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Anti-arrhythmic activities of six indole derivatives of changrolin

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ABSTRACT The indole-derived compounds, which possessed side chains resembling those of changrolin (4-{3',5'-bis[(*N*-pyrrolidinyl)methyl]-4'-hydroxyaniline}-quinazoline) showed potent anti-arrhythmic activity by restoration of sinus rhythm from ouabain-induced tachycardia in guinea pigs. The potency was assessed by comparison of the maintenance time of sinus rhythm recovered from tachyarrhythmias induced by ouabain. The promising compound was MI_2 with piperidyl residue on position 3 &5 of phenol moeity. There was no difference in anti-arrhythmic activities resulting from substitutions between a benzene ring and methyl residue at position 2 of indole, but the latter had weaker parasympatholytic activity. The anti-arrhythmic activity of MI_2 (>60 min) was 2.4 times more potent then changrolin (25 min), but its anti-cholinergic activity was only half of the latter. To compare the suppressive effect on reperfusion-induced arrhythmias by iv MI_2 at different time in relation to the ligation-reperfusion protocol, it was the most effective when administered either 30 min prior to coronary occlusion or at the moment of reperfusion. The compound MI might belong to the I_c group shown by the slowing impulse conduction within the heart.

KEY WORDS indoles; anti-arrhythmia agents; quinazolines; myocardial infarction; ventricular fibrillation

A new type of anti-arrhythmic agents is being developed following the discovery of changrolin[1] originally isolated from a medicinal plant and used as an antimalaria drug which was recently developed to be an orally effective anti-arrhythmic agent. An enthusiasm[2-4] to modify the chemical structure of changrolin has stimulated an attempt to increase the anti-arrhythmic activity and reduce the anti-cholinergic action which might lead to arrhythmogenic effects. We intend to search compounds possessing indole as their skeleton and the side chains of changrolin for strengthening the anti-arrhythmic activity. The optimal arrhythmia-inhibiting effect of MI_2 on ligation-reperfusion induced arrhythmias was also explored.

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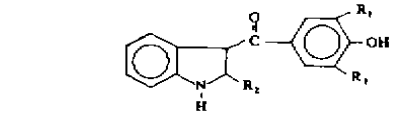
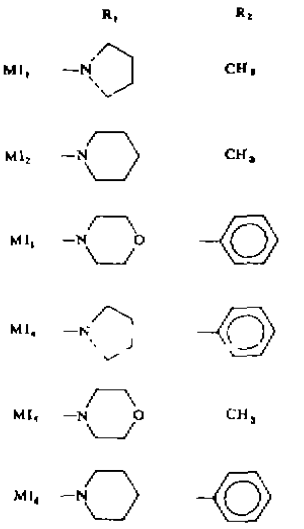
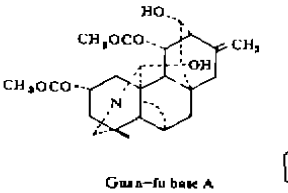
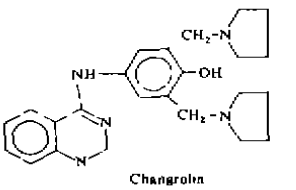
MATERIALS AND METHODS

The indole derivatives were designed and synthesized by the Division of Medicinal









Chemistry of the University. The abbreviations for the six tested compounds are as follows: MI_1, MI_2, MI_3, MI_4, MI_5 and MI_6.

Mexiletine and guan-fu base A were provided by the Department of Organic Chemistry, and phytochemistry of our university, respectively, and changrolin from Shanghai Institute of Pharmaceutical Industry. The tested compounds were dissolved in distilled water and preparations were freshly made before each use. Wistar rats, weighing 195 ± SD 13 g, and guinea pigs, weighing 394 ± SD 37 g, of either sex, were supplied by the Experimental Animal Center in Shanghai and the Animal Vivarium of our University, separately.

Anti-cholinergic activity of the compounds were tested with isolated ileum of guinea pig, suspended in Krebs-Ringer's solution at 37℃ and gassed by oxygen. A final concentration of 0.5 $\mu mol \cdot L^{-1}$ acetylcholine bromide was used to induce a contraction of intestinal loop with preload 0.5 g. Before medication three measurements were made for each sample at intervals of 5 min and the assay was repeated after adding a variety of concentrations of indole derivatives, atropine and disopyramide ranging from 0.001~100 $\mu mol \cdot L^{-1}$. The IC_{50} was measured and used to compare the potency in anti-cholinergic activity with two reference drugs.

