中国药理学报 *Acta Pharmacologica Sinica* 1989 Sep; **10** (5) : 394-398

Prevention of postischemic reperfusion damage on isolated working rat hearts by bopindolol and propranolol

JIN Xing-Zhong, LIU Gui-De

(*Department of Pharmacology, The First Medical College of PLA, Guangzhou 510515, China*)

ABSTRACT Reperfusion after 30-min regional or global ischemia of the isolated working rat hearts did not restore the cardiac functions (as measured by cardiac output and power production), but exacerbated the existing damages. The lipid peroxidation product malondialdehyde (MDA) in the regional and global ischemic-reperfused myocardium increased by 37.6 and 45.2%, respectively. Bopindolol 0.1 μmol/L and propranolol 10 μmol/L protected the myocardium against the postischemic reperfusion damages, accelerated the recovery of cardiac functions during reperfusion and decreased the MDA content in the ischemic-reperfused myocardium. It is postulated that the prevention of cardiac cells from lipid peroxidation injury is related to the protection afforded by the drugs to the ischemic-reperfused hearts.

KEY WORDS adrenergic *beta* receptor blockaders; bopindolol; propranolol; myocardial reperfusion injury; heart function tests; lipid peroxides

Myocardial postischemic reperfusion would be a double-edged sword to ischemic myocardium[1]. The production of oxygen-free radicals occurs as a consequence of the reintroduction of molecular oxygen into previously ischemic tissue during myocardial reperfusion. These substances are chemically reactive and cause myocardial postischemic reperfusion damages by reacting with polyunsturated fatty acids[2]. *Beta*-antagonists, such as propranolol, have been shown to attenuate intracellular calcium load, protect the ischemic-reperfused myocardium from the depletion of high-energy phosphates[3,4]. Nevertheless, what is the effect of *beta*-antagonists on myocardial lipid peroxidation damage caused by oxygen-free radicals during reperfusion remains to be studied. Bopindolol is a new long-acting, non-selective *beta*-adrenoceptor antagonist without membrane stabilizing activity (MSA) and its chemical name is 4-(benzoyloxy-3-tert-butyaminopropoxy)-2-methyl-indole hydrogen malonate[5]. No report was found about its effects on the myocardial postischemic reperfusion damages at present. The aim of the present study was to investigate in isolated working rat hearts if bopindolol or propranolol could protect against lipid peroxidation, prevent myocardial postischemic reperfusion damages and accelerate the recovery of cardiac function during reperfusion.

Received 1988 Jul 13 Accepted 1989 Mar 27

MATERIALS AND METHODS

Hearts were removed from Sprague-Dawley rats, ♂, weighing 298 ± SD 21 g, and placed in a heart chamber which was jacketed by circulating warm water (37℃), and perfused with modified Krebs-Hensleit (K-H) bicarbonate buffer[6]. After 15-min equilibration perfusion, bopindolol or propranolol was added to the perfusate to provide the final concentrations of 0.1 or 10 μmol/L respectively. Fifteen min later, the hearts were made regional ischemia for 30 min and then reperfused for another 15 min[7]. The hearts were made global ischemia for 30 min[8], during which the hearts were superfused with unoxygenated K-H buffer at 37℃ to prevent any drop in temperature due to the cut-off of perfu-

sion. For reperfusion, both left atrial and aortic cannulae were unclamped and the coronary system was perfused retrogradely with oxygenated buffer solution through the aortic cannula at a pressure of 8 kPa. When the hearts resumed powerful contractions (about 10 min after reperfusion), the flow was reversed into working heart mode and the reperfusion continued for another 15 min. At the end of the perfusion sequences, the hearts were removed rapidly and frozen at −20℃ for the measurement of malondialdehyde (MDA) in the myocardium.

Mechanical performances The aortic flow (AF), coronary flow (CF), total cardiac output (TCO = AF + CF), left ventricular systolic pressure (LVSP), $\pm$LV dP/dt_{max} and ECGs were monitered throughout the experiments and recorded at regular intervals. The external work (power production) produced by the left ventricle was measured as the sum of pressure and kinetic power[6].

