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摘要 介绍一种测定生物样品中环丙沙星(Cip)的HPLC方法及其应用实例. 采用Nucleosil C_{18}为固定相, 18∶82 乙腈—磷酸缓冲液 10 mmol·L^{-1}(含硫酸四丁基铵 5 mmol·L^{-1}, pH 2.7)为流动相, 荧光检测. 本法对血清和脑脊液中 Cip 的最低检测浓度分别为 3 ng·ml^{-1}和 5 ng·ml^{-1}, 具有简便、灵敏、准确且样品需量少 (0.1 ml) 等优点.

关键词 环丙沙星; 高压液相色谱法; 药物动力学

药代动力学

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Protective effect of dipfluzine on experimental brain edema in rats

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ABSTRACT The effects of dipfluzine (1-diphenylmethyl-4-(3-(4-fluorobenzoyl))-piperazine, Dip), a new calcium antagonist developed in China, on experimental brain edema in ♀ Wistar rats with bilateral carotid artery ligation were compared with those of cinnarizine (Cin). Dip 25—100 mg·kg^{-1} ip protected the rats against the characteristic signs of global cerebral ischemia that correlate well with the development of brain edema. Its effects were more potent than those of Cin; and the effects of both drugs were more potent by both pretreatment and posttreatment than those by posttreatment alone. Dip 50 mg·kg^{-1} ip attenuated the reduction in cerebral blood flow (CBF) and the infarct size after occlusion, but did not alter CBF before ischemia. These findings suggested that Dip may be potentially useful to treat ischemic brain edema in part by preserving CBF in the ischemic zone.

KEY WORDS dipfluzine; cinnarizine; cerebral infarction; brain edema

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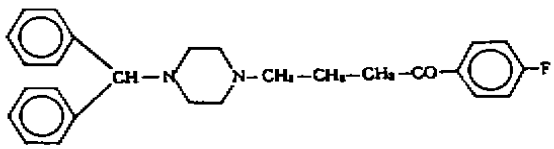
Brain edema is a serious complication in acute cerebrovascular disorders, yet there are few drugs available for its therapeutic intervention. Dipfluzine (1-diphenylmethyl-4-(3-(4-fluorobenzoyl))-piperazine, Dip), a novel diphenylpiperazine calcium channel blocker first developed by Department of Chemistry, Beijing University, has been demonstrated to possess selective and more potent dilatory effects on cerebral vessels than cinnarizine (Cin) did *in vitro* and *in vivo*[1,2]. Accordingly, Dip may be of prophylactic or therapeutic value for ischemic brain edema. The present study was to evaluate the therapeutic effects of Dip and Cin for experimental brain edema induced by bilateral common carotid artery ligation in ♀ Wistar rats.



Dipfluzine

METHODS AND RESULTS

Chemicals Dip and Cin (purities > 99.85 %), synthesized by Department of Chemistry, Beijing University, were dissolved in 2 % tartaric acid solution containing 20 % dimethylacetamide (vehicle). Drugs were injected ip 4 ml · kg^{-1}. Same amount of the vehicle was used as control.

Rat brain edema Brain edema was produced by bilateral carotid artery ligation (BCAL) under light anesthesia by inhaling ether. The common carotid arteries were ligated under a dissecting microscope to avoid any injury to the vagus nerves[3]. Rats were observed at 27 — 30 C room temperature. Rats died within 48 h were immediately decapitated and the rest was decapitated at 48 h after BCAL. After the determination of the wet weight (W), the brain was placed in 110 C for 48 h, and the dry weight (D) was determined. The water content was calculated as (W-D). The dehydrated brain was homogenized with 10 ml of HNO_3 0.75 mol · L^{-1} prepared with deionized water, and placed at 4 C for 3 d. Sodium and potassium contents of the supernatant were measured with a flame photometer[4].

Wistar rats, ♂ and ♀ 20 each, weighing 234 ± 36 g and Sprague-Dawley rats, ♀ and ♂ also 20 each, weighing 228 ± 41 g, were divided into 4 groups. Each group was further divided into ligated and sham-ligated subgroups.

All sham-ligated rats did not develop any signs, while BCAL in ♀ Wistar rats resulted in convulsion within 12 h and in death within 48 h in 35/50 rats. The water, Na, and K contents were also changed. The SD rats and the ♂ Wistar rats were less sensitive to BCAL than the ♀ Wistar rats (Tab 1). Therefore, ♀ Wistar rats were selected as the model of experimental brain edema in this study.

Three administration schedules were studied: the pretreatment series, the pretreatment plus posttreatment series, and the posttreatment series ♀.

