INHIBITORY EFFECT OF MORICIZINE ON REPERFUSION INDUCED TACHYARRHYTHMIAS IN RATS

— A Comparison Study with Disopyramide and Mexiletine —

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Acute ligation of proximal left coronary artery was performed on forty male Sprague-Dawley rats. Five min later, occlusion was released in order to evaluate the effectiveness of the 3 antiarrhythmic drugs in eliminating reperfusion ventricular arrhythmias. The drugs evaluated were moricizine (5 mg/kg), disopyramide (DSP) (5 mg/kg) and mexiletine (MXT) (5 mg/kg), which were administered intravenously 5 min before ligation of the coronary artery.

Compared to control rats that underwent identical experimental procedures, all 3 drugs significantly lowered the mortality rate from 90% of the control group to 20, 20 and 0% for moricizine, DSP and MXT groups. The incidence of ventricular fibrillation (Vf) was also decreased significantly by these drugs. The duration of ventricular tachycardia (VT) and Vf of surviving rats in drug groups were 111.7 ± 35.0 sec, 71.6 ± 29.4 sec and 32.9 ± 14.6 sec for moricizine, DSP and MXT, respectively. Many of the drug treated rats could be restored to the normal sinus rhythm and survived. All 3 drugs slowed the heart rate significantly, but as for the blood pressure only MXT showed significant suppressing effect. In conclusion, moricizine has the same significant preventive effect on reperfusion induced ventricular tachyarrhythmias as DSP and MXT.

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RESearch with reperfusion arrhythmia models is important for determining how to prevent sudden cardiac death, because sudden cardiac death is considered to be related to reperfusion induced ventricular arrhythmias, most likely triggered by dissolution of platelet plugs or sudden resolution of coronary spasm. Therefore animal models are used to investigate the mechanisms and treatment of this kind of arrhythmias.

Moricizine, which is a phenothiazine derivative, is a new class 1 antiarrhythmic drug developed in the USSR. There is some con-

fusion with the classification of moricizine by Vaughan Williams system. In The Cardiac Arrhythmia Suppression Trial (CAST)¹ good results were reported with moricizine. Patients who were treated with flecainide and encainide were more likely to die than those who were receiving placebo, and the drugs were withdrawn from the trial. Only moricizine is still under investigation. However as we have reported² moricizine has no obvious protective effect on early ventricular arrhythmias in rats after coronary artery occlusion. In the present study we evaluated the effectiveness of moricizine on reperfusion induced arrhythmias, another main cause of sudden cardiac death, and

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Fig.1. Experiment protocol

compared it with disopyramide (DSP) and mexiletine (MXT).

METHODS

Forty male Sprague-Dawley rats (200–370 mg) divided into 4 groups: control, moricizine, DSP and MXT group, respectively. All groups had similar body weights. The rats were anaesthetized with pentobarbital sodium (60 mg/kg i.p.). Systemic arterial blood pressure was recorded from the left carotid artery. A catheter was placed in a femoral vein for administering drugs and the trachea was cannulated for artificial ventilation. The ECG was recorded using standard limb lead I. Arterial blood pressure and ECG were continuously monitored on an oscilloscope and recorded (Polygraph MIC-9800, Fukuda Denshi, Tokyo). The chest was opened by left thoracotomy, with the 4th and 5th ribs sectioned approximately 2 mm to the left of the sternum. The rats were ventilated with room air using a stroke volume of approximately 2 ml/100g and at a rate of 54 strokes/min to maintain arterial blood PO₂, PCO₂ and pH within the normal range (Respirator model SN-480-7 Shinanoseisakujo, Tokyo). After opening the pericardium the heart was exteriorized by gentle pressure on the chest wall and a 6/0 silk suture was placed under the left coronary artery (Selye's method³). Then both ends of the ligature were passed through a small plastic tube. The heart was repositioned in the chest and the blood pressure and the ECG were allowed to stabilize for 15 min. Any rat in which this procedure itself produced dysrhythmias or a sustained fall in mean arterial pressure to less than 70 mmHg was discarded from the study at this point. In the preliminary study, 1–5 mg/kg dose of the drugs were tested. Five mg/kg was an adequate dose of each drug to suppress arrhythmia. Therefore, the dose of drugs chosen in the present experiment was 5 mg/kg. As shown in Fig. 1, 5

