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Gradual Reperfusion Lowers the Incidence of Reperfusion-Induced Ventricular Fibrillation in a Cat Model of Regional Ischemia

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Blood flow restoration to ischemic zone of the heart is essential to salvage of ischemic tissue. However, there is a large body of evidence documenting that the reperfusion can induce reperfusion injury like reperfusion-induced malignant arrhythmias. In the present study, employing a cat model of regional cardiac ischemia, we examined if reperfusion rendered in a gradual fashion could lower the incidence of reperfusion-induced ventricular fibrillation (VF), which usually precipitated within a few to several tens of seconds after abrupt reperfusion. The experiments were conducted with male mongrel cats (n=46, 2.5-5 kg). The animals in the control and 30 MIN groups were subjected to an episode of 20- and 30-min left anterior descending coronary artery occlusion, respectively, followed by abrupt reperfusion. The animals in 5 G and 10 G groups received gradual reperfusion over a 5- and 10-min period, respectively, following a 20-min occlusion. The proportion of animals that exhibited VF during the reperfusion phase was 11/15 in the control, 7/10 in the 30 MIN, 5/10 in the 5 G and 2/11 in the 10 G groups. The incidence of VF in the 10 G group was significantly lower than that in the control or 30 MIN group subjected to abrupt reperfusion. These results suggest that the gradual reperfusion is a useful procedure against reperfusion-induced VF.

Key Words: Gradual reperfusion, Reperfusion injury, Reperfusion-induced ventricular fibrillation

INTRODUCTION

Although early in the course of severe ischemia the reversibly injured myocytes can be salvaged by timely reperfusion, the reperfusion has been known to exacerbate the ischemic damage, such as accelerated necrosis (Hearse et al, 1978), lethal reperfusion injury (Hearse et al, 1991), myocardial stunning (Braunwald et al, 1982; Bolli et al, 1990) and reperfusion-induced arrhythmias (Tanaka et al, 1988). However, the mechanisms underlying reperfusion injury are still unknown.

Recently, the increasing use of techniques such as angioplasty and thrombolysis for treatment of ischemic heart disease has afforded impetus to investigate the mechanisms of reperfusion injury. Especially, the fact that the frequency of serious reperfusion-induced arrhythmias is higher in the patients treated by balloon angioplasty than by thrombolytic therapy may provide the clue to resolve the mechanisms underlying reperfusion-induced arrhythmias (Manning & Hearse, 1984; Fine et al, 1987; Hogg et al, 1990; Saran et al, 1990; Fox AAA, 1990). Although several reasons for this difference are proposed in explanation, it may relate to the duration of ischemia and/or the speed of reperfusion. The large-scale studies demonstrated that the incidence of lethal arrhythmias was greatest with reperfusion after brief periods of ischemia about 20 min (Tennant & Wiggers, 1935; Balke et al, 1981; Blumgart et al, 1944; Lown & Wolf, 1971; Sommers & Jennings, 1972). In contrast, a few studies of the relation between the speed of reperfusion and the incidence of reperfusion-induced arrhythmias have been investigated (Sewell et al, 1955; Petropulos & Meijne, 1964; Grech & Ramsdale, 1994). Recently, our study clearly showed that the ventricular premature beat-driven intermittent

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reperfusion (postconditioning) suppressed reperfusion-induced ventricular fibrillation (Na et al, 1996). However, this manipulation, although it is very effective in cats, may be dangerous to apply to the human patients; occluding the coronary artery whenever ventricular premature beats (VPBs) appear is difficult and one may easily miss the beats. Therefore, to make the procedures safe and simple, we employed in the present study a new maneuver of restoring coronary flow in a gradual fashion. The idea that the reduction of blood flow rate during reperfusion alleviates the incidence of reperfusion arrhythmias is not original. Petropoulos & Meijne (1964) reported that maintaining reperfusion at a lower rate reperfusion may attenuate the frequency of serious arrhythmias in dog heart. In contrast, Ibuki et al (1992) demonstrated that stepwise reperfusion did not reduce the incidence of reperfusion-induced VF in the isolated rat heart.

The aim of this study using feline hearts was to see if the gradual reperfusion over a 5- or 10-min period following a 20-min occlusion of the left anterior descending coronary artery (LAD) suppressed reperfusion-induced arrhythmias, which usually follow abrupt restoration of blood flow.

