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Prophylactic effects of *m*-nisoldipine and nisoldipine on reperfusion arrhythmias exacerbated by free radical generating system in Langendorff heart of rat

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ABSTRACT Xanthine-xanthine oxidase (X-XOD, 500 μmol/L + 100 nmol/L) free radical generating system was perfused 10 min prior to coronary artery ligation until the end of the experiment. It exacerbated the reperfusion ventricular fibrillation, reduced the activities of superoxide dismutase and catalase and increased the contents of malondialdehyde in Langendorff heart of rats. *m*-Nisoldipine or nisoldipine (0.05 μmol/L) was perfused 10 min prior to coronary artery ligation until the end of the experiment. They prevented reperfusion arrhythmias exacerbated by X-XOD and decreased the free radicals generated by X-XOD.

KEY WORDS *m*-nisoldipine; nisoldipine; nifedipine; reperfusion; arrhythmia; xanthines; xanthine oxidase; free radicals

It has been suggested recently that regional reperfusion after only a few minutes of ischemia resulted from the ligation of left anterior descending coronary artery in heart of rats can induce reperfusion arrhythmia and free radical formation, and *m*-nisoldipine (*m*-Nis) or nisoldipine (Nis) prevents reperfusion arrhythmias and inhibits free radical formation in heart of rats[1]. Xanthine-xanthine oxidase (X-XOD) pathway was an important source of superoxide radical upon reperfusion[2]. This may be converted to the more reactive hydroxyl radical and then to other free radical species[3]. Thus, these free radicals may cause membrane damage leading to local electrophysiological derangements that trigger serious ventricular arrhythmias[4]. However, it hasn't yet been reported that reperfusion arrhythmia in rats are exacerbated by X-XOD and calcium antagonists prevent this reperfusion arrhythmias.

In order to further research the relations of calcium antagonists, free radicals and reperfusion arrhythmias, the reperfusion-arrhythmogenic effects of the free radicals resulted from the addition of X-XOD to the perfusion fluid and prophylactic effects of m-Nis and Nis on reperfusion arrhythmias exacerbated by X-XOD free radical generating system in Langendorff heart of rats were investigated in this paper.

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MATERIALS AND METHODS

Preparation of experimental ischemia-reperfusion arrhythmias in Langendorff heart of rats 86 Sprague-Dawley male rats weighing 274 ± SD 43 g were lightly anaesthetized with iv sodium pentobarbital 30 mg/kg and their hearts were removed after iv heparin 200 IU. The hearts were immediately arrested by immersion in ice-cold improved Krebs-Henseleit solution (K-H). Langendorff hearts of rats were prepared. Improved K-H solution aerated with 95% O_2 + 5% CO_2 and maintained at 37℃, pH 7.4, was used for perfusion. A constant perfusion pressure was 9.8 kPa. The concentration of K^+ has an important role in the genesis of reperfusion arrhythmias in Langendorff heart of rats[5]. Therefore, in this experiment, the concentration of K^+ in improved K-H solution was 7.5 mmol/L which reduced the incidence of reperfusion arrhythmias in Langendorff heart of rats. This was benifical to investigate the reperfusion arrhythmogenic effects of free radicals resulted from X-XOD free radical generating system. The model of reperfusion arrhythmias were established according to our previous report (coronary artery ligation for 15 min followed by reperfusion for 1 min)[1]. The epicardiogram was recorded by inserting a thin stainless steel wire into the myocardium of the right ventricular free wall as the one electrode and attaching the other to the root of aorta.

At the end of experiment, the left descending coronary artery was ligated again at the original ligating site, and the dilute carbon ink was injected via aorta in order to estimate the weight of ischemic myocardium (nonstained)/total weight of the left ventricle[6]. Any heart in which this radio was less than 30% or more than 50% was discarded.

Perfused hearts were divided into 9 groups: A) control; B) X (10 μmol/L); C) X-XOD (10 μmol/L + 1 nmol/L); D) X-XOD (100 μmol/L + 10 nmol/L); E) X-XOD (500 μmol/L + 100 nmol/L); F) X-boiled XOD (500 μmol/L + 100 nmol/L); G) group E + *m*-Nis (0.05 μmol/L); H) group E + Nis (0.05 μmol/L); I) group E + Nif (0.05 μmol/L). XOD was added just before perfusion with the improved K-H solution.

Measurement of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) After reperfusion for 1 min, reperfused area (R area) and normal area (N area) of myocardium were obtained. The activities of SOD and CAT were measured according to the colorimetric method of nitro blue tetrazolium[7] and the improved ultraviolet colorimetric method[8] respectively. The contents of MDA were measured according to the colorimetric method of thiobarbituric acid[9]. Protein determination was performed by the colorimetric method of phenol reagent[10].

