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Inhibitory effects of nimodipine on platelet aggregation and thrombosis

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KEY WORDS nimodipine; platelet aggregation; thrombosis; thromboxane B_2; 6-ketoprostaglandin F_1 alpha; adenosine diphosphate; carotid arteries

AIM: To study the inhibitory effects of nimodipine (Nim) on rat platelet aggregation and arterial thrombosis *in vivo*. **METHODS**: The aggregation rate of platelets induced by ADP and inhibition rate of Nim were measured by the change of light transmission. Effect of Nim on arterial occlusion time was measured by electric stimulation. Effect of Nim on the contents of 6-keto-$PGF_{1\alpha}$ and TXB_2 in serum was measured by radioimmunoassay. **RESULTS**: Nim 4.5, 9, 18, and 36 mg·kg^{-1}·d^{-1} ig for 4 d restrained the platelet aggregation. The IC_{50} (95 % confidence limits) was 26 (9 – 44) mg·kg^{-1}. Nim 4.5, 9, and 18 mg·kg^{-1}·d^{-1} ig for 4 d markedly prolonged the time of thrombotic occlusion in carotid artery induced by electric stimulation. Nim 9 and 18 mg·kg^{-1}·d^{-1} improved the imbalance of 6-keto-$PGF_{1\alpha}$/TXB_2 in serum after thrombosis. **CONCLUSION**: Nim was a potent inhibitor of platelet aggregation, which was partially concerned with the improved balance of 6-keto-$PGF_{1\alpha}$/TXB_2.

Nimodipine (Nim) dilates cerebral vessels selectively and increases cerebral blood flow[1]. It is widely used to treat patients with subarachnoid hemorrhage. Nim inhibits platelet functions *in vitro*[2]. Here we present the inhibitory effects of Nim on platelet aggregation and on arterial thrombosis *in vivo*.

MATERIALS AND METHODS

Agents Nim (Zhengzhou Chemical Pharmaceutical Factory, 95060120) was suspended with 0.5 % sodium carboxymethylcellulose (CMC). Adenosine diphosphate (ADP) was from Dongfeng Biochemical Technology Corp, Shanghai, China. TXB_2 and 6-keto-$PGF_{1\alpha}$ RIA kits were purchased from Suzhou Medical College. All other reagents were AR grade.

Instruments PAM-2 automatic equibilium platelet aggregating apparatus was from Danyang Electronic Plant, Jiangsu, China. BT87-3 experimental *in vivo* thrombosis detecting apparatus was from Baotou Medical College, Baotou, China.

Platelet aggregation Wistar ♂ rats ($n = 50$) weighing 268 ± s 35 g (provided by He-nan Experimental Animal Center, Zhengzhou, China) were treated with CMC or Nim 4.5, 9, 18, and 36 mg·kg^{-1}·d^{-1} ig for 4 d. After 1 h, the rats were anesthetized with ip urethane 1 g·kg^{-1}. Blood was obtained from carotid artery, anticoagulated with sodium citrate (3.8 %, 1 : 9, vol/vol). PRP and platelet aggregation test were made[3].

Arterial thrombosis and measurements of 6-keto-$PGF_{1\alpha}$/TXB_2 contents in serum Wistar ♂ rats ($n = 40$) weighing 298 ± s 30 g (provided by He-nan Experimental Animal Center, Zhengzhou, China) were treated with CMC or Nim 4.5, 9, 18 mg·kg^{-1}·d^{-1} ig for 4 d. After 1 h, the rats were anesthitized with ip urethane 1 g·kg^{-1}. Bilateral carotid arteries were isolated and one was used for thrombosis test[4]. Blood was obtained from contralateral carotid artery immediately after thrombosis, anticoagulated with Na_2 edetic acid (2 %, 1: 9, vol/vol), spun at 1200 × g for 15 min to get the supernatant for detecting 6-keto-$PGF_{1\alpha}$ and TXB_2 contents with RIA kits[5].

