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dl-3-*n*-Butylphthalide improves regional cerebral blood flow after experimental subarachnoid hemorrhage in rats[1]

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KEY WORDS butylphthalide; subarachnoid hemorrhage; blood flow velocity; cerebral arteries; caudate nucleus; nimodipine

ABSTRACT

AIM: To investigate the effect of *dl*-3-*n*-butylphthalide (*dl*-NBP) on experimental subarachnoid hemorrhage (SAH) in rats. **METHODS**: SAH was induced by injection of autologous artery blood 0.35 mL into lateral ventricle. Regional cerebral blood flow (rCBF) in caudate nucleus was determined by hydrogen clearance method. **RESULTS**: A rapid and marked decrease in rCBF in caudate nucleus was observed 15 min after SAH and the rCBF remained at low level (about 50 % pre-SAH value) within 180 min. *dl*-NBP (50, 100 mg·kg^{-1}, ig) increased rCBF 30 - 180 min after the onset of SAH without significant effect on mean artery blood pressure. *dl*-NBP 100 mg·kg^{-1} increased rCBF in caudate nucleus by 26 % at 15 min and by 36 % at 180 min respectively after SAH. *d*-NBP but not *l*-NBP (10 mg·kg^{-1}, ip) increased rCBF. **CONCLUSION**: *dl*-NBP improves rCBF in caudate nucleus of rats subjected to SAH.

INTRODUCTION

Previous studies have shown that *dl*-3-*n*-butylphthalide (*dl*-NBP) was effective in the treatment of cerebral ischemia. *dl*-NBP had the ability to improve ischemic brain energy metabolism in mice[1], to reduce the infarct size after middle cerebral artery occlusion (MCAO) in rats[2], and to reduce brain edema induced by focal cerebral ischemia[3]. The results of our recent study[4] suggested that therapeutic effects of *dl*-NBP might be due to its ability to increase regional cerebral blood flow (rCBF) in ischemic zone and thus reduced ischemic brain damage.

Subarachnoid hemorrhage (SAH), resulting from the rupture of intracranial aneurysm in humen, may lead to decrease rCBF and cause brain damage. Cerebral ischemia is an important contributor to brain damage and is the primary cause of mortality after SAH[5,6]. Therefore, to increase cerebral blood flow may improve the prognosis of SAH. In this study, we determined to verify possible protective effect of *dl*-NBP on acute stage of SAH and compare the effect of *d*-NBP with *l*-NBP on rCBF.

MATERIALS AND METHODS

Materials *dl*-, *d*-, and *l*-NBP were supplied by Department of Medicinal Synthetic Chemistry of our Institute. Nimodipine was kindly supplied by Beijing Union Pharmaceutical Factory.

Subarachnoid hemorrhage rat model Wistar rats (♂, 267 - 360 g, Grade Ⅱ, Certificate No 01-3008) were anesthetized with 10 % chloral hydrate (400 mg·kg^{-1}, ip). The right femoral artery was cannulated for monitoring artery blood pressure with 2-channel physiologic recorder (LMS-2B, Chengdu, China). The rat was placed in a stereotaxic frame (Jiangwan IC, Shanghai, China). Head temperature was monitored with temperature probe (AP540, *Diamond General Corp, Michigan, USA*) and maintained at 36.5 - 37.5 ℃ with a heating plate and an incandescent lamp (100 W). A burr hole was drilled over the right cortex 2.4 mm posterior to the bregma and 5 mm lateral to the sagittal suture. Then autologous blood 0.35 mL from right femoral artery

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was injected into the lateral ventricle through the burr hole using № 7 needle under the guide of sterotaxic frame to ensure a depth of 5 mm under the dura.