TABLE I THE EFFECTS OF DRUGS AGAINST REPERFUSION ARRHYTHMIAS

	Incidence of Vf	Mortality rate	Duration of VT & Vf (sec)
Saline (n=10)	90%	90%	*
MOR 5 mg/kg (n=10)	40% #	20% ##	111.7±35.0 (n=8)
DSP 5 mg/kg (n=10)	30% #	20% ##	71.6±29.4 (n=8)
MXT 5 mg/kg (n=10)	10% ##	0% ##	32.9±14.6 (n=10)

*In Saline group statistical analysis could not be done because 9 of 10 rats died

$p < 0.05$, ## $p < 0.01$ vs saline control

MOR: moricizine, DSP: disopyramide, MXT: mexiletine, VT: ventricular tachycardia, Vf: ventricular fibrillation. $M \pm SE$.

mg/kg of moricizine, DSP or MXT dissolved in 0.6 cc saline or 0.6 cc saline placebo was administered slowly over 30 sec through the femoral vein 5 min before coronary occlusion. Occlusion was attained by pulling the suture while pressing the tube against the surface of the ventricular wall. Ischemia was maintained for 5 min by clamping the tube and the suture. Unclamping the plastic tube started the reperfusion instantaneously.

The following parameters were assessed to evaluate the dysrhythmic activity in each rat; the mortality rate, incidence of ventricular fibrillation (Vf) and total duration of ventricular tachycardia (VT) and Vf for surviving rats (VT defined as any run of 4 or more ventricular consecutive extrasystoles) during 20 min after release of the clamping.

All the values are expressed as mean $\pm$ S.E. The statistical significance of differences for total duration of VT and Vf and hemodynamic parameters such as heart rate and blood pressure among the groups was calculated by unpaired Student's t-test, and that for incidences of Vf and mortality rate was calculated by Fisher's exact probability method. Paired Student's t-test was used for hemodynamic parameters before and after administration of drugs. All drugs were kindly provided by Du-pont

Fig.2. An example of reperfusion arrhythmia

Fig.3. The antiarrhythmic effects of the drugs. MOR: moricizine, DSP: disopyramide, MXT: mexiletine

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RESULTS

1: Antiarrhythmic effects of moricizine, DSP and MXT (Table I, Fig. 2 and 3)

Fig. 2 shows a typical case of reperfusion induced ventricular arrhythmia. Fifteen seconds after reperfusion, VT, which eventually became Vf was induced.

Table I summarizes effects of the drugs. In 3 drug treated groups, the duration of VT and Vf was shorter, and more rats were restored to normal sinus rhythm and survived. The incidences of mortality and Vf were lowered significantly by the drugs, among which MXT was shown to be the most effective.

2: Hemodynamic effects of drug treatment (Table II)

All 3 antiarrhythmic drugs slowed the heart rate significantly. Blood pressure decreased in both the control and drug-treated groups significantly during the occlusion period, while before occlusion and during 20 min after reperfusion only MXT significantly decreased the blood pressure.

3: Tachyarrhythmia during coronary artery occlusion

Control, DSP and MXT groups had fewer ventricular tachyarrhythmias before reperfusion than the moricizine group. In the moricizine treated group, 6 of 10 rats went into VT 1 min before reperfusion, and the VT duration was 17.5 ± 6.6 sec.

TABLE II HEMODYNAMIC EFFECTS OF THE DRUGS

		HR (bpm)				mBP (mmHg)			
		Saline	MOR	DSP	MXT	Saline	MOR	DSP	MXT
Before drug administration		439±12	452±8	448±12	459±10	91±4	99±4	96±5	100±2
Drug administration	4 min	439±12	399±10*#	417±11*	411±12*#	94±4	94±4	94±6	83±3##
Occlusion	4 min	436±11	378±12	402±9	397±14	45±4	53±6	46±5	47±4
Reperfusion	5 min	—	343±17*#	398±15#	405±13*#	—	98±9	98±5	82±8#
	20 min	—	371±8*#	399±14#	398±12*#	—	95±10	90±5	87±3*#

* $p < 0.05$ (vs saline control), # $p < 0.05$, ## $p < 0.01$ (vs before drug administration)

MOR: moricizine, DSP: disopyramide, MXT: mexiletine, HR: heart rate, mBP: mean blood pressure.
M±SE.

