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Effects of *dl*-3-*n*-butylphthalide on regional cerebral blood flow in right middle cerebral artery occlusion rats[1]

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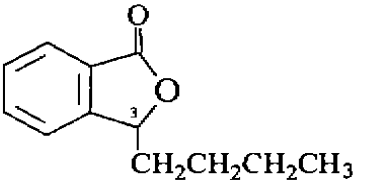
KEY WORDS cerebral ischemia; bood flow velocity; cerebral arteries; corpus striatum; *dl*-3-*n*-butylphthalide; nimodipine

AIM: To study the effect of *dl*-3-*n*-butylphthalide (NBP) on regional cerebral blood flow (rCBF) in forcal cerebral ischemia rats. **METHODS**: In chloral hydrate-anesthetized rat, the proximal portion of right middle cerebral artery (RMCA) was occluded, and H_2 needle electrode was implanted in right striatum. rCBF was monitored in striatum using hydrogen clearance method. **RESULTS**: Ten min after RMCA occlusion (RMCAO), NBP (5, 10, 20 mg·kg^{-1} ip) markedly increased rCBF to striatum ($P < 0.01$). When NBP was given ip 40 min after RMCAO, the increasing effect on rCBF was also observed ($P < 0.05$). However, when NBP was injected ip 60 min after RMCAO, the increasing effect of NBP on rCBF was not found. In NBP-pretreated (ip 40 min before RMCAO) group, rCBF in striatum measured at different time points of 30, 60, 90, 120, 150, and 180 min after RMCAO were increased by 97 %, 107 %, 136 %, 211 %, 173 %, and 317 %, respectively, compared with the percentages of vehicle group. The potency of the effect of Nim (0.5 mg·kg^{-1} ip) was similar to that of NBP (10 mg·kg^{-1} ip). **CONCLUSION**: NBP pre-treatment or post-treatment markedly enhanced the rCBF to striatum in RMCAO rats.

dl-3-*n*-Butylphthalide (NBP), with a color of light yellow and a flavor of celery, was first isolated from the seeds of celery in Europe. Its botanical name is *Apium graveolens* Linn. It is soluble in alcohol, ethyl ether and chloroform, and insoluble in water. NBP was shown to improve brain energy metabolism in complete brain ischemic mice[1], to protect rats from ischemic neurological damage[2], and to attenuate brain edema in RMCAO rat[3]. In the previous studies for the mechanism of action, it was found that NBP decreased glycine and dopamine levels in striatum extracellular fluid in global cerebral ischemic rats[4], and inhibited the increase of the striatum extracellular purine metabolites during ischemia and reperfusion period[5]. The present study was designed to clarify the effect of NBP on rCBF in RMCAO rats.



***dl*-3-*n*-Butylphthalide**

MATERIALS AND METHODS

Adult male Wistar rats of 346 ± 32 g ($n = 120$) were fasted overnight but with water ad libitum. Anesthesia was induced with chloral hydrate 375 mg·kg^{-1} ip. Head and body temperature monitored by AD590 temperature probe (Diamond General Development Corp, USA) was kept at 37.5 ± 0.5 ℃ by a heating lamp and a heating pad. The right femoral artery was cannulated with a PE-50 polyethylene catheter filled with saline and 0.1 % heparin for monitoring the arterial blood pressure (2 channel physiological recorder, LMS-2B model). A model of right middle cerebral artery occlusion (RMCAO) which has previously been shown to provide the similar consequences in regional cerebral blood flow (rCBF) and neuropathological alterations as for human stroke[6] was produced under a surgical microscope[7].

CBF measurements rCBF was measured using hydrogen clearance techniques[8]. A 3-4 mm hole was drilled at 2 mm anterior and 3.5 mm lateral to the bregma. A 770 needle hydrogen electrode was inserted in the right striatum (6.0 mm depth from the dura) with a stereotaxic micromanipulator and a 334 reference electrode was inserted beneath the neck skin. The electrodes were connected to the 1231 Chemical microsensor II (Diamond General Development Corp, USA) and polarized to +650 mV for 0.5 h for stabilization. A PE-50 polyethylene

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catheter was inserted into the nose and located in the nasopharynx for the inhalation of hydrogen. The washout of hydrogen was monitored. rCBF was calculated from the $T_{1/2}$ required for the hydrogen levels to fall from 90 % to 40 % using the formula: $CBF = 0.693 \times 100 / T_{1/2} (mL \cdot s^{-1} \cdot kg^{-1})$.