Drug Nifedipine (Nif), the product of the Shanghai 17 th Pharmaceutical Factory, *m*-Nis and Nis which were obtained from Department of Organic Chemistry, Hebei Medical College were dissolved according to our previous report[11]. X was the product of Sigma Chemical Co. XOD was obtained from Shanghai Institute of Biochemistry, Chinese Academy of Sciences (Batch № 8610306).

RESULTS

Effects of X-XOD free radical generating system on reperfusion arrhythmias, activities of SOD and CAT, and contents of MDA Results were shown in Tab 1 and 2. X or different concentrations of X-XOD compounds were added to the perfused solution respectively 10 min prior to coronary artery ligation until the end of the experiment in experimental groups. Perfusion with X (10 μmol/L) alone or X-XOD (10 μmol/L + 1 nmol/L) neither

Tab 1. Arrhythmogenic effects of xanthine-xanthine oxidase (X-XOD) and prophylactic effects of calcium antagonists (all perfused 10 min prior to coronary artery ligation until the end of the experiment) on reperfusion-induced arrhythmias resulted from coronary artery ligation for 15 min followed by reperfusion for 1 min in Langendorff hearts of rats. $\bar{x}\pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control; $^{\dagger}P>0.05$, $^{\dagger\dagger}P<0.05$, $^{\dagger\dagger\dagger}P<0.01$ *vs* X-XOD (500+0.1 μmol/L).

Drug (μmol/L)	n	Ventricular fibrillation		
		Incidence (%)	Onset (s)	Duration (s)
Control	9	56	29±8	15±18
X (10)	8	62*	30±6*	14±14*
X-XOD (10+0.001)	10	60*	27±7*	17±14*
X-XOD (100+0.01)	9	78*	19±7**	22±13*
X-XOD (500+0.1)	14	93***	18±10**	34±17**
X-boiled XOD (500+0.1)	9	67*	28±9*	17±14*
m-Nisodipine(0.05) +X-XOD (500+0.1)	9	44†††	50±15†††	9±13†††
Nisodipine (0.05) +X-XOD (500+0.1)	9	44†††	43±24†††	14±21††
Nifedipine (0.05) +X-XOD (500+0.1)	9	67†	27±24†	24±22†

increased the incidence of the reperfusion ventricular fibrillation (VF), nor shortened the onset of reperfusion VF and also lengthened the duration of reperfusion VF. Perfusion with X-XOD (100 μmol/L + 10 nmol/L) shortened the onset of reperfusion VF only, but perfusion with X-XOD (500 μmol/L + 100 nmol/L) markedly elevated the incidence of reperfusion VF, shortened the onset of reperfusion VF, and lengthened the duration of reperfusion VF. Perfusion with X-boiled XOD (500 μmol/L + 100 nmol/L) had no effect.

In control group, the activities of SOD and CAT in R area of myocardium were lower than that in N area of myocardium, the contents of MDA in R area of myocardium were higher than that in N area of myocardium. In all groups of X-XOD, the activities of SOD and CAT in R area of myocardium were lower than that in N area of myocardium, the contents of MDA in R area of myocardium were higher than that in N area of myocardium. In two groups of X-XOD (100 μmol/L + 10 nmol/L) and X-boiled XOD (500 μmol/L + 100 nmol/L), the activities of SOD and CAT and the contents of MDA in R or N area of myocardium weren't significantly different from that in control group, but in group of X-XOD (500 μmol/L + 100 nmol/L), the activities of SOD and CAT in R or N area of myocardium were higher than that in control group remarkablely (Tab 2).

Effects of *m*-Nis, Nis and Nif on reperfusion arrhythmia exacerbated by free radical generating system and free radicals The results were shown in Tab 1 and 2. In X-XOD (500 μmol/L +100 nmol/L) group, 13 out of 14 heart of rats (93%) exhibited the reperfusion VF, the onset and duration of reperfusion VF were 18 ± 10 and 34 ± 17 s, respectively. Perfusion with 0.05 μmol/L of *m*-Nis or Nis (perfused 10 min prior to coronary artery ligation until the end of the experiment) reduced the incidence of reperfusion VF significantly, shortened the duration of reperfusion VF, and lengthened the onset of reperfusion VF induced by perfusion with X-XOD (500 μmol/L + 100 nmol/L) free radical generating system. The same concentration of Nif was of no effect on this reperfusion VF.

