1-INH reduces the amount of necrosis during cardiac ischemia reperfusion in a feline model (Buerke *et al.*, 1998). Other studies have shown the ability of C1-INH to improve cardiac function following ischemia (Klima *et al.*, 2000; Shandelya *et al.*, 1993). In our study, we tested the ability of exogenous C1-INH, delivered during reperfusion, to prevent extensive damage to myocardial tissue and preserve cardiac function.

Our data demonstrate the ability of C1-INH to inhibit the complement system, as shown by markers of complement system inhibition. MDA, glutathione and NO were the three markers used to test C1-INH ability. C1-INH was also shown to preserve and even improve cardiac function as heart rates were similar in both the control and experimental groups. Moreover, aortic pressure and contractility were higher post-reperfusion in the experimental group as compared to the control group, thus illustrating the improved heart function that C1-INH can provide.

MDA was chosen as an index of free radical activity as it is the end product of lipid peroxidation reaction specific to unsaturated fatty acids and free radicals that is present during ischemia/reperfusion injury (Cicardi *et al.*, 1995). High levels of MDA are indicative of lipid

peroxidation. One of the findings in this study was the significantly lower values of tissue MDA in the experimental group compared to that in the control group, supporting the inhibiting ability of C1-INH on the complement system cascade leading to lipid peroxidation. Lack of a similar change in perfusate MDA levels showed that oxidative stress was limited to tissue level (Blaustein *et al.*, 1989).

The second marker of complement system inhibition, glutathione, is an endogenous substance activated during reperfusion injury to neutralize the deteriorating effects of reactive oxygen intermediates (Schlauer *et al.*, 2004). Endogenous cardiac glutathione is a protective agent against myocardial dysfunction during short term myocardial ischemia. Lower tissue levels of glutathione signify its use against myocardial damage, while higher levels signify that a different protective mechanism was used to defend against tissue injury (Yoshida *et al.*, 1997). We found that tissue levels of glutathione were preserved in the experimental group and depleted in the control group, thus supporting the fact C1-INH was effective in reducing ischemia/reperfusion injury (Sinclair *et al.*, 1990; Mathey *et al.*, 1994; Lefer and Lefer, 1993).

NO tissue levels were also measured as a third indicator of complement system inhibition by C1-INH. NO release is normally lower during ischemia/reperfusion injury since it is consumed by oxidative radicals (Lefer and Lefer, 1993). We found that perfusate NO levels were significantly higher in the experimental group as compared to the control group. With this third marker, we concluded that C1-INH was able to inhibit the complement system and prevent tissue injury, specifically lipid peroxidation and radical formation, during ischemia/reperfusion injury.

Although tissue MDA, tissue glutathione and perfusate NO levels indicated the success of C1-INH, perfusate levels of MDA and glutathione were similar between the control and experimental groups. This may be due to the difficulty in obtaining perfusate samples. Additionally, further studies should be conducted to confirm the ability of C1-INH to inhibit the complement system using other markers of tissue injury.

During CPB and reperfusion, tissue injury can be attributed to complement system activation. Both reperfusion injury and the intrinsic activation of the complement system damage the myocardium during peri/post-operative periods despite continuous blood perfusion (Lefer and Lefer, 1993). In this study, the inhibitory effects of C1-INH on complement activation and the classical pathway of the complement system have been demonstrated by MDA, glutathione and NO levels,

markers of ischemia/reperfusion injury. Perfusion, with C1-INH was observed to increase cardiac contractility and aortic pressure, as well as to protect myocardium from reperfusion injury due to lipid peroxidation and antioxidant systems. Evaluation of C1-INH as a potential therapeutic agent in large animal models are warranted.

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