Protocol Rats were divided into 5 groups: (a) sham group: the same surgical operation but without RMCAO; (b) vehicle control group: RMCAO and 0.05 % Tween-80; (c) NBP-post-treated groups: NBP was injected ip after RMCAO; (d) NBP-pre-treated group: NBP (10 $mg \cdot kg^{-1}$) was injected ip 40 min before RMCAO; (e) nimodipine (Nim) groups: Nim 0.5 $mg \cdot kg^{-1}$ was injected ip 40 min before or 10 min and 40 min after RMCAO. Regional CBF was monitored for 3h after RMCAO.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and compared with paired t-test within group or grouped t-test between groups.

RESULTS

Mean arterial blood pressure (MABP) and head temperature No change in MABP was found in treatment with NBP at the dose of 5, 10, and 20 mg $\cdot kg^{-1}$ ip, while MABP decreased by 26.1 % 15 min after Nim 0.5 $mg \cdot kg^{-1}$ ($P < 0.05$). The head temperature was remained at 37 ℃ throughout the experiments (Tab 1).

Effects of NBP on rCBF in NBP-post-treated rats rCBF in the sham group to right striatum was 8.7 ± 1.8 $mL \cdot s^{-1} \cdot kg^{-1}$ ($n = 6$) during 3 h experiment. Its value was closed to the normal rCBF level (8.3 ± 1.5 $mL \cdot s^{-1} \cdot kg^{-1}$, $n = 6$). The rCBF in the vehicle group decreased to 2.3 ± 1.5 $mL \cdot s^{-1} \cdot kg^{-1}$ 60 min following RMCAO, then gradually reduced to 1.0 ± 1.0 $mL \cdot s^{-1} \cdot kg^{-1}$ by the end of 3 h ($n = 6$). The average rCBF value during the entire ischemic period was 1.7 ± 0.7 $mL \cdot s^{-1} \cdot kg^{-1}$, decreasing about 81 % compared with that of the sham group. Administration of NBP (5, 10, 20 $mg \cdot kg^{-1}$ ip) or Nim (0.5 $mg \cdot kg^{-1}$ ip) 10 min after RMCAO produced marked increases in CBF to striatum. The average rCBF were 4.3 ± 0.4, 5.3 ± 0.7, 4.3 ± 0.7 $mL \cdot s^{-1} \cdot kg^{-1}$ for 5, 10 and 20 $mg \cdot kg^{-1}$ of NBP, respectively, and 4.93 ± 0.17 $mL \cdot s^{-1} \cdot kg^{-1}$ for Nim group (0.5 $mg \cdot kg^{-1}$), which were increased by 159 %, 220 %, 160 %, and 196 %, respectively, compared with the value of vehicle group. The effect of NBP on rCBF lasted for 3 h after RMCAO (Tab 2).

Tab 1. **MABP and head temperature (HT).** $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* **before medication.**

Drug (ip)	Dose/ $mg \cdot kg^{-1}$	Rats	Time after RMCAO/ min	Time after medication/min	MABP/kpa	HT/ ℃
NBP	10	6	0	0	12.5 ± 0.7	37.5
		6	15	5	12.7 ± 1.6^a	37.5^a
		6	30	20	12.7 ± 0.7^a	37.8^a
		5	60	50	12.5 ± 0.9^a	37.6^a
Nim	0.5	6	0	0	12.3 ± 1.0	37.9
		6	15	5	9.8 ± 0.7^b	37.5^a
		6	30	20	12.0 ± 0.3^a	37.8^a
		6	60	50	12.8 ± 0.7^a	37.9^a

Treatment with NBP at the dose of 5 and 10 $mg \cdot kg^{-1}$ ip produced an increasing effect on rCBF with dose dependent manner, while the increasing effect at higher dose of 20 $mg \cdot kg^{-1}$ was not found to be in a dose-dependent manner (Tab 2).