Ventricular tachycardia (VT) was produced in guinea pigs by iv ouabain 90 $\mu g \cdot kg^{-1}$ followed by repeating 15 $\mu g \cdot kg^{-1}$ iv at intervals of 15 min until the appearence of the first ventricular ectopic beat which will spontaneously develop into VT, followed by ventricular fibrillation (VF), if not treated effectively. The tested compounds were injected iv immediately after VT developed fully. An effective response to the tested drug was recognized if ventricular ectopic beats were totally suppressed resulting in the recovery of sinus rhythm. The duration of sinus rhythm maintained was taken as an index for evaluation of its anti-arrhythmic potency. Sixty minutes were the cut off time and no further observation was needed.

Rat chest was opened under pentobarbital anesthesia and the left coronary artery was underlined by a thread[5] which was led out of the chest through a plastic tube. The coronary blood flow will be blocked by tension applied. The ischemia-reperfusion profile was consisted of 10 min occlusion followed by reperfusion. The MI_2 3 or 5 $mg \cdot kg^{-1}$ was injected iv at the 8th min of 10 min occlusion protocol. Saline and mexiletine were used as negative and positive control, respectively.

The time dependent property of the anti-arrhythmic effect was checked by administering MI_2 3 $mg \cdot kg^{-1}$ iv either at the beginning of reperfusion, or 5 and 30 min prior to coronary occlusion.

Tachyarrhythmias induced in the ischemic and reperfusion period were assessed by using a scoring system designed as follows:

0 — No arrhythmia, 1 — Occasional ventricular ectopic beats (VEBS), 2 — Frequent VEBS, bigeminal or trigeminal, 3 — Short runs of VT, 4 — Sustained VT, 5 — Ventricular flutter, 6 — VF, 7 — Death.

Bradyarrhythmias were classified and scored by dividing the cardiac cycle with 0.2 s.

Influences of MI_2 on surface ECG was conducted by repeatedly iv MI_2 2 $mg \cdot kg^{-1}$ at intervals of 5 min. The length of RR, PR, QRS complex and QTc interval was monitored and administration was continued until the QRS complex was distorted greatly. The t test was used for statistical analysis.

RESULTS

Anti-arrhythmic action on ouabain-induced ventricular arrhythmias Most of the compounds were effective in suppressing ventricular tachycardia to restore sinus rhythm in guinea pig models. Among the compounds

Tab 1. Anti-arrhythmic activities of indole derivatives on ventricular tachycardia in guinea pigs induced by ouabain infusion. Potency of compounds was calculated based on the time for keeping sinus rhythm after being converted from VT, and efficacy of changrolin was taken as an unity. Cut off at 60 min. $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* saline.

Compounds	*n*	Restoration of sinus rhythm, min	To develop VF, min	Potency
Saline	6	0	8.0 ± 7.0	–
MI_1	3	0*	20.0 ± 1.4**	0
MI_2	7	60 ± 0***	60 ± 0***	2.4
MI_3	9	47 ± 27***	60 ± 0***	1.9
MI_4	3	13 ± 17*	18 ± 20*	0.5
MI_5	2	8 ± 11*	41 ± 47*	0.3
MI_6	5	48 ± 27***	50 ± 30*	1.9
Changrolin	6	25 ± 21**	33 ± 24**	1
Guan-fu base A	6	27 ± 19**	29 ± 18**	1.1
Mexiletine	6	50 ± 24***	58 ± 29***	2.0

studied MI_2 was the most potent. It was about 2.4 fold as potent as that of changrolin, superior to guan-fu base A and equivalent to mexiletine (Tab 1).

Anticholinergic activity The cholinolytic activity of MI_2 was assessed as 1 / 4300 of atropine and 1 / 4.3 of disopyramide, and also weaker than MI_3. Thus the compound was chosen to be further tested for its anti-arrhythmic activities (Tab 2).

Tab 2. Anti-cholinergic activities of indole derivatives assessed by inhibiting ileum contraction caused by acetylcholine. $n=4$, $\bar{x} \pm$ SD. Taking MI_2 as an unity to assess potency.

Compounds	IC_{50}, μmol · L^{-1}	Potency
MI_1	48 ± 25	0.25
MI_2	31 ± 6	1
MI_3	7.9 ± 2.5	3.9
MI_6	14 ± 13	2.2
Disopyramide	7.2 ± 1.4	4.3
Atropine	0.0064 ± 0.0028	4 306
Changrolin		2.1*

* from reference (4)

Inhibiting reperfusion-induced arrhythmias in rats In the control group, the incidence of arrhythmias was high following reopening of the left coronary artery after 10 min occlusion, the scores being 4.5–4.9 during the first minute, and maintained over 3 min and declined gradually. A dose of MI_2 3 mg · kg^{-1} iv at 8 min after occlusion was not as effective as 5 mg · kg^{-1} to decrease the score of arrhythmias. Mexiletine 3 mg · kg^{-1} iv was not sufficient to lower the score, either.

