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Acute toxicity of dipfluzine and its effects on isolated vascular smooth muscle

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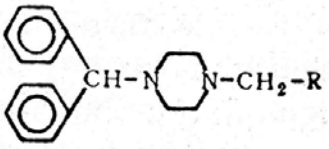
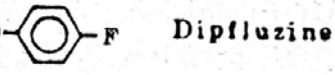
ABSTRACT Dipfluzine (Dip) is a new derivative of cinnarizine (Cin) first developed by Department of Chemistry, Beijing University. Dip showed a dose-dependently inhibitory effect on both KCl- and NE-induced contraction in the rabbit aortic rings. It was more effective in suppressing the contractile response evoked by KCl than that by NE. Dip also inhibited the KCl-induced contraction in porcine basilar, coronary and radial arteries. Their pD_2' values were 5.7 ± 0.6, 5.4 ± 0.4 and 4.6 ± 0.5 respectively. The selectivity of Dip for vasodilation was proved by higher pD_2' value of the basilar artery than that of the coronary and radial arteries, and this selectivity of Dip was more significant than that of Cin. The acute iv LD_{50} of Dip and Cin in mice were 37 and 36 mg/kg, respectively.

KEY WORDS dipfluzine; cinnarizine; thoracic aorta; basilar artery; coronary vessels; calcium channel blockers; vascular smooth muscle

Dipfluzine (Dip), a new diphenylpiperazine compound, was first developed by Department of Chemistry, Beijing University, China, according to the characteristics of molecular formula of cinnarizine (Cin) and droperidol. Our previous studies[1] had shown that Dip possessed a dose-dependent depressive effect on blood pressure and femoral resistance, and a potent antagonistic effect on contraction induced by $CaCl_2$ in isolated central artery of rabbit ear. In order to examine the pharmacological characteristics of Dip, selectivities on various vascular smooth muscle preparation of Dip and Cin and their acute toxicity were compared in this paper.



R -CH=CH- Cinnarizine



Dipfluzine

MATERIALS AND METHODS

Dip and Cin supplied by Department of Chemistry, Beijing University, were dissolved in tartaric acid solution 100 mmol/L separately as the stock solution, and further diluted with tartaric acid 10 mmol/L daily before use. The same concentrations of tartaric acid solution (solvent, pH 3.1)

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were used in parallel as control experiments.

Preparation of rabbit aortic rings Rabbits of either sex weighing 2.2 ± SD 0.5 kg were killed by a heavy blow to the nucha. The descending thoracic aorta was cut into rings 3–4 mm wide[2]. These rings were suspended in 20 ml of Krebs–Henseleit (K–H) solution, at 35℃, pH 7.4 ± 0.5 and aerated with 95% O_2 + 5% CO_2. The tension of the rings was recorded isometrically by electromechanical transducers connected to XWT–204 model potentiometric recorder. The rings were loaded with an initial tension of 3 g for 1 h in the K–H solution which was renewed every 15 min. After the equilibration period, KCl or NE were added cumulatively to the K–H solution to test their contractile effects on the rings and to draw the cumulative concentration–response curves (CRC)[3]. The procedure described above was repeated in the presence of Dip, Cin or solvent, in which the aortic rings were incubated for 15 min. The pD_2' values for Dip and Cin against NE or KCl were calculated[4].

Preparation of isolated porcine arterial rings Basilar artery (BA), coronary artery (CA) and radial artery (RA) were taken immediately after slaughter and immersed in ice-cold O_2-saturated modified Tyrode solution[5]. The BA, CA and RA were cut into 5, 3–4 and 3–4 mm wide respectively, suspended in 20 ml of modified Tyrode solution (35℃) aerated with 95% O_2 + 5% CO_2, and stretched to an initial tension of 1, 4 and 2 g performed by 2 steps (interval 30 min), respectively.