Tab 2. Effects of X-XOD and calcium antagonists (all perfused 10 min prior to coronary artery ligation until the end of the experiment) on the activities of superoxide dismutase, catalase and the contents of malondialdehyde of normal area (N) or reperfused area (R) of reperfused arrhythmic hearts resulted from coronary artery ligation for 15 min followed by reperfusion for 1 min in Langendorff heart of rats. n = 9 except X-XOD (500 +0.1 μmol/L) group (n = 14). $\bar{x}\pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* N; $^{\dagger}P>0.05$, $^{\dagger\dagger\dagger}P<0.01$ *vs* control; $\ddagger P>0.05$, $\ddagger\ddagger P<0.05$, $\ddagger\ddagger\ddagger P<0.01$ *vs* X-XOD (500+0.1 μmol/L); $\S P>0.05$, $\S\S P<0.05$, $\S\S\S P<0.01$ *vs m*-nisodipine; $^{\|\|}P<0.05$, $^{\|\|\|}P<0.01$ *vs* nisodipine. The parameters were measured in the homogenate of the myocardium.

Drug(μmol/L)	Superoxide dismutase (IU/mg protein)		Catalase (nmol/mg protein·min)		Malondialdehyde (nmol/mg protein)	
	N	R	N	R	N	R
Control	112±13	98±11**	843±47	762±83**	0.30±0.02	0.38±0.10***
X-XOD(100+0.01)	110±9†	91±12***†	849±45†	747±77***†	0.31±0.06†	0.40±0.08**†
X-XOD(500+0.1)	93±11†††	71±10***†††	764±58†††	591±112***†††	0.41±0.10†††	0.53±0.12***†††
X-boiled XOD(500+0.1)	108±11†	92±13**†	864±49†	783±63***†	0.29±0.03†	0.38±0.11**†
m-Nisodipine (0.05) +X-XOD(500+0.1)	118±8‡‡‡	112±14*‡‡‡	884±41‡‡‡	866±62*‡‡‡	0.31±0.04‡‡	0.34±0.15*‡‡‡
Nisodipine(0.05) +X-XOD(500+0.1)	109±6§†††††	103±11*‡‡‡§	906±52§†††††	874±83*‡‡‡§	0.32±0.04§††††	0.35±0.11*‡‡‡§
Nifedipine (0.05) +X-XOD (500+0.1)	101±9§§‖†	79±14**‡§§§‖‖	803±75‖§§§†	644±93***‡§§§‖‖	0.40±0.03‖§§†	0.50±0.12**‡§§§‖‖

In *m*-Nis and Nis groups, the difference between the activities of SOD and CAT and the contents of MDA in R area and N area of myocardium weren't significant ($P>0.05$); but in *m*-Nis and Nis groups, the activities of SOD and CAT in R or N area of myocardium were higher and the contents of MDA in R or N area of myocardium were lower than that in X-XOD (500 μmol/L + 100 nmol/L) group. In Nif group, the activities of SOD and CAT in R area were markedly lower and the contents of MDA in R area were higher than that in N area of myocardium. There was no significant difference between the activities of SOD and CAT and the contents of MDA in R or N area of Nif group and that of X-XOD (500 μmol/L + 100 nmol/L) group. The effects of *m*-Nis on the activities of SOD and CAT and the contents of MDA in myocardium were the same as that of Nis ($P>0.05$); but in Nif group the activities of SOD and CAT in R or N area were lower and the contents of MDA in R or N area were higher than that in *m*-Nis or Nis group ($P<0.05$ or 0.01).

DISCUSSION

The changes of the activities of SOD and CAT and the contents of MDA reflect indirectly the amounts of free radical formation[1]. Thus, in higher concentration of X-XOD (500 μmol/L + 100 nmol/L) group the amounts of free radical formation were higher than other groups. Perfusion with X-boiled XOD (500 μmol/L + 100 nmol/L), the amounts of free radical formation did not increase because boil inactivated XOD. Perfusion with lower concentration of X-XOD (100 μmol/L +10 nmol/L)

also did not increase the amounts of free radical formation significantly. The reperfusion arrhythmogenic effects of X-XOD free radical generating system were consistent with its effects on free radical formation, lower concentration (100 μmol/L + 10 nmol/L) of X-XOD or higher concentration (500 μmol/L +100 nmol/L) of X-boiled XOD did not elevate the incidence of reperfusion VF; but the higher concentration of X-XOD (500 μmol/L +100 nmol/L) exacerbated the reperfusion VF remarkablely. Thus, the amounts of free radical formation play an important role in the genesis of reperfusion arrhythmias. The present study showed also that the significant difference of free radical formation between R area and N area of myocardium was an important factor to initiate reperfusion arrhythmias.

The effective concentration of *m*-Nis or Nis to prevent reperfusion arrhythmias in heart of rats[1], also decreased the incidence of reperfusion VF elevated by X-XOD free radical generating system and the free radicals generated by X-XOD in Langendorff heart of rats, but the same concentration of Nif had no effect. Irita *et al*[12] found that Nis inhibited the free radical formation independently of affecting calcium influx. *m*-Nis or Nis prevents reperfusion arrhythmias by lowering cell calcium[1]. Thus, these results suggest that *m*-Nis or Nis prevents reperfusion-induced arrhythmias by scavenging free radicals and lowering cell calcium.

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