DISCUSSION

Several factors have been reported to affect the incidence and severity of reperfusion arrhythmia.⁴ The duration of the preceding ischemia seems to be one of the most important factors. In dogs and perhaps in man, the incidence and severity of reperfusion arrhythmia is highest after occlusion of the coronary artery for 20–30 min.⁵ In rats, occlusion of the coronary artery could induce severe ventricular tachyarrhythmias,⁶ and both shorter and longer periods of occlusion produced less severe arrhythmias. In the present study, Vf occurred in 9 of 10 control rats with reperfusion after occlusion for 5 min. We have reported that phase 1 arrhythmia (early occlusion arrhythmia) in the rat frequently starts after about 5 min of occlusion and the number of PVC reached a peak around 10 min after ligation of the left coronary artery. To clearly separate reperfusion arrhythmia from occlusion arrhythmia, we reperfused the ischemic myocardium 5 min after occlusion in this experiment protocol.

The amount of myocardium at risk (ie, ischemic) that becomes reperfused also affects the reperfusion arrhythmia. Austin et al⁷ described the close relationship between the occurrence of Vf and the amount of myocardium at risk following coronary artery occlusion. We have been doing this kind of experiment, thus the ischemic or infarction size was almost constant. The good reproducibility may also depend on the fact that rat has few collateral vessels⁸

The presence and severity of antecedent coronary artery occlusion tachyarrhythmias

is another factor which influences reperfusion arrhythmia. It was reported that the more severe the antecedent occlusion arrhythmia, the more severe the reperfusion arrhythmia which would be induced later.⁵ In this study, during the ischemic period, the control, DSP and MXT groups had a few ventricular arrhythmias, while 6 of 10 rats given moricizine had VT 1 min before reperfusion, although there should have been more severe ventricular tachyarrhythmias in the moricizine group according to reports in the literature. This study revealed that for the majority of rats in the moricizine group ventricular arrhythmias after reperfusion could be restored to normal sinus rhythm. The mortality and incidence of Vf decreased significantly compared to the control group.

We reported previously that moricizine has no obvious protective effect on phase 1 arrhythmias, while DSP and MXT have significant antiarrhythmic effects. In this study, moricizine administered before occlusion had a protective effect on reperfusion arrhythmia like DSP and MXT. It is hard to explain why moricizine can prevent reperfusion arrhythmias in the present experiment. Hemodynamically, moricizine was reported to have no depressant effect on cardiac contractility, and in our study, it slowed the heart rate significantly like DSP and MXT, therefore the hemodynamic effects of moricizine can not explain the different results seen in occlusion and reperfusion arrhythmia experiments.

The mechanism of reperfusion arrhythmia has been reported to be different from that of occlusion arrhythmia,⁹ because washout of accumulated ions and metabolites may

influence ischemic injury of the reperfused area. It was reported that DSP is a class 1a antiarrhythmic drug and MXT belongs to type 1b, while moricizine has both 1a and 1b antiarrhythmic characteristics.¹⁰ However, Vaughan Williams¹¹ reported that moricizine should be placed in the 1c category. It is not yet known whether the different results are due to the different mechanisms of occlusion arrhythmia and reperfusion arrhythmia or caused by the different electrophysiological characteristics of these 3 antiarrhythmic drugs.