METHODS

Animal preparation

Adult mongrel male cats (n=46, 2.5~5 kg) were anesthetized by intramuscular injection of α-chloralose (60 mg/kg b.w.). The antecubital vein was cannulated for administration of muscle relaxant. For artificial respiration by a volume cycled respirator (Model 645, Harvard Apparatus Ltd., USA), tracheostomy was performed. Following injection of muscle relaxant (pancronium bromide, 0.7~1 mg/kg), positive pressure respiration with room air was started with a stroke volume of ~10 ml/kg and at a rate of ~15~20 strokes/min. During this artificial respiration, the end-tidal pCO_2 was 29~36 mmHg as measured by a capnometer (Model 2200, Traverse Medical Monitors, USA). The electrocardiogram (ECG) was recorded on a physiograph (Model 79 E, Grass Inst. Co., USA) at a chart speed of 25 mm/sec. The ECG recording was through the surface Lead II. The core body temperature was maintained at 37±0.5°C using servo-controlled heating pad (Model 50-7129, Harvard Apparatus Ltd., USA).

Regional ischemia/reperfusion model

A left thoracotomy was performed by removing the ribs 5 and 6. After incision of the pericardium, the LAD was isolated from the surrounding tissue, and care was taken to avoid damaging nerves coursing parallel to the LAD.

To induce regional ischemia, vessel clip with a screw was placed around the isolated artery. Reperfusion was instituted by releasing the clip.

Experimental protocols

The animals in abruptly reperfused groups (CONT and 30 MIN groups) were subjected to an episode of 20- and 30-min coronary artery occlusion, respectively, followed by abrupt reperfusion. The animals in gradually reperfused groups (5 G and 10 G groups) received the gradual reperfusion over a 5- and 10-min period following a 20-min occlusion, respectively. The gradual reperfusion was instituted by the release of vessel clip controlled by a screw. The experimental protocols described above are schematically illustrated in Fig. 1.

The experimental procedures in the present study were in accordance with the guidelines set by the Korea University College of Medicine Animal Research Policies Committee.

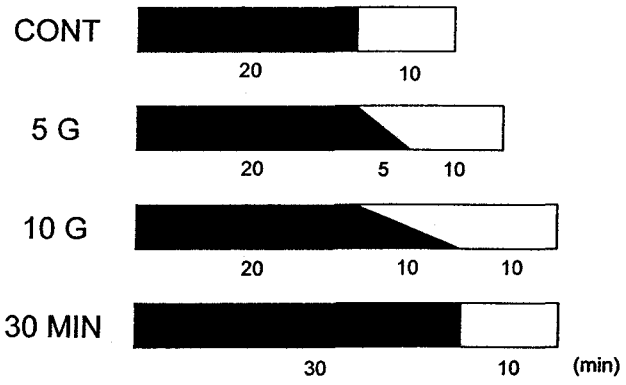

Fig. 1. Schematic diagram illustrating the experimental protocols. Animals in control group (CONT) were subjected to abrupt reperfusion following a 20-min episode of occlusion of the left anterior descending coronary artery. Animals in experimental groups were subjected to either the gradual reperfusion over a 5-min period following the 20-min occlusion (5 G group), the gradual reperfusion over a 10-min period following the 20-min occlusion (10 G group), or the abrupt reperfusion following the 30-min occlusion (30 MIN group). Black bars; occlusion, white bars; reperfusion.

Classification and analysis of arrhythmias

The ventricular arrhythmias were classified into 3 types according to Lambeth conventions (Riva et al, 1988): 1) discrete premature QRS complexes were defined as VPBs, 2) a run of four or more consecutive ventricular premature beats as ventricular tachycardia (VT) and 3) an electrical signal that did not show discrete individual QRS deflections as VF. VF lasting for longer than 5 minutes was considered to indicate cardiac death; in the present study, we have not tried to resuscitate the animals showing VF. The incidence of different types of arrhythmias during occlusion and reperfusion phases was analyzed. Four animals, which showed VF before the reperfusion was started, were excluded from the data pool.

Statistical tests

Fisher exact test was used for comparison of data from different animal groups. $P < 0.05$ was considered significant.

RESULTS

Arrhythmias occurring during reperfusion phase

CONT group: Out of 15 cats in the CONT group that were subjected to a 20-min ischemic insult, 13 (86.7%), 11 (73.3%) and 11 (73.3%) showed VPBs, VT and VF (Fig. 2) during the subsequent reperfusion phase, respectively. These arrhythmias occurred within a few to several tens of seconds of reperfusion.