Pretreatment series Wistar rats, ♀, n = 60, weighing 248±36 g were equally divided into 6 groups at random; Three groups received ip daily one dose of vehicle, Dip, and Cin, respectively, for 6 d before BCAL. Another 3 groups received ip daily one dose of vehicle, Dip, and Cin, respectively, for 3 d prior to BCAL. The daily dose of dip and Cin was 100 mg · kg^{-1}. The results showed that

Tab 1. Model sensitivity to bilateral carotid artery ligation (BCAL). n = 10, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ vs sham-ligated.

Rats	Sex	BCAL	Rats showed convulsion in 12 h	Rats died in 48 h	Contents in brain H_2O %	Na^+ μmol/g dry wt	K^+ μmol/g dry wt
Sprague-Dawley	♂	—	0	0	77.8±0.5	183±16	369±21
		+	0	0	78.2±1.4	200±38	375±58
	♀	—	0	0	77.0±0.8	200±16	357±24
		+	1	0	76.8±0.4	202±45	345±18
Wistar	♂	—	0	0	77.8±0.8	193±20	360±24
		+	0	0	77.9±0.4	183±13	351±18
	♀	—	0	0	77.1±1.1	212±16	354±29
		+	7[b]	7[b]	79.2±1.5[c]	280±51[c]	318±21[c]

pretreatment of Dip and Cin, ip for either 3 or 6 d, reduced markedly the incidences of convulsion and mortality and alleviated the increased brain water and Na contents. Dip and Cin ip for 6 d also prevented from decrease in brain K content, but those for 3 d did not (Tab 2).

Pretreatment plus posttreated series Wistar ♀ rats ($n=70$) weighing 234 ± 41 g were randomly divided into 7 groups: Three groups of them received ip 2 doses of Dip 25, 50, and 100 mg·kg^{-1}, respectively, at 30 min before and 2.5 h after BCAL; other 4 groups were given the vehicle 4 ml·kg^{-1}, Cin 25, 50, and 100 mg·kg^{-1} respectively according to the above protocol. The results showed that Dip attenuated dose-dependently the changes of the ischemic brain edema in rats, and Cin improved all changes at 100 mg·kg^{-1}, reduced the increase of brain water and Na contents at 50 mg·kg^{-1}.

In 13 groups above, the correlations between brain water content and incidence of convulsion ($r=0.95$, $P<0.01$), mortality ($r=0.96$, $P<0.01$), Na ($r=0.67$, $P<0.05$), or K ($r=0.88$, $P<0.01$) content were significant.

Posttreatment series Wistar ♀ rats ($n=30$) weighing 241 ± 39 g were divided into 3 groups: Dip 50 mg·kg^{-1}, Cin 50 mg·kg^{-1}, and the vehicle, respectively, were injected ip 30 min and 3.5 h after BCAL. The results showed that posttreatment of Dip 50 mg·kg^{-1} ip alone lowered the brain water content, but Cin did not produce any noticeable effect on ischemic brain edema in rats (Tab 2).

Effects on cerebral blood flow (CBF) and infarct size Two groups of 10 ♀ Wistar rats each weighing 231 ± 29 g were anesthetized with ip urethane 1.25 g·kg^{-1}. Both common carotid arteries were isolated from vagus nerve, and loosely encircled with silk thread. A small hole was drilled in the skull 2.5 mm lateral to the midline and 1.5 mm posterior to

Tab 2. Effects of dipfluzine (Dip) and cinnarizine (Cin) on ischemicbrain edema in ♀ Wistar rats. $n=10$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ vs vehicle.

Pretreatment /mg·kg^{-1}	Posttreatment /mg·kg^{-1}	Incidence of convulsion in 12 h %	Mortality by 48 h %	Contents in Brain H_2O %	Sodium μmol/g dry wt	Potassium μmol/g dry wt
6 d Vehicle		80	60	79.5±1.5	224±47	279±89
Dip 100		20^b	0^b	78.3±0.7^b	176±25^b	358±17^c
Cin 100		20^b	10	78.4±0.5^b	187±13^b	360±38^c
3 d Vehicle		70	60	79.5±1.2	213±48	320±26
Dip 100		10^b	10	78.3±0.8^b	165±20^c	329±20
Cin 100		10^b	10	78.1±0.7^b	170±14^c	346±23
Vehicle	Vehicle	80	70	79.6±1.3	280±54	300±44
Dip 25	25	40	10^b	78.4±0.7^b	224±55^b	342±36^b
50	50	20^b	10^b	78.1±0.9^c	208±32^c	369±36^c
100	100	20^b	0^c	77.9±0.5^c	200±18^c	369±30^c
Cin 25	25	50	30	78.9±1.2	234±52	333±29
50	50	30	30	78.5±0.9^b	234±30^b	351±63
100	100	20^b	10^b	78.1±1.0^c	194±26^c	369±54^c
	Vehicle×2	70	70	79.3±1.2		
	Dip 50×2	60	50	78.1±1.2^b		
	Cin 50×2	80	70	78.6±1.3		