The antiarrhythmic properties of moricizine have been demonstrated in a variety of experimental animal models,^{12,13} but few articles deal with its effect on reperfusion arrhythmia. Since sudden cardiac death is thought to be mainly associated with phase 1 (early occlusion) and reperfusion arrhythmias, it is interesting that the same dosage of moricizine can suppress reperfusion arrhythmia while having no effect on early occlusion arrhythmia. Moricizine is a phenothiazine derivative, and chlorpromazine was demonstrated to reduce an accelerated phospholipid degradation and membrane dysfunction in the coronary ligated heart of rats.¹⁴ Chlorpromazine was also reported to reduce myocardial cellular damage resulting from the calcium overload.¹⁵ These cardioprotective actions against metabolic disturbance may play an important role in prevention of reperfusion-induced ventricular arrhythmias. Though experimental models differ from clinical situations, the present findings on the animal model may help clinicians to recognize differences in the pathophysiologic mechanisms of ventricular arrhythmias during various phases of evolving myocardial ischemia and infarction.

REFERENCES

1. THE CARDIAC ARRHYTHMIA SUPPRESSION TRIAL (CAST) INVESTIGATORS: Increased mortality due to encainide or flecainide in a randomized trial of arrhythmia suppression after myocardial infarction. *N Eng J Med* 1989; **321**: 406–412
2. ZHU SH, KOMORI S, TAMURA K, HASHI-MOTO K: The effect of pretreatment with moricizine on early arrhythmia resulting from myocardial ischemia in rats. *Jpn Circ J* 1992; **56**: (in press)
3. SELYE H, BAJUSZ E, GRASSOS S, MENDELL P: Simple techniques of the surgical occlusion of coronary vessels in the rat. *Angiology* 1960; **11**: 398–407
4. KARAGUEUZIAN HS, MANDEL WJ: Electrophysiologic mechanisms of ischemic ventricular arrhythmias. Experimental-clinical correlation. In: Mandel WJ ed. *Cardiac Arrhythmias. Their Mechanisms, Diagnosis and Management*. 2nd ed. Philadelphia, J. B. Lippincott Company 1987; pp 460–462
5. BALKE CW, KAPLINSKY E, MICHELSON E, NAITO M, DREIFUS LS: Reperfusion ventricular tachyarrhythmias: Correlation with antecedent coronary artery occlusion tachyarrhythmias and duration of myocardial ischemia. *Am Heart J* 1981; **101**: 449–455
6. MANNING AS, HEARSE DJ: Reperfusion induced arrhythmia: mechanisms and prevention. *J Mol Cell Cardiol* 1984; **16**: 497–518
7. AUSTIN M, WENGER TL, HARREL FE: Effect of myocardium at risk on outcome after coronary artery occlusion and release. *Am J Physiol* 1982; **243**: H340–H345
8. MAXWELL MP, HEARSE DJ, YELLON DM: Species variation in the coronary collateral circulation during regional myocardial ischemia: a critical determinant of the rate of evolution and extent of myocardial infarction. *Cardiovasc Res* 1987; **21**: 737–746
9. CORR PB, POGWIZD SM: Mechanisms contributing to arrhythmogenesis during early myocardial ischemia, subsequent reperfusion, and chronic infarction. *Angiology* 1988; **39**: 684–699
10. BIGGER JT JR: Cardiac electrophysiologic effects of moricizine hydrochloride. *Am J Cardiol* 1990; **65**: 15D–20D
11. VAUGHAN WILLIAMS EM: Classification of the antiarrhythmic action of moricizine. *J Clin Pharmacol* 1991; **31**: 216–221
12. DANILO P, LANGAN WB, ROSEN MR, HOFFMAN BF: Effects of the phenothiazine analog, EN-313, on ventricular arrhythmias in the dog. *Eur J Pharmacol* 1977; **45**: 127–139
13. ROZENSHTRAUKH LV, ANYUKHOVSKY EP, BELOSHAPKO GG, DREMIN SA: Effect of tetrodotoxin and ethmozine on arrhythmias of the dog heart isolated in the late stage of experimental myocardial infarction. *Byul Eksp Biol Med* 1980b; **9**: 446–448
14. CHIEN KH, CRIE JS, BECKER RS, WILDENTHAL K: Influence of chlorpromazine on lysosomal alterations during myocardial ischaemia. *Cardiovasc Res* 1983; **17**: 407–414
15. SCHAFFER SW, BURTON KP, JONES HP, OEI HH: Phenothiazine protection in calcium overload induced heart failure; a possible role for calmodulin. *Am J Physiol* 1983; **244**: H328–H334