Biochemical assay Myocardial leakage of creatine phosphokinase (CPK) to the coronary effluent was used as a sign of ischemic-reperfused cell damages and assayed in a SBA-300 biochemical autoanalyzer (Gilford, USA). ΔCPK = postischemia-preischemia value. Samples of the whole layer of myocardium for measurement of MDA were cut from the anterior regions of the left ventricle of the globally ischemic-reperfused hearts and in the regionally ischemic-reperfused hearts, cut respectively from the ischemic and normal regions of the left ventricle which were identified by methylene blue staining method in the preliminary experiments. The tissue samples (about 120 mg) were minced and homogenized ultrasonically. The amount of MDA in the homogenates was assayed by the thiobarbituric acid test at 532 nm[9].

Drugs Bopindolol crystals (Sandoz) was dissolved in double-stilled water before use. The propranolol ampoules were used.

The statistical evaluations were performed according to *t*-test. All values are expressed as $\bar{x} \pm SD$.

RESULTS

Protective effects of bopindolol and propranolol on the regionally ischemic-reperfused myocardial damages The regionally ischemic-reperfused control experiments ($n = 12$) were performed to estimate the extent of myocardial recovery from 30-min regional ischemia after 15 min reperfusion. The mechanical function were markedly impaired and CPK-release was increased by 30 min coronary artery ligation (CAL). During 15 min reperfusion, no restoration of the myocardial functions was seen and CPK-release further increased in this period. Bopindolol 0.1 μmol/L did not affect significantly the mechanical performances of the normal heart preparations 13 min after it was used. Propranolol 10 μmol/L had a marked inhibitory effect on the heart with HR, AF, CF, TCO and W_T (power production) decreased by 11.0, 8.9, 7.8, 9.0 and 10.0% respectively at the same time. There was no significant difference of the mechanical performances of the hearts in drug-treated groups and the ischemic-control group after CAL. However, the mechanical recoveries of the hearts in drug-treated groups were much better than that in the control group 2 min after reperfusion. Fifteen min after reperfusion, LVSP, +LV$dP/dt_{max}$, AF, TCO, CF and W_T restored to 88, 84, 82, 86, 104 and 77% of the preligation values respectively in bopindolol group and to 88, 90, 69, 81, 96 and 70% of preligation values respectively in propranolol group. HR remained unchanged in bopindolol-treated hearts but was lowered by propranolol during perfusion time. The CPK-release induced by regional ischemia and reperfusion was markedly reduced by either bopindolol or

Tab 1. Effects of bopindolol ($n=7$) and propranolol ($n=5$) on LVSP, AF, W_T, $+LVdP/dt_{max}$, TCO, CF and ΔCPK-release of the isolated working rat hearts with 30-min coronary artery ligation (CAL) followed by 15-min reperfusion. $\bar{x}\pm SD$, $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Perfusion time (min)	Before CAL		CAL		Reperfusion	
	15	28	45	58	62	75
LVSP (kPa)						
Control	13.2±0.4	13.5±0.7	9.9±0.9	10.1±1.1	9.9±1.3	10.1±1.2
Bopindolol	13.6±1.6	13.3±0.5	10.0±0.8*	10.0±0.8*	11.8±0.7**	12.0±0.7***
Propranolol	14.5±0.8	14.1±0.9	10.7±0.7*	10.7±0.5*	12.4±0.7***	12.8±0.7***
AF(ml/min)						
Control	40±7	39±8	22±8	23±7	16±7	19±8
Bopindolol	43±5	42±5	26±4*	26±3*	33±4***	35±3***
Propranolol	42±5	38±5	21±4*	22±3*	28±3***	29±4***
W_T(mW)						
Control	12.3±2.0	12.3±1.8	5.0±2.9	5.5±2.8	5.0±1.7	6.0±1.9
Bopindolol	13.0±1.8	12.7±1.5	5.7±0.9*	5.8±1.0*	9.3±1.6***	10.0±1.5***
Propranolol	13.2±2.2	12.2±2.2	5.4±0.7*	5.5±0.7*	8.5±1.0***	9.3±1.1***
$+LVdP/dt_{max}$(kPa/s)						
Control	409±22	409±22	295±27	296±27	282±30	305±27
Bopindolol	410±18	395±15	286±25*	282±22*	340±29**	352±21***
Propranolol	435±17	432±22	291±9*	292±8*	361±30***	390±15***
TCO(ml/min)						
Control	54±9	54±9	31±9	32±9	30±7	33±9
Bopindolol	57±7	57±6	32±4*	32±4*	47±5***	50±4***
Propranolol	55±8	50±8	29±5*	30±3*	41±6***	43±5***
CF(ml/min)						
Control	13.9±1.5	13.9±1.5	8.7±0.7	8.7±0.7	13.8±1.8	13.8±1.8
Bopindolol	14.1±2.4	14.6±2.6	8.3±1.3*	8.3±1.3*	14.5±2.4*	14.6±2.3*
Propranolol	13.4±3.0	12.4±3.0	8.2±1.8*	8.2±1.8*	12.8±2.6**	13.3±1.9**
ΔCPK-release(IU/L)						
Control		26±12		56±13	81±18	142±28
Bopindolol		30±8*		32±14***	31±14***	58±17***
Propranolol		28±12*		25±8***	40±8***	44±14***

