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BN-063, a newly synthesized adenosine A_1 receptor agonist, attenuates myocardial reperfusion injury in rats

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Abstract

To assess the efficacy of the newly synthesized selective adenosine A_1 receptor agonist, BN-063 (1-cyclopropylisoguanosine), against myocardial reperfusion injury, 31 rats underwent 45 min of left coronary artery occlusion and 1 h of reperfusion. Animals were randomly assigned to four groups: control, I0.5-R0.5, in which BN-063 (0.5 mg/kg i.v. bolus) was administered during both ischemia and reperfusion, R-0.5 and R-1.0, in which BN-063 was administered only during reperfusion at 0.5 and 1.0 mg/kg, respectively. The area at risk was determined by intravascular injection of blue dye during coronary artery occlusion, which was performed by retightening the ligature at the end of reperfusion, and infarct size was determined by incubation of heart slices in nitro blue tetrazolium chloride. A significant reduction in infarct size, as a percentage of the area at risk, was noted with all three BN-063 treatment groups (control: 63.5 ± 4.0%, I0.5-R0.5: 39.6 ± 3.7%, R-0.5: 37.5 ± 3.5%, R-1.0: 38.1 ± 5.2%). However, the I0.5-R0.5 group did not shown a more beneficial effect than the other two BN-063-treated groups. In addition, BN-063 exerted a protective effect on the number of ventricular premature contractions associated with reperfusion (control: 906 ± 52, I0.5-R0.5: 325 ± 61, R-0.5: 321 ± 95, R-1.0: 340 ± 46). The results of this study demonstrate that BN-063, through activation of adenosine A_1 receptors, exerts antiarrhythmic and anti-infarct effects during myocardial ischemia-reperfusion. Therefore, BN-063 would be useful clinically in the treatment and prevention of acute myocardial infarction.

Keywords: Myocardial ischemia; Reperfusion injury; Adenosine A_1 receptor agonist; Infarct

1. Introduction

Early reperfusion can salvage some of the previously ischemic myocardium, produced by occlusion of the coronary artery, and result in a reduction in the degree of ischemia-induced myocardial injury. It has been suggested that lethal injury to viable myocardial and endothelial cells after reperfusion would minimize the potential benefit of reperfusion (Braunwald and Kloner, 1985). The significant enhancement of myocardial salvage by the administration of some pharmacological agents, e.g. adenosine, following regional myocardial ischemia strongly supports the importance of 'reperfusion injury'.

In recent studies, adenosine has been intensively investigated, particularly in the field of myocardial ischemia-reperfusion. Adenosine is an endogenous nucleoside which is present in relatively high concentrations at the time of reperfusion (Rochette et al., 1979; Van Gilst et al., 1986). It has numerous physiological properties, by acting on cell surface receptors, and may attenuate reperfusion injury. Exogenous adenosine has been shown to reduce reperfusion injury and limit infarct size in canine hearts (Babbitt et al., 1990; Pitarys et al., 1991). Improved postischemic ventricular function has also been observed after global ischemia in isolated perfused hearts treated with adenosine (Bolling et al., 1989, 1990). Recently, adenosine has also been implicated in preconditioning. Pretreatment with an adenosine antagonist can block the infarct size-limiting effect of preconditioning and pretreatment with a selective adenosine A_1 receptor agonist can mimic the beneficial effects of preconditioning (Liu et al., 1991). The mechanisms of these protective effects of adenosine are unclear. They may include coronary arteriolar vasodilatation (Wilson et al., 1990), inhibition of neutrophil activation (Cronstein et al., 1986),

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blockade of platelet aggregation (Tanabe et al., 1984) and inhibition of the release of catecholamine and renin (Richardt et al., 1987). Additional nonreceptor-mediated actions of adenosine, such as being a substrate for nucleotide synthesis, could also restore the cellular energy charge during reperfusion after myocardial ischemia (Reibel and Rovetto, 1979).