Acute toxicity tests 100 mice (50 ♀ and 50 ♂) weighing 20.2 ± SD 1.6 g were divided into 10 groups according to a stratified random sampling, and iv with Dip or Cin 30 ml/kg. The activities and death rates of mice were observed for 72 h. The LD_{50} values and their 95% confidence limits of Dip and Cin were calculated[6].

RESULTS

Effects on rabbit aortic rings Both Dip and Cin concentration dependently attenuated the contraction of aortic rings induced by NE or KCl (Fig 1). The pD_2' values against NE and KCl were 2.4 ± 0.6 and 4.8 ± 0.3 for Cin, and 4.3 ± 0.5 and 5.4 ± 0.4 for Dip respectively. Both Dip and Cin were more effective in depressing contractile response evoked by KCl than that by NE, but all pD_2' values of Dip against NE and KCl were significantly higher than those of Cin ($P<0.05$ and 0.01).

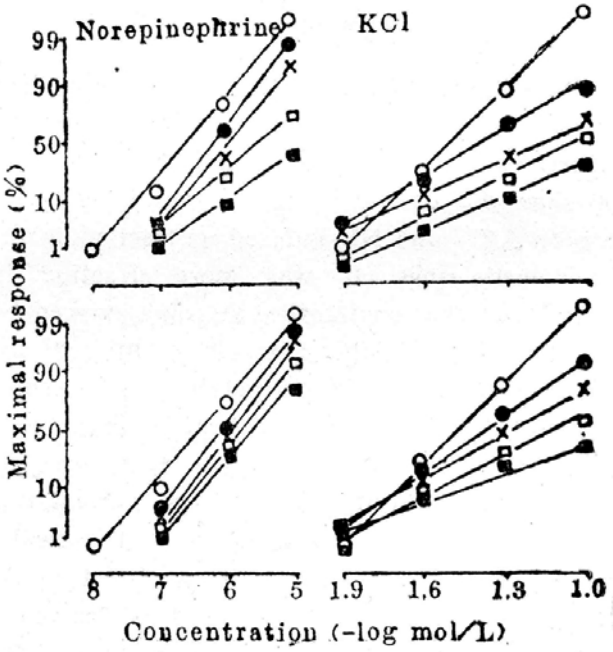

Fig 1. Contractions of isolated rabbit aortic rings after norepinephrine (NE) and KCl in the absence (○) or presence of dipfluzine (Dip, upper panels) and cinnarizine (Cin, lower panels), (●) 0.05 mmol/L, (×) 0.5 mmol/L, (□) 5 mmol/L, (■) 50 mmol/L. $n=6$, $\bar{x}$ ± SD.

Effects on porcine arterial rings Both Dip and Cin showed potent inhibitory effects on KCl-induced contractions of BA, CA and RA rings (Fig 2). The order of pD_2' values against KCl for Dip and Cin in different porcine arterial rings was all the same, that is BA>CA>RA. The pD_2' values for Dip to inhibit KCl-induced BA

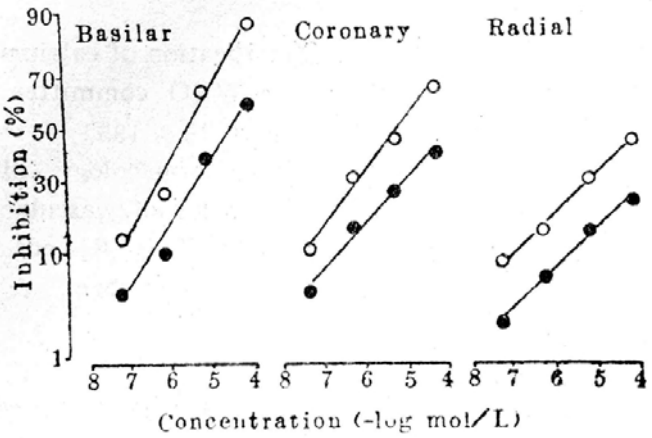

Fig 2. Effects of Dip (○) and Cin (●) on isolated porcine basilar ($n=6$), coronary ($n=6$) and radial ($n=5$) artery rings contracted with KCl 84.7 mmol/L. $\bar{x} \pm$ SD.

and CA contractions were significantly higher than that of RA ($P<0.01$). The pD_2' value for Cin against KCl-induced contraction of BA was significantly higher than those of CA and RA ($P<0.01$). The pD_2' values for Dip against KCl-induced contractions of BA and CA were higher than those for Cin significantly ($P<0.05$ and 0.01) (Tab 1).