30 MIN group: Out of 10 cats in 30 MIN group that were subjected to a 30-min ischemic insult, 9 (90%), 7 (70%) and 7 (70%) showed VPBs, VT and VF (Fig. 2) during the subsequent reperfusion phase, respectively. These arrhythmias also occurred within a few to several tens of seconds of reperfusion. The proportion of animals that showed VPBs, VT or VF in the 30 MIN group was not significantly different from that in the CONT group.

5 G group: Of 10 cats subjected to a 5-min gradual reperfusion after the 20-min ischemic insult, 10 (100%), 10 (100%) and 5 (50%) exhibited VPBs, VT and VF (Fig. 2) during the reperfusion phase, respectively. The proportion of animals that showed VF in the 5 G group was smaller than that in the CONT group, although this difference did not reach the significant level.

10 G group: Of 11 cats subjected to a 10-min gradual reperfusion after a 20-min ischemic insult, 11 (100%), 7 (64%) and 2 (18%) exhibited VPBs, VT and VF (Fig. 2) during the reperfusion phase, respectively. The proportion of animals that showed VF in the 10 G group was significantly smaller than that in the CONT or 30 MIN group ($P < 0.05$, Fig. 2).

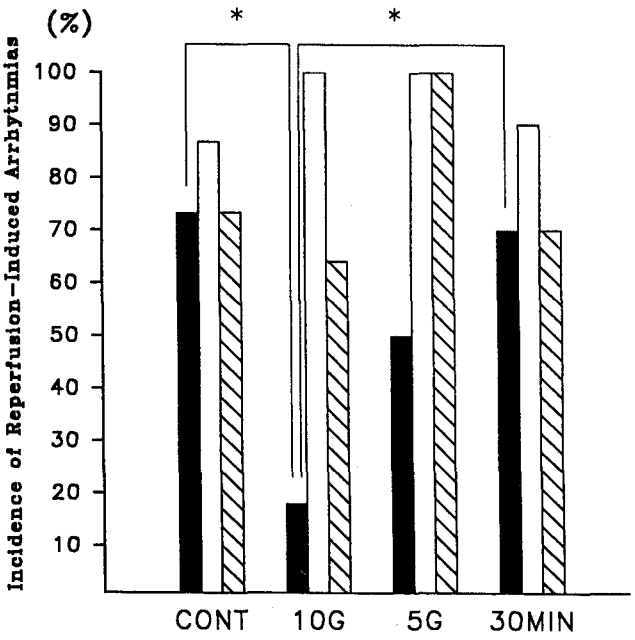

Fig. 2. Percentage of animals in each group that exhibited reperfusion-induced VPBs (hatched bars), VT (white bars) or VF (black bars). *, $p < 0.05$ (Fisher exact test). Abbreviations are the same as in Fig. 1.

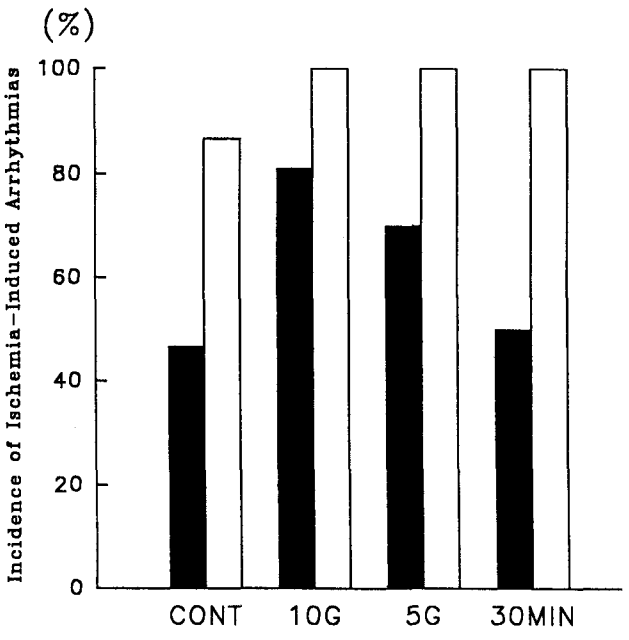

Fig. 3. Incidence of ischemia-induced arrhythmias. Percentage of animals in each group that exhibited ischemia-induced VT (black bars) or VPBs (white bars). Abbreviations are the same as in Fig. 1.

Arrhythmias occurring during ischemia phase

To see whether the incidence of reperfusion arrhythmias correlates with that of the preceding ligation arrhythmias, we compared the four groups for the VT and VPB observed in the occlusion phase.

CONT group: Out of 15 cats in the CONT group that were subjected to a 20-min ischemic insult, 13 (86.7%) showed VPBs, and 7 (46.7%) showed VT during a 20-min occlusion period (Fig. 3).