Measurement of rCBF The rCBF in caudate nucleus was determined by H_2 clearance method. Another burr hole, 3 mm anterior to the bregma and 2.5 mm right to the sagittal suture, was made over the right hemisphere. The #760 needle electrode, previously polarized to +600 mV, was stereotaxically inserted through the hole to a depth of 6 mm into the brain which corresponds to the right caudate nucleus. Reference electrode was placed subcutaneously at the front part of head. Hydrogen was administered to the animals by inhaling through the nose with a PE-50 tube and the current generated by the oxidation of H_2 at electrode tip was monitored through diamond electrode chemical microsensor (Diamond General Corp. Michigan, USA). rCBF values were determined from H_2 clearance curves using the equation: $rCBF = 10 \times 0.693 \div T_{1/2} \times 100$ ($mL \cdot min^{-1} \cdot kg^{-1}$). The rats were divided into 10 groups: (1) Control group: surgical operation only without injection of saline or blood into lateral ventricle, $n = 6$; (2) Saline group: received normal saline 0.35 mL injection, icv, $n = 6$, The group was studied to exclude the possible effect of injected volume on rCBF; (3) Vehicle 1 group: 2 $mL \cdot kg^{-1}$ of sesame oil ig 5 min after the onset of SAH, $n = 6$; (4) *dl*-NBP 50 $mg \cdot kg^{-1}$, ig, $n = 6$; (5) *dl*-NBP 100 $mg \cdot kg^{-1}$, ig, $n = 6$; (6) Vehicle 2 group: saline 2 $mL \cdot kg^{-1}$ ip 5 min after SAH, $n = 8$; (7) *d*-NBP 10 $mg \cdot kg^{-1}$, ip, $n = 6$; (8) *l*-NBP 10 $mg \cdot kg^{-1}$, ip, $n = 6$; (9) *dl*-NBP 20 $mg \cdot kg^{-1}$, ip, $n = 6$; (10) Nimodipine (Nim) 0.25 $mg \cdot kg^{-1}$, ip, $n = 6$. All drugs were given 5 min after the beginning of SAH.

Statistical analysis The difference between two groups were analyzed with t test.

RESULTS

Mean artery blood pressure (MAP) There was a rapid rise in mean artery blood pressure following injection of blood 0.35 mL into lateral ventricle (data not shown) and then the MAP fell slowly. The MAP at 15 min was slightly higher than preinjection value ($P > 0.05$), *dl*-NBP 50 – 100 $mg \cdot kg^{-1}$ (ig), *d*- or *l*-NBP 10 $mg \cdot kg^{-1}$ (ip), *dl*-NBP 20 $mg \cdot kg^{-1}$ (ip) or Nim 0.25 $mg \cdot kg^{-1}$ exert no significant effect on MAP (Tab 1).

rCBF In vehicle group during 180 min after SAH, there was an immediate and marked reduction in rCBF, falling at 15 min to 53 % ± 8 % of preinjection value and remaining low at 52 % ± 8 % by 180 min. The rCBF values in receiving saline icv decreased at 15 min to 90 % ± 13 % and at 30 min to 87 % ± 10 % respectively, and thereafter returned to preinjection values (Tab 2).

dl-NBP (50, 100 $mg \cdot kg^{-1}$, ig) significantly increased rCBF after SAH insult. After treatment with *dl*-NBP 50 or 100 $mg \cdot kg^{-1}$, the rCBF in caudate nucleus was increased 60 or 15 min after SAH. *dl*-NBP 100 $mg \cdot kg^{-1}$ increased rCBF in caudate nucleus

Tab 1. Mean artery blood pressure (kPa) in rats subjected to subarachnoid hemorrhage, and treated with 3-*n*-butylphthalide (NBP) or nimodipine (Nim). $n = 6-8$ rats. $\bar{x} \pm s$.

Group	Dose/ $mg \cdot kg^{-1}$	Time after subarachnoid hemorrhage/min							
		Before	15	30	60	90	120	150	180
Control		13.3±1.3	13.9±1.5	13.8±1.7	13.8±1.5	13.7±1.4	13.7±1.2	13.6±1.1	13.7±1.4
Saline (icv)		11.9±1.1	11.4±1.7	13.3±1.6	13.8±1.9	12.9±1.2	12.9±1.3	12.4±1.8	12.3±1.9
Vehicle 1		12.9±1.3	14.1±1.3	14.1±1.2	13.1±0.5	12.8±0.5	12.6±1.2	12.3±1.2	11.5±1.3
dl-NBP (ig)	50	12.3±0.6	13.6±0.9	13.5±1.0	14.1±0.5	13.9±1.3	13.6±1.5	12.9±1.2	12.7±1.2
	100	12.6±1.2	13.1±1.1	13.3±1.1	14.1±0.9	14.5±0.7	14.2±1.3	13.9±1.5	14.0±1.7
Vehicle 2		11.7±1.2	12.5±1.7	13.1±1.9	12.9±2.3	12.2±1.9	12.9±2.1	13.0±1.7	11.7±1.5
d-NBP (ip)	10	11.5±1.0	13.1±1.1	13.5±1.1	12.9±1.3	12.9±1.7	12.0±1.9	12.5±2.1	14.8±1.9
l-NBP (ip)	10	11.5±1.3	12.5±1.7	12.7±1.4	13.0±1.4	12.6±2.3	12.1±2.5	12.1±2.6	12.4±2.8
dl-NBP (ip)	20	11.8±0.9	12.8±1.0	12.7±0.7	12.5±0.7	12.2±0.8	11.6±1.1	11.3±0.8	10.7±1.0
Nim (ip)	0.25	12.2±1.8	13.6±2.7	12.9±3.2	13.3±3.1	13.5±3.1	13.1±3.1	11.9±3.2	12.0±3.5