Two adenosine receptor subtypes, A_1 and A_2 receptors, are linked to the cardioprotective effects of adenosine against ischemic reperfusion injury. It has been elucidated that the protective actions of adenosine A_1 and A_2 receptor agonists are similar to those of adenosine (Norton et al., 1992). Since adenosine A_2 receptor activation can result in potentially harmful side effects (e.g., hypotension), the administration of an adenosine A_1 receptor agonist would be clinically appealing (Norton et al., 1992). Accordingly, the object of the present study was to further evaluate the protective effect of a newly synthesized adenosine A_1 receptor agonist, BN-063, against reperfusion injury.

BN-063 (1-cyclopropylisoguanosine) is a newly synthesized analogue of doridosine (1-methylisoguanosine) (Chern et al., 1991), a naturally occurring nucleoside isolated early in 1980 from aqueous ethanolic extracts of the sponge *Tedania digitata* (Cook et al., 1980). On the basis of our in vivo and receptor-binding studies (Tao et al., 1993), BN-063 is a selective adenosine A_1 receptor agonist. This study assessed the effect of BN-063 on reperfusion injury induced by 45 min of left coronary artery ligation followed by 1 h of reperfusion.

2. Materials and methods

2.1. Animal preparation

Adult Sprague-Dawley rats of either sex weighing 250–300 g were anesthetized with intraperitoneal urethane (1.2 g/kg). Tracheotomy was performed and an intubating cannula was connected to a rodent ventilator. The animals were ventilated artificially with room air. Respiratory rate was synchonised with the rat's spontaneous rate (60–80 strokes/min, 1 ml/100 g). Arterial blood pH and blood gases were maintained within normal physiological limits (pH: 7.35–7.45; P_{CO_2}: 30–35 mm Hg; P_{O_2}: 85–100 mm Hg) by adjusting the respiratory rate and tidal volume. The left femoral artery and vein were cannulated for the measurement of arterial blood pressure and heart rate via a Statham pressure tranducer and a Biotechnometer (Gould, USA) and for the administration of drugs, respectively. Electrocardiograms were recorded from standard lead II limb leads, with a positive electrode connected to the left hind leg, a negative electrode to the right foreleg and a ground electrode to the left foreleg. An oscilloscope electrocardiogram monitor (DSO 420, Gould, USA) was used to display the electrocardiogram continuously throughout the experiment. All signals, including the electrocardiogram and hemodynamic data, were recorded on chart paper.

After a left-side thoracotomy was performed at the fifth intercostal space, the pericardium was incised and the heart was exteriorized. A ligature (6/0 silk suture) was placed around the left main coronary artery close to its origin. The thread was then made into a knot as an occluder and another thread was tied to the former knot as a releaser. The ends of both threads were brought outside the thoracic cavity. Thus, the occlusion could be tightened or loosened by pulling the thread of the releaser. The coronary artery was occluded for 45 min, followed by 1 h of reperfusion.

2.2. Experimental protocol

The animals were randomly assigned to one of four treatment groups at the beginning of the study. There was an even spread of the sexes among the groups. (1) Control group: injections of saline at 10 min before occlusion and 3 min before reperfusion; (2) I0.5–R0.5 group: two injections of the novel adenosine A_1 receptor agonist BN-063 (1-cyclopropylisoguanosine; 0.5 mg/kg) were given as an intravenous bolus 10 min before occlusion and 3 min before reperfusion. (3) R-0.5 group: BN-063 (0.5 mg/kg i.v. bolus) was administered 3 min prior to reperfusion only. (4) R-1.0 group: BN-063 (1.0 mg/kg i.v. bolus) was administered 3 min prior to reperfusion only. The choice of the doses used was based on in vivo data (Tao et al., 1993; Lee et al., 1994). After the ligature was tied, blood pressure, heart rate and electrocardiograms were continuously monitored throughout the experimental period. Early ventricular arrhythmias, which occur within 30 min of the onset of myocardial ischemia, were assessed by the total number of ventricular premature contractions, and the incidence of ventricular tachycardia and ventricular fibrillation.