To observe the changes in rCBF before and after administration of NBP in the same RMCAO rat, the rCBF measured at 30 min after RMCAO was taken as a control value (before treatment), then the drugs were

Tab 2. **Effects of NBP or Nim on rCBF to striatum in RMCAO rats.** $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **vehicle.**

Group	n	Regional cerebral blood flow/$mL \cdot s^{-1} \cdot kg^{-1}$ Time after RMCAO (min) 60	90	120	180
Sham-occlusion	6	9.0 ± 1.2^c	8.2 ± 1.0^c	8.3 ± 1.8^c	9.5 ± 2.8^c
Vehicle	13	2.3 ± 1.5	1.8 ± 1.5	1.5 ± 1.3	1.0 ± 1.0
NBP (5 $mg \cdot kg^{-1}$)	6	4.7 ± 1.2^b	4.3 ± 1.2^b	4.3 ± 0.8^c	3.8 ± 0.8^c
NBP (10 $mg \cdot kg^{-1}$)	6	6.2 ± 1.3^c	5.8 ± 1.2^c	4.5 ± 0.7^c	5.0 ± 1.5^c
NBP (20 $mg \cdot kg^{-1}$)	6	5.2 ± 0.8^c	4.8 ± 0.4^c	3.7 ± 0.7^c	3.7 ± 1.0^c
Nim (0.5 $mg \cdot kg^{-1}$)	6	5.0 ± 1.5^b	5.0 ± 1.8^c	4.5 ± 1.0^c	4.3 ± 1.5^c

given at 40 or 60 min after ischemic insult. The rCBF was recorded at the different time point of 60, 90, 120, 150, and 180 min after RMCAO. The significant increase in rCBF was found in NBP 10 or 20 mg·kg^{-1} groups compared with control value at 60 min after RMCAO (3.3 ± 1.3 *vs* 1.3 ± 1.5, 2.8 ± 0.5 *vs* 1.8 ± 0.5 mL·s^{-1}·kg^{-1}, $n = 6$). There was no significant improving effect on rCBF after treatment with NBP 1 mg·kg^{-1} or Nim 0.5 mg·kg^{-1} or when NBP (10 mg·kg^{-1}) was injected ip 60 min after RMCAO (Tab 3).

Effects of NBP on rCBF in NBP-pre-treated rats NBP (10 mg·kg^{-1} ip) or Nim (0.5 mg·kg^{-1} ip) pre-treatment 40 min before RMCAO was shown to increase in rCBF in striatum in the ischemic hemisphere, the average value of rCBF was 4.5 ± 0.3 for NBP group, 4.3 ± 0.5 for Nim group, and 1.8 ± 0.7 mL·s^{-1}·kg^{-1} for control group, respectively. The effect of NBP at the dose of 10 mg·kg^{-1} on rCBF was similar to that of Nim (0.5 mg·kg^{-1}) ($P > 0.05$, $n = 6$) (Fig 1).

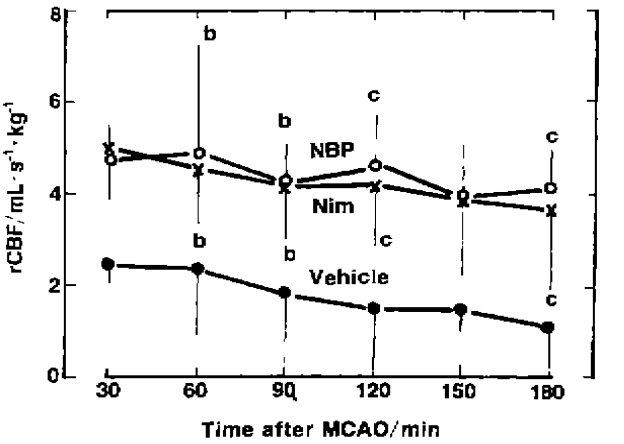

Fig 1. Effects of NBP (10 mg·kg^{-1} ip) and nimodipine (0.5 mg·kg^{-1} ip) given 40 min before ischemia on rCBF to right striatum in right MCAO rats. $n = 6$, $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* vehicle.

DISCUSSION

This study explores the effects of different doses of NBP on rCBF in RMCAO rats under the similar conditions to the previous study in which NBP attenuates ischemic brain damage[2]. The major findings of this study are that: (1) A decrease in striatum rCBF after RMCAO could be improved by NBP pre-treatment and post-treatment, especially when given ip 10 min after RMCAO. (2) The curative effect of NBP relates to the therapeutic time of NBP. The better effect was obtained when NBP given as early as possible after ischemic insult. (3) Nim (0.5 mg·kg^{-1}), ip 10 min after RMCAO, also produced a marked improvement effect on rCBF, however, Nim (0.5 mg·kg^{-1}, ip) had no effect on rCBF when given delayed at 40 min after RMCAO. NBP (10 mg·kg^{-1}), in contrast to Nim, had a longer therapeutic window; (4) The increasing effect on rCBF was not found in a dose-dependent manner at larger dose of NBP (20 mg·kg^{-1}). The main cause might be that NBP is a raceme of l-NBP and d-NBP which had different effects on rCBF, according to our preliminary study, l-NBP and d-NBP had an opposite effect on NO synthase activity, especially at a larger dose (in press).