30 MIN group: Of 10 cats in the 30 MIN group, 10 (100%) had VPBs, and 5 (50%) had VT during a 30-min occlusion period (Fig. 3).

5 G group: Of 10 cats in the 5 G group, 10 (100%) showed VPBs, and 7 (70%) showed VT during the 20-min occlusion period (Fig. 3).

10 G group: Of 11 cats in the 10 G group, 11 (100%) showed VPBs, and 9 (81.8%) showed VT during the 20-min occlusion period (Fig. 3).

The proportion of animals having VPBs or VT during the occlusion phase in the CONT group was not statistically different from those in the 5 G, 10 G or 30 MIN group.

DISCUSSION

These results clearly demonstrated that gradual reperfusion after a regional myocardial ischemia reduced the incidence of reperfusion-induced VF. Reperfusion-induced arrhythmias, occurring very soon after the start of reperfusion, may be a cause involved in sudden cardiac death (Tzivoni et al, 1983; Corr & Witkowski, 1983). Recently, our report showed that the postconditioning is very effective in preventing reperfusion-induced VF and as good as ischemic preconditioning (Na et al, 1996). The postconditioning maneuver was timed by the emergence of reperfusion-induced VPBs; when two or three VPBs occurred consecutively, the coronary artery was occluded, and when the sinus rhythm seemed to be maintained for >5 seconds, it was released. However, it is not easy to occlude the artery just before the emergence of consecutive four VPBs (i.e., VT), which is easy to change into VF. In the present study, to make it easier and safer practically, we employed the maneuver involving the restoration of coronary blood flow in a gradual fashion.

The present study, with feline hearts of regional ischemia, demonstrated that the gradual reperfusion over a 10-min period following a 20-min ischemic insult reduced significantly the incidence of VF that is usually provoked by abrupt reperfusion, while the gradual reperfusion during 5-min slightly, not significantly, reduced the incidence of VF. These results suggest that the incidence of reperfusion-induced VF appears to be related to the blood flow rate of reperfusion. In addition, the incidence of reperfusion-induced VF in the 10 G group was also statistically different from that in the 30 MIN group. This result allows us to confirm that the reduction of incidence of reperfusion-induced VF in the 10 G group is not caused by the partial ischemia provided during gradual reperfusion, but by gradual restoration of coronary blood flow itself.

However, Ibuki et al (1992) demonstrated that stepwise restoration of coronary flow, i.e., flow rate increases by 20% per minute, following myocardial ischemia does not reduce the incidence of reperfusion-induced VF in the isolated rat heart. Presently, we can not explain the discrepancy between these two results. However, the difference in the species (rat versus cat) or between the isolated perfused heart versus in vivo model seems to be a possible cause. Alternatively, stepwise reflow, unlike gradual reflow employed in the present study, could not suppress the vulnerability to reperfusion-induced VF.

Based on the present results alone, we can not draw any firm conclusion for the mechanisms underlying the suppressive effects of gradual reperfusion against reperfusion-induced VF. One possible mechanism, however, is that gradual restoration of coronary blood flow reduces the rate of washout of antiarrhythmic components (potassium and protons) accumulated during ischemia (Kusama et al, 1990). Another possible mechanism is that gradual flow decreases the rate of formation of reactive oxygen intermediates which are known to cause reperfusion-induced arrhythmias (Podzuweit et al, 1989).

To date, the correlation between ischemia- and reperfusion-induced arrhythmias still remains controversial. In canine hearts, the susceptibility to reperfusion VF was positively related to the occurrence of coronary artery ligation arrhythmias (Balke et al, 1981). However, in the feline preparation, a lack of correlation between reperfusion VF and ischemic arrhythmias was demonstrated (Zuanetti et al, 1985). This disparity may be partly due to species difference. In this study, with a feline heart of regional ischemia, the incidence of VPBs or VT during occlusion was not statistically different across four groups. These data suggest that the low incidence of reperfusion VF in the 10 G group is not caused by difference in the severity of ligation arrhythmias.

In summary, these results suggest that the gradual reperfusion as a useful procedure against reperfusion

VF is an alternative to VPB-driven intermittent reperfusion, which might be dangerous to apply to the human patients. Although the data from this study are not sufficient to elucidate the mechanisms underlying the suppressive effects of gradual restoratioin of coronary blood flow against reperfusion VF, these results afford a further basis for the understanding and management of reperfusion-induced malignant arrhythmias.

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