2.3. Measurement

Hemodynamics

Measurements of heart rate and mean arterial blood pressure were made in all groups at baseline, immediately before the sustained occlusion, 10 min after occlusion, 5 and 30 min after reperfusion, and at the end of the experiment. An indirect index of myocardial oxygen consumption was provided by calculation of the product of the systolic blood pressure and heart rate.

Arrhythmias

The primary end point of this study was infarct size. However, we also analyzed records of the electrocardiograms for the incidence of ventricular tachycardia

Table 1
Mortality

Group	Number of rats			Mortality (%)
	Total	Survival	Death	
Control	12	8	4	33.3
BN-063 I0.5-R0.5	8	8	0	0
BN-063 R-0.5	11	7	4	36.4
BN-063 R-1.0	12	8	4	33.3

I0.5-R0.5: BN-063 was administered (0.5 mg/kg i.v. bolus) prior to both ischemic and reperfusion periods; R-0.5: BN-063 was given at 0.5 mg/kg prior to reperfusion only; R-1.0: BN-063 was given at 1.0 mg/kg prior to reperfusion only.

and ventricular fibrillation during ischemia and for the total number of ventricular premature contractions during ischemia and reperfusion.

Area at risk and infarct

At the end of the experiment, the coronary artery was occluded and 0.5 ml methylene blue (3%) was injected intravenously to denote the area at risk. The heart was then excised and the atria were removed. The entire ventricular area was sectioned into four 2–3 mm thick slices from the apex to the base and incubated in nitroblue tetrazolium chloride (20 min, 37° C). This solution stained the normal myocardium purple while the infarct portion remained pale. The areas of risk and infarct were traced by hand on transparent paper. The traced areas were then measured by computerized planimetry.

2.4. Drugs

The BN-063 was prepared according to the method described previously by Chern et al. (1991) and was dissolved in normal saline.

2.5. Statistics

The measurements of hemodynamics, total number of ventricular premature contractions, area at risk and infarct are expressed as group mean ± S.E.M. values. The data for ventricular tachycardia and ventricular fibrillation during the 45 min of occlusion are reported as incidences. The Yates corrected chi-square test was used to analyze the differences in the incidence of arrhythmias and mortality between the control and BN-063-treated groups. The other parameters were compared by a one-factor analysis of variance. If this analysis indicated significant differences among the group means, the control group was compared with each of the treatment groups by means of the Newman-Keuls method. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Mortality and exclusions

A total of 43 rats were initially used in this study, 12 of which were excluded due to intractable ventricular fibrillation. Thus a total of 31 rats were used for data analysis: 7 in the R-0.5 group and 8 in the control, I0.5-R0.5 and R-1.0 groups. As shown in Table 1, the mortality of control, R-0.5 and R-1.0 group was 33.3, 36.4 and 33.3%, respectively. Treatment with BN-063 during ischemia-reperfusion (I0.5-R0.5 group) reduced the mortality to zero. There was no significant difference between groups.

3.2. Hemodynamic parameters

The hemodynamic data are summarized in Table 2. No hemodynamic changes were seen in the control