The thrombosis was indicated by occlusion time (OT) which was determined by the sudden decrease of the artery surface temperature. OT prolongation (%) = (treated OT – control OT)/control OT.

Statistical analysis Data were expressed as $\bar{x}$ ± s and evaluated by t test.

RESULTS

Platelet aggregation *in vivo* Nim obviously inhibited the aggregation induced by ADP (final concentration: 4 μmol·L^{-1}). The IC_{50} (95 % confidence limits) was 26 (9 – 44) mg·kg^{-1} (Tab 1).

Arterial thrombosis and 6-keto-$PGF_{1\alpha}$/TXB_2 in serum Nim potently prolonged the OT at the doses of 4.5, 9, and 18 mg·kg^{-1} (Tab 2).

In thrombotic control group, serum TXB_2 contents increased and 6-keto-$PGF_{1\alpha}$ contents decreased dramatically, also the ratio of 6-keto-$PGF_{1\alpha}$/TXB_2 reduced markedly. Nim 9 and 18 mg·kg^{-1} improved

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the ratio obviously, but 4.5 mg·kg^{-1}·d^{-1} had no effect on the imbalance (Tab 3).

Tab 1. Effects of nimodipine on rat platelet aggregation induced by ADP *in vivo*. $n=10$, $\bar{x}\pm s$. $^cP<0.01$ *vs* control.

Nim/mg·kg^{-1}	Platelet aggregation/%	Inhibition/%
0	51 ± 5	–
4.5	43 ± 7^c	17 ± 7
9	35 ± 11^c	30 ± 14
18	29 ± 6^c	43 ± 6
36	23 ± 7^c	55 ± 10

Tab 2. Effects of nimodipine on thrombosis induced by electric stimulating carotid artery *in vivo* in rats. $n=10$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control.

Nim/mg·kg^{-1}	Occlusion time/min	Prolongation/%
0	14.1 ± 0.9	–
4.5	15.9 ± 1.1^b	13 ± 5
9	17.1 ± 1.2^b	22 ± 6
18	18.7 ± 1.3^c	33 ± 7

Tab 3. Effects of nimodipine on TXB_2 and 6-Keto-$PGF_{1\alpha}$ contents in rat serum. $n=10$, $\bar{x}\pm s$. $^cP<0.01$ *vs* blank. $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* trombotic.

Drug, mg·kg^{-1}	TXB_2, ng·L^{-1}	6-Keto-$PGF_{1\alpha}$, ng·L^{-1}	6-Keto-$PGF_{1\alpha}$/TXB_2, %
Blank	101 ± 32	91 ± 36	89 ± 21
Thrombotic	497 ± 147^c	34 ± 18^c	8 ± 4^c
Nim 4.5	419 ± 148^d	39 ± 19^d	10 ± 4^d
9	356 ± 93^e	48 ± 18^d	15 ± 8^e
18	298 ± 101^f	53 ± 19^e	20 ± 9^f

DISCUSSION

The results characterized Nim as a potent inhibitor of platelet aggregation and arterial thrombosis. Nim suppressed platelet aggregation induced by ADP *in vivo* in a dose-related manner. At the doses of inhibiting platelet aggregation *in vivo*, Nim markedly prolonged OT. The potent antithrombotic effect of Nim also showed dose-dependent manner. These findings were in accordance with results from other dihydropyridines (DHP), calcium-entry blockers[4, 6, 7].