Tab 1. pD_2' values of Dip and Cin on isolated porcine artery rings against KCl. $\bar{x} \pm$ SD. **$P<0.05$, ***$P<0.01$ *vs* radial artery; †† $P<0.05$, ††† <0.01 *vs* Cin.

Artery	n	Cinnarizine	Dipfluzine
Radial	5	4.2±0.5	4.6±0.5
Coronary	6	4.4±0.3	5.4±0.4**†††
Basilar	6	5.0±0.4**	5.7±0.6***††

Acute toxicity tests Intoxicated mice showed muscle tremor and reeling gait a few minutes after iv Dip and Cin. In toxic dosage groups, tremor, tonic and clonic convulsion were seen 1–2 min after iv. Convulsion took place repeatedly until death, which usually occurred in 2 min after iv. The mice alive after 10 min usually survived. The LD_{50} values and their 95% confidence limits were 37 (33–41) mg/kg for Dip and 36 (31–41) mg/kg for Cin.

DISCUSSION

The potential calcium antagonistic effects of Dip with KCl to open the "potential-dependent calcium channels" (PDC) and NE to open the "receptor-operated calcium channels" (ROC) were assessed in the isolated rabbit aortic rings which were the most suitable preparation for investigating calcium antagonists[2]. Relaxing effects of Dip on the vessels contracted by KCl and NE were qualitatively similar to Cin and significantly more potent than those of Cin. In addition, Cin is 251 times, and Dip is 12 times more effective against KCl-induced contraction than that caused by NE if $\log^{-1}pD_2'$ value is used as the criterion of comparison. And Dip is 79 times as potent as Cin for inhibiting NE-induced contraction. These data show that Dip possesses more potent antagonistic effects on both PDC and ROC although its selectivity for PDC is lower. Therefore, it may possess α-receptor-blocking activity like droperidol, a parent compound of Dip, besides its antagonistic effect for ROC. The vasodilation of Dip probably comprises 3 components with PDC-, ROC- and α-receptor-blocking action, the third one remains to be established. May be the vasodilation of Dip also relates to its inhibiting calmodulin-dependent effects[7].

This study showed that Cin selectively relaxed cerebral vessels, which was in agreement with the literature[8]. And Dip tends to more potent inhibitor to BA and CA than Cin, but the acute toxicity of Dip was close to that of Cin. These results indicate that Dip not only retains the characteristics of selectivity of Cin to dilate cerebral vessel, but also has an advantage over Cin in the potency to dilate the arteries contracted by KCl and the selectivity to relax CA. Therefore, Dip may be a potential new diphenylpiperazine calcium antagonist with some therapeutic advantages in cardiovascular and cerebral vessel diseases.

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双苯氟嗪的急性毒性及其对离体血管平滑肌的作用

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提要　双苯氟嗪(Dip)为桂利嗪(Cin)的新衍生物。Dip 抑制 NE 和 KCl 所致兔主动脉环收缩，抑制 KCf 所致收缩显著强于抑制 NE；抑制 KCl 所致猪基底动脉、冠脉及桡动脉收缩的 pD_2' 值分别为 5.7±0.6，5.4±0.4 和 4.6±0.5，对基底动脉选择性最高，且显著强于 Cin，Dip iv LD_{50} 与 Cin 相近，分别为 37 和 36 mg/kg。

关键词　双苯氟嗪；桂利嗪；胸主动脉；基底动脉；冠状血管；钙通道阻滞剂；血管平滑肌