Table 2
Summary of hemodynamic parameters during experimental protocol

Group	Baseline	Ischemia	Time after reperfusion (min)		
			5	30	60
Mean blood pressure (mm Hg)					
Control	79 ± 3	74 ± 2	73.7 ± 2	72 ± 2	72 ± 2
BN-063 I0.5-R0.5	80 ± 4	69 ± 5	55 ± 4 [a]	57 ± 3 [a]	59 ± 3 [a]
BN-063 R-0.5	78 ± 4	74 ± 3	65 ± 4 [a]	69 ± 3	69 ± 3
BN-063 R-1.0	79 ± 2	75 ± 2	56 ± 3 [a]	60 ± 4 [a]	60 ± 4 [a]
Heart rate (beats / min)					
Control	303 ± 14	302 ± 14	326 ± 16	322 ± 15	330 ± 16
BN-063 I0.5-R0.5	291 ± 11	225 ± 14 [a]	229 ± 20 [a]	239 ± 30 [a]	241 ± 30 [a]
BN-063 R-0.5	307 ± 13	298 ± 12	180 ± 26 [a]	244 ± 30 [a]	247 ± 30 [a]
BN-063 R-1.0	283 ± 22	302 ± 13	199 ± 24 [a]	214 ± 22 [a]	217 ± 23 [a]
Rate-pressure product (× 1000)					
Control	37.0 ± 2.2	32.5 ± 2.6	39 ± 2.5	38 ± 2.6	37 ± 2.0
BN-063 I0.5-R0.5	38.3 ± 2.8	24.6 ± 1.2 [a]	22 ± 3.0 [a]	24 ± 3.0 [a]	25 ± 4.0 [a]
BN-063 R-0.5	36.8 ± 1.4	32.0 ± 1.8	21 ± 3.0 [a]	25 ± 3.0 [a]	25 ± 3.0 [a]
BN-063 R-1.0	37.1 ± 1.9	33 ± 3.0	19 ± 3.0 [a]	20 ± 2.0 [a]	20 ± 2.3 [a]

Ischemia: 10 min after occlusion; values are expressed as means ± S.E.M.; [a] $P < 0.05$ vs. control group. BN-063 = 1-cyclopropylisoguanosine.

Table 3
The effect of BN-063 on ischemic and reperfusion arrhythmias in the rat

Group	n	Ischemia			Reperfusion		
		PVC (count)	VT (%)	VF (%)	PVC (count)	VT (%)	VF (%)
Control	8	174 ± 16	100	62.5	906 ± 52	0	0
BN-063 I0.5-R0.5	8	42 ± 9	12.5 [a]	0 [a]	325 ± 61 [a]	0	0
BN-063 R-0.5	7	196 ± 12	100	71.4	321 ± 95 [a]	0	0
BN-063 R-1.0	8	183 ± 10	100	62.5	340 ± 46 [a]	0	0

n: number of animals; PVC: premature ventricular contractions, VT: ventricular tachycardia, VF: ventricular fibrillation; I0.5-R0.5: BN-063 was administered at 0.5 mg/kg prior to both ischemic and reperfusion periods; R-0.5: BN-063 was given at 0.5 mg/kg prior to reperfusion; R-1.0: BN-063 was given at 1.0 mg/kg prior to reperfusion. Values are given as means ± S.E.M. [a] $P < 0.05$ vs. control group.

group. Pretreatment with BN-063 0.5 mg/kg prior to occlusion caused a significant decrease in heart rate and rate-pressure product during ischemia, but did not alter mean blood pressure. During the reperfusion period, all three BN-063-treated groups showed a significant decrease in mean blood pressure, heart rate and rate-pressure product. The reduction of mean blood pressure in the R-0.5 group recovered 10–15 min after reperfusion.

3.3. Arrhythmias

Ligation of the left coronary artery resulted in ventricular arrhythmias, which commenced within 4–5 min of occlusion and were manifested as ventricular premature contractions, ventricular tachycardia and ventricular fibrillation. All animals showed marked ventricular arrhythmias during the ischemic period except those of the I0.5-R0.5 group (Table 3). Treatment with BN-063 (0.5 mg/kg) prior to occlusion significantly reduced the total number of ventricular premature contractions and the occurrence of ventricular tachycardia and ventricular fibrillation when compared with the control group.

In the reperfusion period, 64.5% of the rats had a ventricular premature contraction, but no animals showed ventricular tachycardia or ventricular fibrillation. BN-063 administered prior to reperfusion resulted in a significant reduction in the total number of ventricular premature contractions when compared with the control group (control: 906 ± 52, I0.5-R0.5: 325 ± 61, R-0.5: 321 ± 95 and R-1.0: 340 ± 46). However, the total number of ventricular premature contractions did not differ significantly between the BN-063-treated groups.