Mechanisms of inhibitory effects of Nim on platelet aggregation and thrombosis remains to be clarified. Series of studies of DHPs on antiplatelet revealed that inhibiting transmembrane Ca^{2+} influx; decreasing serotonin secretion; increasing cAMP concentration in platelet *etc* contributed to their antiplatelet effects[7, 8]. 6-keto-$PGF_{1\alpha}$ and TXB_2 are stable metabolites of PGI_2 and TXA_2, respectively. Imbalance of PGI_2/TXA_2 leads to platelet aggregation and arterial thrombosis[9]. Here we confirmed that electric stimulating carotid artery caused imbalance of 6-keto-$PGF_{1\alpha}$/TXB_2 and led to thrombosis in artery. Nim at the doses of 9 and 18 mg·kg^{-1} significantly improved the ratio of 6-keto-$PGF_{1\alpha}$/TXB_2 after thrombosis, which revealed that its antithrombosis was concerned with influence on the balance of PGI_2/TXA_2. At the dose of 4.5 mg·kg^{-1}, Nim showed no effective on the ratio of 6-keto-$PGF_{1\alpha}$/TXB_2, the same dose of Nim, however, prolonged the OT obviously. Therefore it indicated that other mechanisms involved in antithrombotic activity of Nim.

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尼莫地平对血小板聚集和血栓形成的抑制作用

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关键词 尼莫地平；血小板聚集；血栓形成；血栓素 B_2；6-酮前列腺素 $F_{1\alpha}$；腺苷二磷酸；颈动脉

目的：研究尼莫地平（Nim）对大鼠体内血小板聚集和动脉血栓形成的影响。**方法**：比浊法测定血小板的聚集率和抑制率；电刺激法测定 Nim 对体内动脉血栓形成的影响，放免法测定 Nim 对血浆 6-酮前列腺素 $F_{1\alpha}$ 和血栓素 B_2（6-keto-$PGF_{1\alpha}$/TXB_2）含量的影响。**结果**：Nim 4.5，9，18 和 36 $mg\cdot kg^{-1}\cdot d^{-1}$ ig 4 d 可显著抑制血小板的聚集。IC_{50}（95 % 可信限）为 26（9 - 44）$mg\cdot kg^{-1}$。Nim 4.5，9，18 $mg\cdot kg^{-1}\cdot d^{-1}$ ig 4 d 可显著延长电刺激诱导的颈动脉血栓形成时间。Nim 9 和 18 $mg\cdot kg^{-1}$ 可明显改善血浆中 6-keto-$PGF_{1\alpha}$/TXB_2 的比值。**结论**：Nim 抗血栓作用部分与改善 6-keto-$PGF_{1\alpha}$/TXB_2 比值有关。

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Skeletal effects of constant and terminated use of sodium risedronate in ovariectomized rats[1]

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KEY WORDS diphosphonates; ovariectomy; osteoporosis; sodium risedronate; tibia

AIM: To study the skeletal effects of constant and terminated use of sodium risedronate (Ris) treatment in the ovariectomized (Ova) rats. **METHODS**: Ris 5 $\mu g\cdot kg^{-1}$, sc, twice a wk. The proximal tibial metaphysis (PTM) were processed undecalcified for quantitive bone histomorphometry. **RESULTS**: (1) Placebo-treated (normal saline) Ova rats were characterized by decreased trabecular area (TA) on d 60, d 81, and d 150 compared with aging controls, and bone resorption was over formation with high bone turnover. (2) Ova rats were treated with Ris for 60, 81, and 150 d (Ris-on) increased (TA 217 %, 108 %, and 101 %) respectively, *vs* Ova rats and depressed bone turnover indices to aging control level, but bone mass did not maintain at high level in 150-d group as in the early stage. (3) Ova rats were pretreated with Ris for 60 d and then terminated (Ris-on/off), followed by sequential sacrifice of rats on 21 and 90 d. Withdrawal on 21 d showed the same results as the match-age Ris-on group. Withdrawal on 90 d still maintained cancellous bone mass at a high level *vs* 150 d Ris-on groups (+ 26 %) and aging control group (+ 27 %). **CONCLUSION**: Regimen of Ris 60 d on then 90 d off prevented the development of osteoporosis in Ova rats.

Sodium risedronate (Ris) shows a very strong antiresorption effect and low turnover associated with decreasing bone formation and lowering activation in animals[1-5] and humans[6]. Remodeling is very important to repair the microdamage. Prolonged depression of bone remodeling increased the risk of fracture. The purpose of this study was to determine

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