the bregma on the right side. A Teflon-coated platinum electrode (diameter 0.2 mm) was stereotaxically inserted into the right parietal cortex to a depth of 1 mm. A reference electrode of Ag/AgCl for CBF was placed on the nucha. A 2-channel polarographic amplifier system was used to measure the hydrogen concentration. Regional CBF was determined by 5% H_2 gas inhalation for 2 to 3 min followed by desaturation. Through an A-D converter, the signal of hydrogen clearance was analyzed by a microcomputer using a program designed by our department. The monoexponential clearance curves were imitated by the least square method[5], both original and imitative hydrogen clearance curves were displayed automatically. Regional CBF was calculated using the 3 min initial slope index of the curves and the formula: CBF (ml/100 g·min^{-1}) = 69.3/$T_{1/2}$. Rectal temperature was maintained at 37.0—38.0 C with a heating pad.

CBF of rats were measured 5 min before and 30 min after ip vehicle or Dip 50 mg·kg^{-1}. After BCAL, CBF were measured again 5, 60, and 120 min. At 48 h, rats were killed and the cerebrums were dissected out. Coronal slices 5 mm in thickness were incubated in 20 ml of 1 % solution of triphenyltetrazolium chloride (TTC) at 37 ℃ for 30 min[6]. The nonstained (pallor) areas were regarded as infarcted, and expressed as percentage of the total area of the slice. The infarct size in each slice was determined by averaging the nonstained areas on the 2 surfaces of each slice.

Results showed that Dip 50 mg·kg^{-1} attenuated the reduction of CBF for 2 h after BCAL and reduced the infarct size at 48 h after BCAL. However, it did not increase the CBF of rats before BCAL (Tab 3).

Tab 3. Effects of dipfluzine on CBF and infarct size in ♀ Wistar rats subjected to BCAL. $n=10$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ vs 0.

		Dip/mg·kg^{-1} 0	50
Infarct size, %		42±8	25±5^c
Cerebral blood flow, ml·kg^{-1}·min^{-1}			
Before ischemia		560±110	620±110
After ischemia	5 min	340±70	440±50^c
	60 min	300±70	410±50^c
	120 min	320±60	400±70^b

DISCUSSION

Although the BCAL has been known to be insufficient to induce progressive global cerebral ischemia in the great majority of rat strains, it resulted in an accumulation of sodium ions and a depletion of potassium ions, which may lead to symptoms of global cerebral ischemia, such as edema, convulsion, and even death. However, there were significant species and sex differences in the susceptibility to the model of brain edema and reported data were paradoxical[3,7,8,9]. Thus, rats susceptible to cerebral ischemia following BCAL were first selected in the present study. Our results revealed that only in ♀ Wistar rats the method of BCAL yielded a reproducible change in brain edema indexes. The finding was in accordance with those in Sprague-Dawley, CFY[7] and Long Evans rats[8], but did not in spontaneously hypertensive rats[8]. Therefore, these results were insufficient to determine whether the influence of gonadal hormones was responsible for the sex differences in susceptibility to brain edema following BCAL[8]. However, the correlations between brain edema and the symptoms or ionic derangement demonstrated that the establishment of a model of brain edema in rats was technically feasible.

Brain edema, which is related to an altered sodium and potassium ions homostasis, is considered to be one of the most important causes of mortality in ischemic brain disease.

In the experimental model of brain edema in ♀ Wistar rats. Dip given either before or after BCAL provided marked protection against the symptoms and electrolyte alterations after BCAL. But the effects were less potent when posttreatment alone was carried out. It is likely that an insufficient amount of the drugs was delivered to the brain areas once the drugs were administered after BCAL. In addition, the therapeutic actions of Dip on brain edema appeared to be more potent than those of Cin, in accordance with the results of our previous studies in which Dip revealed more potent dilatory effect on cerebral vessel than Cin *in vitro* and *in vivo*[1,2]. Considering together with Dip also improved CBF after BCAL, it is suggested that Dip exerts beneficial effects on brain edema partially by increasing CBF to the ischemic regions.

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双苯氟嗪对大鼠实验性脑水肿的保护作用

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R965.2

摘要 用结扎双侧颈总动脉雌性 Wistar 大鼠比较双苯氟嗪(Dip)与桂利嗪(Cin)对实验性脑水肿的作用. Dip 25—100 mg·kg^{-1} ip 可对抗脑水肿引起的大鼠脑缺血性症状，作用强于 Cin，两药预处理加后处理作用也强于单独后处理作用. Dip 50 mg·kg^{-1}ip 能减小缺血脑的梗死范围和 CBF 降低，但并不改变缺血前 CBF，提示 Dip 可能通过维持缺血区的 CBF 而治疗缺血性脑水肿.

关键词 双苯氟嗪；桂利嗪；脑梗死；脑水肿

脑梗塞