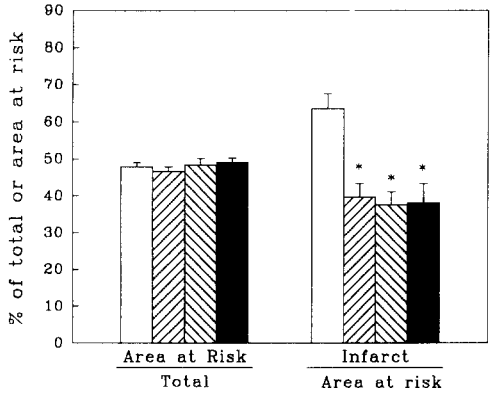

Fig. 1. Effect of the selective adenosine A_1 receptor agonist BN-063 on infarct size, expressed as a percentage of risk region, in rats undergoing 45 min of left coronary artery occlusion followed by 1 h of reperfusion. First columns: control, $n = 8$; second columns: I0.5-R0.5 group, in which BN-063 was given at 0.5 mg/kg during both ischemic and reperfusion periods, $n = 8$; third columns: R-0.5 group, in which BN-063 was given at 0.5 mg/kg during reperfusion only, $n = 7$; fourth columns: R-1.0 group, in which BN-063 was given at 1.0 mg/kg during reperfusion only, $n = 8$. The results are expressed as the means ± S.E.M., $^*P < 0.05$ as compared with the control value (normal saline).

3.4. Infarct size

All control and BN-063-treated hearts showed clearly demarcated areas of infarction as a consequence of 45 min of ischemia followed by 1 h reperfusion, as assessed by the *p*-nitro blue tetrazolium staining technique. No significant differences in the area at risk, expressed as a percentage of the total left ventricle, were noted among the groups (Fig. 1). Infarct size, expressed as a percentage of the area at risk, was 63.5 ± 4.0% in the control group. A significant reduction in infarct size was noted with all BN-063 treatments when compared with control (I0.5-R0.5: 39.6 ± 3.7%, R-0.5: 37.5 ± 3.5%, R-1.0: 38.1 ± 5.2%). The results of the I0.5-R0.5 group were not significantly different from those of the R-0.5 and R-1.0 groups.

4. Discussion

Our previous study demonstrated that BN-063 is a selective adenosine A_1 receptor agonist (Tao et al., 1993). The bradycardia and transient depressor response (3–5 min) of BN-063 were shown after intravenous administration. These hemodynamic effects of BN-063 were almost completely blocked by pretreatment with 1,3-dipropyl-8-cyclopentylxanthine, a selec-

tive adenosine A_1 receptor antagonist. These results suggest that the depressor component is due to a decreased cardiac output (resulting from the reduced heart rate), which is then adjusted by a reflex vasoconstriction, and that it is not an adenosine A_2-mediated vasodilator effect.

The fact that adenosine reduces the severity of ischemia and reperfusion induced ventricular arrhythmias was shown in vivo in the anesthetized animal coronary artery ligation model (Wainwright and Parratt, 1988). Adenosine A_1 receptors have been suggested to be responsible for the antiarrhythmic properties of adenosine (Boachie-Ansah et al., 1993; Wainwright and Parratt, 1993; Lee et al., 1994). In the present study, we demonstrated that the adenosine A_1 receptor agonist BN-063 decreased the ventricular arrhythmias resulting from ischemic insult. The occurrence of ventricular premature contractions resulting from reperfusion insult also significantly decreased in all BN-063-treated groups. These results indicate that adenosine A_1 receptor agonists indeed exert an antiarrhythmic effect against ventricular arrhythmias induced by ischemia or reperfusion. The beneficial effects of BN-063 could be of considerable clinical use in the prevention and treatment of ischemia-reperfusion induced arrhythmias.