Bradyarrhythmic scores following the release of coronary artery occlusion also were elevated, however, neither MI_2 nor mexiletine

Tab 3. Effect of MI_2 administered iv 8 min after left coronary artery occlusion on reperfusion-induced arrhythmia in anaethetized rats (A scoring system was used for evaluation of arrhythmia). Number of rats is in parentheses. $\bar{x} \pm$ SD, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

	Control (36)	MI_2 3 mg · kg^{-1} (6)	MI_2 5 mg · kg^{-1} (6)	Mexiletine 3 mg · kg^{-1} (6)
Time after ligation, min				
5	1.2 ± 1.9	1.5 ± 2.1*	1.0 ± 2.5*	2.2 ± 2.2*
Time after reperfusion, s				
10	4.5 ± 1.8	3.3 ± 2.7* (−26%)	1.6 ± 2.5** (−65%)	3.8 ± 2.8* (−15%)
30	4.9 ± 1.2	3.3 ± 2.7* (−32%)	2.0 ± 2.5*** (−59%)	4.3 ± 2.1* (−12%)
90	4.9 ± 0.9	3.3 ± 2.7* (−32%)	1.3 ± 1.6*** (−73%)	3.2 ± 1.8* (−36%)
120	4.1 ± 2.2	3.0 ± 3.0* (−10%)	1.8 ± 2.4** (−57%)	3.0 ± 1.9* (−26%)
180	4.0 ± 1.0	1.3 ± 1.5** (−67%)	1.9 ± 2.3* (−53%)	3.3 ± 2.0* (−21%)
240	3.6 ± 2.4	2.8 ± 1.3* (−22%)	0.8 ± 1.8** (−78%)	3.2 ± 1.8* (−11%)

Tab 4. Effect of MI_2 administered at 2 min prior to reperfusion on ventricular fibrillation after 10 min occlusion of left coronary artery in anesthetized rats. $^{*}P>0.05$, $^{**}P<0.05$ *vs* **control.**

Groups	n	VF, %	Arrhythmia, %
Control	7	67	100
Mextiletine 3 mg · kg^{-1}	7	57*	86*
MI_2 3 mg · kg^{-1}	6	29*	71*
MI_2 5 mg · kg^{-1}	7	17**	50**

was able to reduce the figures (Tab 3).

The incidence of VF on reperfusion was also declined by MI_2 5 mg · kg^{-1} iv (Tab 4).

Time-dependent effect against reperfusion induced arrhythmias The antiarrhythmic effect was most effective as iv administered at the moment of reopening the coronary artery. The scores of arrhythmia were also markedly reduced if administered 5 or 30 min prior to the occlusion of the left coronary. In the group administered 8 min after the occlusion, little improvement was seen in the arrhythmic scores (Tab 5).

Effects of MI_2 on the surface ECG in guinea pigs Along with the increase in dosage of MI_2, the heart rate was not altered significantly. The increments in the width of QRS complex was remarkable in parallel with increasing dosage (parameters of linear regression, $a=96.8$, $b=8.8$, $r=0.469$, $P<0.01$), and so was the prolongation of QTc interval ($a=18.9$, $b=2.0$, $r=0.563$, $P<0.01$). There was no change in PR interval.

DISCUSSION

The substitution of piperidyl residue increases the potency to a greater extent than that by a pyrrolidyl group in anti-arrhythmic activity suggesting that pyrrolidyl residue[3,4] at position 3 & 5 of phenol moiety is not optimal and can be changed for further improvement. It seems that substitutions at position 2 of indole nucleus does not alter anti-arrhythmic activity. However, a more negative charge by substitution of methyl residue produces rather low para-sympatholytic effect.

The time-dependent inhibitory activity on cardiac arrhythmias[6] could be explained by the difference in local effective drug concentration in the ischemic myocardium where

Tab 5. Time dependent anti-arrhythmic effect, assessed by a scoring system of MI_2 (3 mg · kg^{-1}, iv) on reperfusion-induced arrhythmia (ligation for 10 min, followed by reperfusion) in anesthetized rats. $\bar{x}\pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control.**

