Myocardial Salvage by Chimyl Alcohol: Possible Role of Peroxisomal Dysfunction in Reperfusion Injury

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INTRODUCTION

A growing body of evidence supports the role of membrane phospholipids in myocardial ischemia reperfusion injury. For example, reperfusion of ischemic myocardium is associated with the loss of membrane phospholipids in concert with the accumulation of lysophosphoglycerides and free fatty acids, especially arachidonic acid.[1,2] Mammalian hearts contain two types of phospholipids, diacyl and ether glycerolipids, also known as plasmalogens. Several recent reports indicate that heart contains significant amount of plasmalogens and that the loss of plasmalogens occur in the pathogenesis of ischemic heart disease.[3]

Loss of plasmalogens can occur either due to the breakdown of membrane phospholipids or due to the impairment of plasmalogen synthesis. The biosynthesis of plasmalogens is known to occur in two different subcellular organelles: peroxisomes and microsomes. The initial two enzymes responsible for the introduction of the ether bond in ether phospholipids are localized in peroxisomes, which work in sequence to catalyze the acylation of dihydroxyacetone phosphate and removal of the fatty acid by a long chain alcohol derived from acyl CoA (FIG. 1). The subsequent enzymatic steps for the formation of plasmalogens take place in the microsomes. Thus, an alkyl glycerol such as chimyl alcohol, can serve as a precursor for the formation of plasmalogens in the microsomes. This study examined whether supplementation of the heart with chimyl alcohol prior to ischemia could reduce the ischemia reperfusion injury.

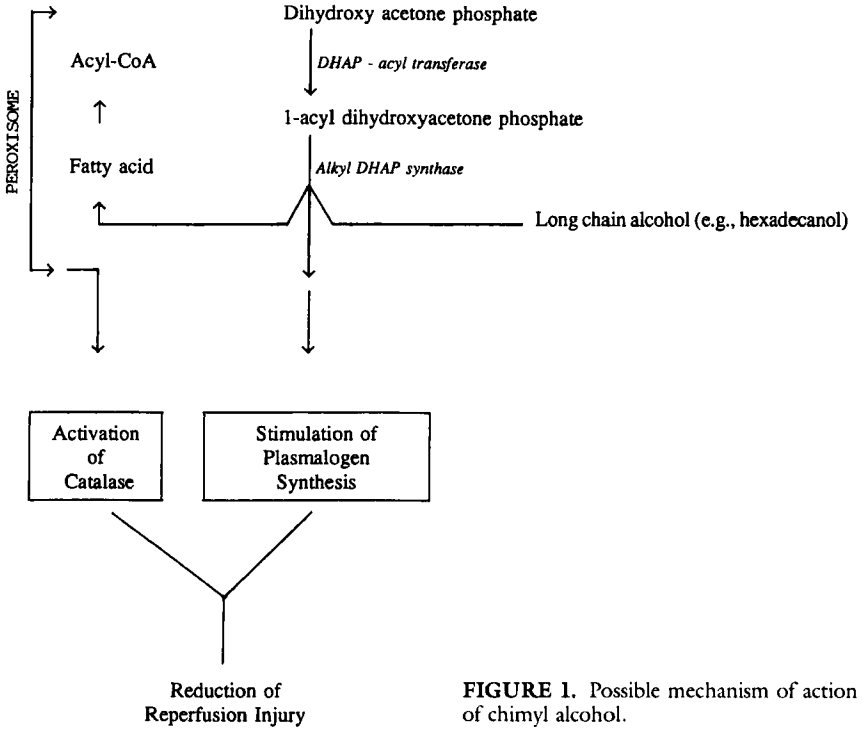

FIGURE 1. Possible mechanism of action of chimyl alcohol.

MATERIALS AND METHODS

Sprague-Dawley rats weighing approximately 300 g were anesthetized with pentobarbital (60 mg/kg, i.p.); hearts were quickly removed and perfused by Langendorff technique as described previously.[4] Experiments were divided into two groups: the experimental group received 50 μM chimyl alcohol prior to ischemia while the control group received equivalent amount of saline. After 15 min of perfusion with or without the chimyl alcohol, the heart was arrested at normothermia by terminating the coronary flow for 30 min followed by 30 min of reperfusion.

Left ventricular developed pressure (LVDP) and the coronary flow were measured to monitor the myocardial function,[5] creatine kinase (CK) release was quantified as a measure of tissue injury,[5] and malonaldehyde formation measured to monitor the oxidant stress.[6] In addition, peroxisomal catalase activity was also measured as described previously.[7]

RESULTS

As shown in FIGURE 2, coronary flow is significantly higher in the postischemic chimyl alcohol–treated heart as compared to the matched control. The recovery of