Intravenous infusion of adenosine in the early reperfusion period was observed to attenuate myocardial infarct size in either the canine model or rabbit model (Babbitt et al., 1990; Pitarys et al., 1991; Norton et al., 1992). Norton et al. (1992) have reported that the protective effects of adenosine are receptor mediated and that both adenosine A_1 and A_2 receptor agonists afford similar degrees of protection. Current understanding of the pathogenesis of myocardial reperfusion injury suggests that activation of adenosine A_2 receptors results in vasodilation, inhibition of platelet aggregation and thomboxane A_2 release, and inhibition of superoxide production from neutrophils, and that these are likely mechanisms to account for the protective effects of adenosine. But there is a question related to safety since activation of adenosine A_2 receptors induces a 'stress reaction' resulting in potentially deleterious side effects such as hypotension. Therefore, it is obvious that there is great potential for the development of new drugs which act selectively as agonists at adenosine A_1 receptor sites. The results shown in Fig. 1 demonstrate that treatment with BN-063 resulted in a significant reduction in infarct size. BN-063 administered during the reperfusion period only (R-0.5 and R-1.0 groups) enhanced myocardial salvage in the present study. This result confirmed the report by Norton et al. (1992), which demonstrated that, when administered during the reperfusion period, intravenous cyclopentyladenosine, a selective adenosine A_1 receptor agonist, significantly reduced the infarct size. The results also show that BN-063 is equally efficacious whether administered pre- and post-ischemically or during the reperfusion period only. It seems that the process of irreversible injury was not affected by the pharmacological intervention during ischemia. The adenosine A_1 receptor-mediated reduction in myocardial infarct size elicited by BN-063 may be exerted primarily during reperfusion, not during ischemia. This concept is not in accordance with the report by Zhao et al., which indicated that adenosine A_1 receptor-mediated cardioprotection by endogenous adenosine is expressed primarily during the ischemic period, with little or no effect on infarct size expressed during reperfusion (Zhao et al., 1994). However, preischemic administration of BN-063 significantly reduced the incidence of ischemia-induced ventricular arrhythmias. These effects are useful to enhance survival.

BN-063 treatment during ischemia-reperfusion (I0.5-R0.5 group) led to severe bradycardia and subsequent hypotension during the reperfusion period and may have resulted in worsening of myocardial ischemia. It may be possible to override the benefit of administration of BN-063 prior to ischemia. These hemodynamic effects may also be responsible for the failure to observe a substantially significant effect with the high dose of BN-063 (R-1.0 group) administered during the reperfusion period.

However, stimulation of the adenosine A_1 receptor results in various actions that may prove to be beneficial against the deleterious consequences of reperfusion. First, inhibition of norepinephine release from sympathetic nerve endings and a reduction of renin release would decrease the vasoconstriction of the reperfused bed (Richardt et al., 1987). Second, inhibition of norepinephine release from sympathetic nerve endings and a decrease in lipolysis could reduce oxygen-derived free radical generation via autoxidation of catecholamine and formation of lipid hydroperoxides, respectively (Fredholm, 1985; Forman et al., 1993). Third, stimulation of adenosine A_1 receptors could ameliorate reperfusion injury by opening ATP-sensitive potassium channels associated with hyperpolarization of myocardial cells, consequently reducing Ca^{2+} influx (Kirsch et al., 1990; Forman et al., 1993). Progressive calcium overload has been postulated to be an important mechanism in the pathogenesis of reperfusion injury. Fourth, myocardial ischemia is associated with increased levels of endogenous catecholamine and therefore with an increase in myocardial oxygen consumption through stimulation of β-adrenoreceptor (Carlsson et al., 1985). Both the chronotropic and dromotropic effects of adenosine A_1 receptor activation on the conducting system would result in a decrease in myocardial oxygen consumption (Pelleg and Belardinelli, 1993). Fifth, activation of the adenosine

A_1 receptor could increase the cellular uptake of glucose by enhancing anaerobic glycolysis and decreased intracellular lactate production and acidosis via an antilipolytic effect (Mainwaring et al., 1988; Fredholm, 1985).

In conclusion, BN-063, a selective adenosine A_1 receptor agonist, suppressed ventricular arrhythmias induced by myocardial ischemia, limited infarct size and attenuated ventricular premature contractions, which were the only form of arrhythmia observed in the reperfusion period. BN-063 could be a useful agent in the prevention and treatment of arrhythmias associated with acute myocardial infarction.

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