Tab 2. Effects of *dl*-3-*n*-butylphthalide (*dl*-NBP, ig) and nimodipine (Nim, ip) on rCBF (mL·min·kg^{-1}) in caudate nucleus in rats subjected to subarachnoid hemorrhage. $n=6$ rats. $\bar{x} \pm s$.
$^bP<0.05$, $^cP<0.01$ *vs* corresponding vehicles.

Group	Dose/ mg·kg^{-1}	Time after subarachnoid hemorrhage/min							
		Before	15	30	60	90	120	150	180
Control		480 ± 30	490 ± 70	480 ± 70	460 ± 50	460 ± 70	450 ± 50	480 ± 60	480 ± 30
Saline (icv)		420 ± 30	380 ± 50	370 ± 40	400 ± 60	430 ± 40	420 ± 40	400 ± 40	400 ± 40
Vehicle		440 ± 50	230 ± 40	210 ± 30	220 ± 30	230 ± 10	220 ± 30	230 ± 40	220 ± 30
dl-NBP	50	450 ± 60	240 ± 50	250 ± 50	260 ± 30^b	270 ± 30^c	280 ± 30^c	280 ± 40^b	280 ± 30^b
	100	440 ± 30	290 ± 40^b	270 ± 30^c	280 ± 30^c	310 ± 60^c	300 ± 30^c	310 ± 20^c	300 ± 40^c
Nim	0.25	490 ± 40	300 ± 30	310 ± 70^b	300 ± 80^b	320 ± 40^c	300 ± 40^c	330 ± 60^c	330 ± 80^c

by 26 % at 15 min and by 36 % at 180 min after SAH respectively. Nim 0.25 mg·kg^{-1} ip also showed an significant increase in rCBF (Tab 2).

The different effects on rCBF between *d*-NBP and *l*-NBP were shown (Fig 1). *d*-NBP 10 mg·kg^{-1} ip displayed significant effect on rCBF, increasing to 61 % ± 11 % at 30 min and to 75 % ± 8 % at 3 h ($P<0.05$, $P<0.01$ *vs* vehicle group). However, *l*-NBP 10 mg·kg^{-1} failed to display the effect as *d*-NBP. The rCBF in caudate nucleus of rats treated with *dl*-NBP 20 mg·kg^{-1} ip also showed the similar increasing effect as that of *d*-NBP treated group.

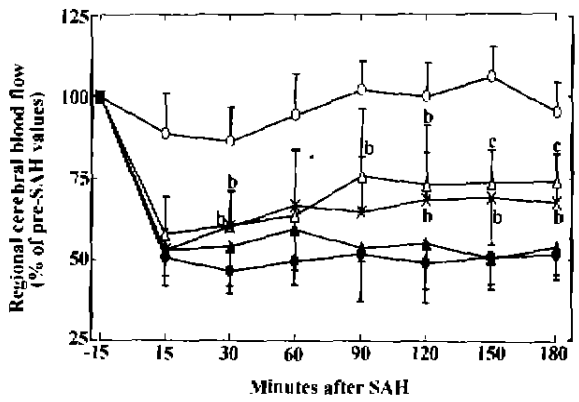

Fig 1. Effect of *d*-, *l*-, and *dl*-3-*n*-butylphthalide (NBP) (10, 10, and 20 mg·kg^{-1} ip respectively) on rCBF in caudate nucleus in rats subjected to subarachnoid hemorrhage (SAH). (○) saline (icv), (●) vehicle, (×) *dl*-NBP, (△) *d*-NBP, (▲) *l*-NBP. $n=6$ rats. $\bar{x} \pm s$.
$^bP<0.05$, $^cP<0.01$ *vs* Vehicle group.

DISCUSSION

The results of this study showed that *dl*-NBP increased the rCBF in caudate nucleus of rat subjected to SAH without any significant effect on MAP, suggesting that NBP might possess selective dilating effects on CNS microvasculature. This result was consistent with previous study of our laboratory which showed that NBP attenuated the decreasing effect on striatal blood flow induced by middle cerebral artery occlusion insults[4].