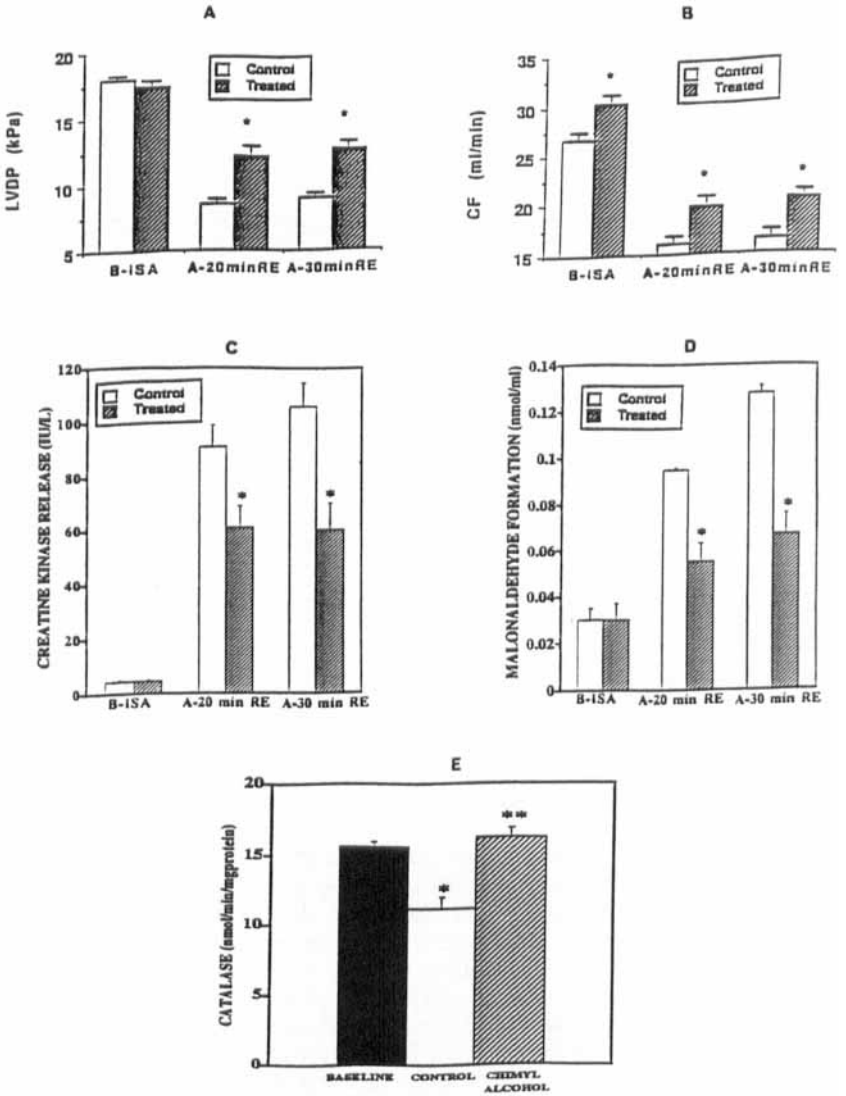

FIGURE 2. Effect of chimyl alcohol on (**A**) left ventricular developed pressure (LVDP); (**B**) coronary flow (CF); (**C**) creatine kinase (CK) release from the heart in the perfusate; (**D**) malonaldehyde (MDA) formation; and (**E**) catalase. Isolated rat heart was subjected to 30 min of ischemia followed by 30 min of reperfusion. B-ISA = before ischemia; A-20 min RE = 20 min reperfusion following 30 min ischemia; A-30 min RE = 30 min reperfusion following 30 min ischemia. Results are expressed as means ± SE of at least 6 different animals in each group. * $p < 0.05$ compared to control group.

LVDP during the reperfusion phase was also significantly better in the experimental group (9 ± 1 versus 13 ± 2 mm Hg). The CK release from the postischemic heart was increased significantly from both the control and chimyl alcohol groups, but the amount of CK release was much lower in the treated group as compared to the control group (60 ± 10 versus 106 ± 9 IU/l) after 30 min of reperfusion, suggesting that chimyl alcohol treatment reduced the reperfusion injury.

Since reperfusion of ischemic myocardium is associated with the development of oxidant stress, MDA formation was monitored as a presumptive marker for oxidant stress. As seen in FIGURE 2, the amount of MDA in the postischemic heart was increased steadily in both groups, but the increase was significantly lower in the chimyl alcohol group (0.166 ± 0.008 versus 0.08 ± 0.002 nmol/ml), suggesting reduction of oxidant stress by chimyl alcohol. To further explore the possible mechanism for the reduction of oxidant stress, the activity of the antioxidant enzyme catalase was measured in the peroxisomes. The peroxisomal catalase was significantly lowered in the postischemic heart (11.26 + 0.8 versus 15.6 + 0.35 nmol/min/mg protein) (FIG. 2). Treatment with chimyl alcohol restored the catalase activity to the pre-ischemic baseline level (16.24 + 0.67 nmol/min/mg protein).

DISCUSSION

The results of this study indicate that chimyl alcohol, a precursor of ether-linked phosphoglyceride biosynthesis, can reduce myocardial ischemia reperfusion injury possibly by restoring the catalase activity and by reducing the reperfusion-induced oxidant stress. It may be possible that ischemia-reperfusion causes reduction/inhibition of one or more of the peroxisomal enzymes necessary for plasmalogen biosynthesis. Secondly, ischemia-reperfusion might lead to structural disorder in the peroxisomes, thus leading to the loss of enzymes and/or substrates necessary for plasmalogen synthesis. As depicted in FIGURE 1, chimyl alcohol can supply the necessary substrate to the microsome for the plasmalogen synthesis by bypassing the first two enzymatic steps of the plasmalogen synthesis in the peroxisome. These results thus suggest peroxisomal disorder in the postischemic myocardium.

The reduction of peroxisomal catalase activity in the postischemic heart and restoration of this antioxidant enzyme by chimyl alcohol further supports the role of peroxisomes in the ischemia-reperfusion injury. Decrease in peroxisomal catalase activity following an ischemic insult was also observed in other organs such as kidney.[8] It seems reasonable to assume that chimyl alcohol preserves the catalase activity in heart by enhancing the plasmalogen synthesis, since these ether lipids have been found to directly scavenge the oxygen free radical,[9] which are known to be produced during the reperfusion.[10]

SUMMARY

The results of this study suggest that reperfusion of ischemic myocardium may lead to the peroxisomal disorder both functionally and biochemically. An alkyl glycerol such as chimyl alcohol can protect the ischemic heart from the reperfusion injury probably by enhancing the plasmalogen synthesis.

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