Time	Control (36)	30 min before ligation	5 min before ligation	8 min after ligation	At the moment of reperfusion
Time after ligation, min					
5	1.2±1.9	1.0±1.7*	2.2±2.4*	1.5±2.1*	0.3±0.8*
Time after reperfusion, s					
10	4.5±1.8	0.8±1.3*** (−82%)	2.0±2.3* (−56%)	3.3±2.7* (−27%)	0.5±1.2*** (−89%)
30	4.9±1.2	1.3±1.6** (−73%)	1.8±2.0** (−63%)	3.3±2.7* (−33%)	0.8±1.3*** (−84%)
50	4.5±1.1	1.8±2.9* (−60%)	2.0±2.3** (−56%)	4.7±1.2* (+4%)	1.2±1.2*** (−73%)
90	4.9±0.9	1.7±2.3** (−65%)	2.3±2.7* (−53%)	3.3±2.7* (−33%)	0.7±1.0*** (−86%)
180	4.0±1.0	1.7±2.3** (−58%)	1.0±1.7*** (−75%)	1.3±1.5** (−68%)	0.5±0.8*** (−63%)
240	3.6±2.3	0.7±1.6** (−81%)	0.5±0.6** (−86%)	1.7±1.0* (−53%)	0.2±0.4*** (−94%)
300	2.1±2.9	0.8±1.6* (−62%)	0.2±0.4* (−90%)	0.7±0.8* (−67%)	0* (−100%)

arrhythmias are generated. The drug concentrations are expected to be higher in groups in which the drug studied is administered prior to occlusion or on reperfusion than that administered during the occlusion period.

The classification of the antiarrhythmic agent MI_2 could also be evaluated by the slowing of intraventricular impulse conduction and listed into the Ic group[7,8] same as changrolin.

REFERENCES

1 Li LQ, Qu ZX, Wang ZM, Zheng YL, Ding GS, Hu GJ, *et al*. Studies on a new anti-arrhythmic agent changrolin — 4-{3',5'-bis[(*N*-pyrrolidinyl)methyl]-4'-hydroxyaniline}-quinazoline. *Sci Sin* 1979; 22 : 1220

2 American Hospital Supply Corp. (USA): Acc-9358. *Drugs Fut* 1986; 11 : 169

3 Stout DM, Matier WL, Barcelon-Yang C, Renyaolds RD, Brown BS. Synthesis and antiarrhythmic and parasympatholytic properties of substituted phenols. 2. Amides. *J Med Chem* 1984; 27 : 1347

4 Stout DM, Matier WL, Barcelon-Yang C, Renyaolds RD, Brown BS. Synthesis and antiarrhythmic and parasympatholytic properties of substituted phenols. 3. Modifications to the linkage region. *J Med Chem* 1985; 28 : 295

5 Dai DZ, Kuang LX, Chen L, Huang ML, Zhang DL, Zhang XA. Influence of propranolol withdrawal on the induction of sudden death in chronically infarcted rats by isoproterenol challenge. *Acta Pharmacol Sin* 1987; 8 : 242

6 Kane KA, Parratt JR, Williams FM. An investigation into the characteristics of reperfusion-induced arrhythmia in the anaesthetized rat and their susceptibility to anti-arrhythmic agents. *Br J Pharmacol* 1984; 82 : 349

7 Amery WK, Aerts T. Lorcainide. In: Scriabine A, ed. *New Drugs annuals: Cardiovascular drugs*. NY: Raven Press, 1983; 109-32

8 Mitchell LB, Winkle RA. Enceinide. In: Scriabine A, ed. *New Drugs annuals: Cardiovascular drugs*. NY: Raven Press, 1983; 93-107

常咯啉的6个吲哚衍生物的抗心律失常活性

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提要 以抑制豚鼠哇巴因诱发室速而转为窦律的维持时间，比较心律失常的强度. 3',5'-哌啶基的 MI_2 最强(>60 min)，比常咯啉(25 min)强 2.4 倍. 抗胆碱作用亦减弱. 在不同时间给药，比较对再灌诱发心律失常的抑制作用，在再灌的即刻或结扎前 30 min 给药，抑制作用最强. 由于抑制室内传导，MI_2 可能属于 Ic 类.

关键词 吲哚类；抗心律失常药；噻唑啉类；心肌梗死；心室纤颤

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Effects of intratracheal instillation of fenvalerate on the ultrastructures of pulmonary alveolar macrophages in rat

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ABSTRACT On 1 d after instillation of fenvalerate (Fen) 0.19, 0.93, 4.66, and 23.3 mg · kg^{-1}, and on 30 min, 4 h, 1 d, 4 d, and 7 d after instillation of Fen 4.66 mg · kg^{-1} by a single intratracheal instil- lation, respectively, the ultrastructural changes in rat pulmonary alveolar macrophages (PAM) were observed, and the toxicity indices (TI) were calculated. It was found that the ineffective dose of Fen was 0.19 mg · kg^{-1}, and the threshold dose was $\leqslant$ 0.93 mg · kg^{-1} as well as the serious

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