Several actions of *dl*-NBP may contribute to its increasing effect on rCBF. Previous report[7] suggested that oxyhemoglobin which capture NO might contribute to cerebral vasospasm in SAH. We had found that *d*-NBP increased but *l*-NBP decreased the activity of constitutive NO synthase (cNOS) (data to be published). The increasing effect of *d*-NBP on rCBF in this study may be partly explained by activation of cNOS and thus increasing the production of NO[8], which dilated cerebral microvessels. *l*-NBP, though inhibited cNOS and led to reduce production of NO[8], did not aggravate the reduction of rCBF induced by SAH. Moreover, *dl*-NBP, containing the same quantity of *d*-NBP and *l*-NBP, exerted the similar effect of *d*-NBP on rCBF. One of the possible mechanisms of these results might be due to the different metabolism between *d*-NBP and *l*-NBP.

Another contributing action of *dl*-NBP to improvement in rCBF may be its effect on the metabolism of arachidonic acid (AA). We have found that *dl*-NBP increased the ratio of PGI_2/TXA_2 in brain

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tissue after middle cerebral artery occlusion and reperfusion in rats[9], which may contribute to improve the microcirculation in brain.

The present study showed the therapeutic effect of NBP on SAH and its action scope in the treatment of stroke was increased, however, the action mechanisms of NBP should be further investigated for clear explanation of its therapeutic effects.

REFERENCES

1 Feng YP, Hu D, Zhang LY. Effect of *dl*-3-*n*-butylphthalide (NBP) on mouse brain energy metabolism in complete brain ischemia induced by decapitation. Acta Pharm Sin 1995; 30: 741 – 4.

2 Liu XG, Feng YP. Protective effect of *dl*-3-*n*-butylphthalide on ischemic neurological damage and abnormal behavior in rats subjected to focal ischemia. Acta Pharm Sin 1995; 30: 896 – 903.

3 Deng WB, Feng YP. Effect of *dl*-3-*n*-butylphthalide on brain edema in rats subjected to focal cerebral ischemia. Chin Med Sci J 1997; 12: 102 – 6.

4 Yan CH, Zhang JT, Feng YP. Effects of *dl*-3-*n*-butylphthalide on striatum cerebral blood flow in normal and middle cerebral artery occlusion in rats. Chin J Pharmacol Toxicol 1998; 12: 36 – 9.

5 Bederson JB, Germano IM, Guarino L. Cortical blood flow and cerebral perfusion pressure in a new noncraniotomy model of subarachnoid hemorrhage in the rat. Stroke 1995; 26: 1086 – 92.

6 Broderick JP, Brott TG, Duldner JE, Tomsick T, Leach A. Initial and recurrent bleeding are the major cause of death following subarachnoid hemorrhage. Stroke 1994; 25: 1342 – 7.

7 Martin W, Villani GM, Jothianandan D, Furchgott RF. Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. J Pharmacol Exp Ther 1985; 232: 708 – 16.

8 Yan CH, Feng YP. Effects of *d*-3-*n*-butylphthalide and *l*-3-*n*-butylphthalide on extracellular NO level and intracellular cGMP level in primary cultured rat cortical neurons. Acta Pharmacol Sin 1998; 33: 418 – 23.

9 Chong ZZ, Feng YP. Effects of *dl*-3-*n*-butylphthalide on production of TXB_2 and 6-keto-PGF_1 in rat brain during focal cerebral ischemia and reperfusion. Acta Pharmacol Sin 1997; 18: 505 – 8.

丁基苯酞改善大鼠实验性蛛网膜下腔出血后局部脑血流[1]

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关键词 丁基苯酞；蛛网膜下腔出血；血流速度；脑动脉；尾状核；尼莫地平

目的：探讨丁基苯酞（*dl*-3-*n*-butylphthalide、*dl*-NBP）对大鼠蛛网膜下腔出血（SAH）的可能治疗作用。**方法**：侧脑室注射自体动脉血造成蛛网膜下腔出血模型，氢清除法测定尾核局部脑血流（rCBF）。**结果**：蛛网膜下腔出血后 15 min，rCBF 即快速降至注血前的 52 %，并且在 180 min 内基本上维持在该水平。*dl*-NBP 50，100 mg·kg^{-1}(ig)皆可提高 SAH 后 30 – 180 min 内的 rCBF；而 *dl*-NBP 100 mg·kg^{-1}在 15 min 时即可将 rCBF 提高26 %，180 min 时达 36 %。结果还发现 *d*-NBP（10 mg·kg^{-1}，ip）提高 rCBF，但 *l*-NBP（10 mg·kg^{-1}，ip）则无明显作用。**结论**：丁基苯酞改善 SAH 后 rCBF。

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