Note: LVSP = left ventricular systolic pressure; AF = aortic flow; W_T = total work; TCO = total cardiac output; CF = coronary flow; CPK = creatine phosphokinase.

propranolol (Tab 1). In response to poly-unsatured peroxidative breakdown, the cardiac formation of MDA in the ischemic-reperfused regions was much more than that in the normal regions (without ischemia). Bopindolol 0.1 μmol/L almost abolished the difference while propranolol 10 μmol/L did not. But in comparison to the control hearts, propranolol reduced significantly the cardiac formation of MDA in the ischemic-reperfused areas (Tab 2).

Tab 2. Effects of bopindolol ($n=7$) and propranolol ($n=5$) on the content of malondialdehyde (MDA, nmol/g wet wt) in regionally ischemic-reperfused myocardium. $\bar{x}\pm SD$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* normal area, $^{\dagger\dagger\dagger}P<0.01$ *vs* ischemic-reperfused area of control.

Group	Normal area	Ischemic-reperfused area
Control	170±20	234±50***
Bopindolol	165±43	179±30*††
Propranolol	167±19	201±30**††

Protective effects of bopindolol and propranolol on the globally ischemic-reperfused myocardial damages Conditions of global ischemia were used to avoid the complications due to an altered regional distribution of flow. The contractility, cardiac output and coronary flow decreased almost to zero after global ischemia. Reperfusion for 15 min only restored LVSP, $+LVdP/dt_{max}$, $-LVdP/dt_{max}$, AF, CF and W_T to 38, 41, 31, 40, 74 and 20% of the pre-ligation values repectively in the control hearts, while the recoveries of the above parameters in the drug-treated hearts were much better and CPK-release was less than those in the control hearts (Tab 3). The amount of MDA formed in the globally

Tab 3. **Recovery of mechanical performances and CPK-release of the isolated working rat hearts 15 min after the start of reperfusion following 30-min global ischemia.** $n = 5$, $\bar{x} \pm SD$. $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* **control.**

Parameter	Before global ischemia			15 min after reperfusion		
	Control	Bopindolol	Propranolol	Control	Bopindolol	Propranolol
HR(bpm)	247±10	252±14	242±55	233±14	251±15*	223±28*
LVSP(kPa)	14.9±1.1	15.6±0.6	16.5±1.0	5.6±1.1	12.9±0.9***	13.2±1.4***
$+LVdP/dt_{max}$ (kPa/s)	485±24	494±42	510±32	207±57	393±25***	397±44***
$-LVdP/dt_{max}$ (kPa/s)	328±50	357±44	373±37	99±31	229±11***	257±40***
AF (ml/min)	41±4	47±5	44±44	16±3	35±3***	30±3***
CF (ml/min)	14.2±2.3	15.0±1.2	13.8±1.3	10.2±2.3	14.6±1.1*	13.3±1.5*
W_T (mW)	13.7±2.3	16.2±1.6	15.6±2.2	2.6±1.4	10.6±1.4***	9.6±1.9***
CPK-release (IU/L)	26±4	23±4	31±14	169±34	92±4***	96±13***

ischemic-reperfused hearts was about 241 ±18 nmol/L, larger than that in the normal hearts (166 ± 16 nmol/L, n = 5, $P < 0.01$). Bopindolol and propranolol decreased significantly the formation of MDA in the globally ischemic-reperfused myocardium (to 186 ± 22 and 210 ± 16 nmol/L respectively, $n = 5$, $P < 0.05$).