One of the strategies of treatment for patients with regional ischemia due to the sudden occlusion of the major cerebral artery is to minimize the neurological deficit by increasing the cerebral blood flow in the ischemic penumbra area to the level at which neuronal function can be maintained or recovered[19]. In our

Tab 3. Effects of NBP or Nim ip on rCBF to striatum in RMCAO rats. Drugs were given 40 min (# 60 min) after RMCAO. $n = 6$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Group	Regional cerebral blood flow/mL·s^{-1}·kg^{-1} Time after RMCAO (min)					
	30/control	60	90	120	150	180
NBP (1 mg·kg^{-1})	2.0 ± 1.5	1.5 ± 1.2^a	1.3 ± 1.2^a	1.3 ± 1.2^a	1.3 ± 1.0^a	1.3 ± 1.2^a
NBP(10 mg·kg^{-1})	1.3 ± 1.5	3.3 ± 1.3^b	2.8 ± 0.8^c	1.7 ± 1.5^a	1.8 ± 1.7^a	1.3 ± 0.7^a
NBP(20 mg·kg^{-1})	1.8 ± 1.5	2.8 ± 0.5^b	2.3 ± 0.8^a	2.3 ± 0.7^a	2.8 ± 0.7^b	2.3 ± 0.5^a
Nim (0.5 mg·kg^{-1})	2.3 ± 0.5	2.5 ± 0.7^a	2.7 ± 0.7^a	2.5 ± 0.7^a	2.4 ± 0.4^a	2.7 ± 0.7^a
NBP(10 mg·kg^{-1})#	1.67 ± 0.27	1.8 ± 0.4^a	2.0 ± 0.7^a	1.7 ± 1.0^a	1.8 ± 1.0^a	1.7 ± 0.8^a

studies, the CBF of striatum in vehicle group decreased to an average of 1.7 ± 0.7 mL·s^{-1}·kg^{-1}, which is the upper limit of cellular membrane failure. While CBF in NBP (10 mg·kg^{-1})-pre-treated group increased to 4.4 ± 0.4 mL·s^{-1}·kg^{-1}; and in NBP (10 mg·kg^{-1})-post-treated group to 5.3 ± 0.7 mL·s^{-1}·kg^{-1} (administration of NBP at 10 min after RMCAO), which were much higher than the synaptic transmission failure level[10]. Our findings suggest that the improvement effect of NBP on rCBF may contribute to protective effect against cerebral ischemic injury.

In conclusion, NBP can significantly increase rCBF following RMCAO in rats. Further research work of NBP on NO, PGI_2, and TXA_2 is being undertaken.

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dl-3-*n*-丁基苯酞对右大脑右中动脉阻断大鼠纹状体脑血流的影响[1]

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关键词 脑缺血；血流速度；脑动脉；纹状体；*dl*-3-*n*-丁基苯酞；尼莫地平

目的：观察 *dl*-3-*n*-丁基苯酞（NBP）对右大脑右中动脉阻断（RMCAO）大鼠缺血区局部脑血流（rCBF）的影响. **方法**：氢清除法动态监测 RMCAO 大鼠 rCBF 变化. **结果**：RMCAO 后 10 min ip NBP（5，10，20 mg·kg^{-1}）可明显增加 rCBF（与溶剂对照组相比 $P < 0.01$），40 min 给药有类似作用但作用较弱（与给药前相比 $P < 0.05$），60 min 给药不能增加 rCBF（与给药前相比 $P > 0.05$）. 此外，RMCAO 前 40 min ip NBP 也可使 RMCAO 后不同时间点 rCBF 明显增加. 尼莫地平（0.5 mg·kg^{-1}，ip）与 NBP（10 mg·kg^{-1}，ip）具有相似的作用. **结论**：NBP 预防给药或治疗给药能使 RMCAO 后减少的 rCBF 明显增加.