DISCUSSION

There is still no certain explanation as to bopindolol and propranolol in protecting isolated working rat heart muscle against the deleterious effects of postischemic reperfusion. One possible explanation is that direct *beta*-adrenergic blockade may be involved because propranolol 10 μmol/L exihibited negative chronotropic and inotropic effects on the heart preparation used in our study. Alternatively, MSA of propranolol might be involved[8] because a comparatively higher drug concentration (10 μmol/L) was used in the present study. However, it seems that neither the direct *beta*-adrenergic blockade nor MSA can explain satisfactorily the beneficial effects of bopindolol on the ischemic-reperfused myocardium because it is without MSA and did not alter significantly the mechanical performances of the heart preparations in 0.1 μmol/L drug concentration. In view of the dramatic increase in cellular calcium on reperfusion[10], we speculated that both bopindolol and propranolol attenuated the effect of calcium overload and thus protect the ischemic-reperfused myocardium.

In conclusion, bopindolol or propranolol used before ischemia protects the myocardium against some of the mechanical and metabolic damages caused by postischemic reperfusion. Although the 2 drugs differ in some of their pharmacological properties (bopindolol being a partial *beta*-adrenergic agonist and without MSA), their abilities to protect the ischemic-reperfused hearts may involved a common mechanism: to diminish the cellular lipid peroxidation induced by oxygen free radicals. Under these conditions, it is possible to envisage a sequence of events whereby some of the membrane and mitochondrial functions and cellular homeostasis will be better maintained, which will certainly be beneficial to the ischemic-reperfused myocardium.

REFERENCES

1 Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? *J Clin Invest* 1985; 76 : 1713

2 Hess ML, Manson NH. Molecular oxygen: friend and foe. The role of the oxygen free radical system in the calcium paradox and

ischemic/reperfusion injury. *J Mol Cell Cardiol* 1984; 16 : 969
3 Ichihara K, Abiko Y. Effects of diltiazem and propranolol on irreversibility of ischemic cardiac function and metabolism in the isolated perfused rat heart. *J Cardiovasc Pharmacol* 1983; 5 : 745
4 Nayler WG, Ferrari R, Williams A. Protective effect of pretreatment with verapamil, nifedipine and propranolol on mitochondrial function in the ischemic-reperfused myocardium. *Am J Cardiol* 1980; 46 : 242
5 Berthold R, Weite R, Weber H. Pharmacological studies with bopindolol: a new long acting β-adrenoceptor antagonist. *Br J Pharmacol* 1981; 74 : 829P
6 Jin XZ, Liu GD. Prevention of regional ischemic-reperfused myocardial damages in the isolated working rat hearts by puerarin. *J Med Coll PLA* 1987; 2 : 335
7 Kannengiesser GJ, Opie LH, van der Werff TJ. Impaired cardiac work and oxygen uptake after reperfusion of regionally ischeamic myocardium. *J Mol Cell Cardiol* 1979; 11 : 197
8 Arnim TV, Welman E. Propranolol in ischemic-reperfused heart : dissociation of beta-adrenergic blocking and protective effect. *Ibid* 1981; 13 : 521
9 Guarnieri C, Flamigni F, Caldarera CM. Role of oxygen in the cellular damage induced by re-oxygenation of hypoxic heart. *Ibid* 1980; 12 : 797
10 Higgins AJ, Blackburn KJ. Prevention of reperfusion damage in working rat hearts by calcium antagonists and calmodulin antagonists. *Ibid* 1984; 16 : 427

波吲洛尔及普萘洛尔对大鼠离体工作心脏缺血后再灌注损伤的保护作用

金行中、刘桂德 （第一军医大学药理教研室，广州 510515，中国）

提要 大鼠离体工作心脏局部或全心缺血 30 min 后再灌注不仅不能使心脏功能恢复反而加重已有的心肌损伤。局部或全心缺血后再灌注心肌内脂质过氧化产物丙二醛分别增加 37.6 和 45.2%。波吲洛尔 0.1 μmol/L 和普萘洛尔 10 μmol/L 能保护缺血再灌注心肌，促进再灌时心脏功能的恢复，降低缺血后再灌注心肌内丙二醛含量。两药对缺血后再灌注心肌的保护作用可能与其防止缺血后再灌时心肌脂质过氧化损伤有关。

关键词 肾上腺素β受体阻滞剂；波吲洛尔；普萘洛尔；心肌再灌注损伤；心脏功能试验；脂质过氧化物

征订《中国药理学报十年累积索引》

本刊创刊十年编辑了一本《十年累积索引 1980—1989》，内含关键词索引、KEYWORD INDEX 和作者索引，是读者查阅本刊论文的最佳工具书。

本刊《十年累积索引》将于今年 11 月出版，定价 8.10 元。需要者请于 1989 年 10 月 20 日前（以邮戳为准）汇款至 200031 上海市岳阳路 319 号《中国药理学报》编辑部。