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Differential effects of new breviscapine on arachidonic acid metabolisms in blood cells and in endothelial cells

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ABSTRACT New breviscapine (NB) is the soluble sodium and calcium salts of 4′-scutellarin-7-glucuronide extracted from the Chinese herb *Erigeron breviscapus* (Vant) Hand-Mazz. It inhibited platelet thromboxane B_2 production without alteration of HETE. It also inhibited 6-ketoprostaglandin $F_{1\alpha}$ production by endothelial cells. For leukocytes, NB did not affect thromboxane B_2 production. However, it potentiated the effect of calcimycin in stimulating LTB_4 formation. These results indicate that NB exerts differential effects on the arachidonic acid metabolism in different blood cells and endothelial cells.

KEY WORDS flavones; new breviscapine; blood platelets; leukocytes; vascular endothelium; thromboxane B_2; prostaglandins F; hydroxyeicosatetraenoic acids; leukotrienes B

Many non-steroidal anti-inflammatory drugs inhibited the cyclooxygenase pathway of arachidonic acid metabolism. The sensitivities of the cyclooxygenase of different cell types seemed to be quite diverse[1,2]. On the other hand, non-steroidal anti-inflammatory drugs increased leukotriene formation in neutrophils[3]. New breviscapine (NB) is the soluble sodium and calcium salts of 4′-scutellarin-7-*o*-glucuronide, a compound extracted from a Chinese herb *Erigeron breviscapus* (Vant) Hand-Mazz[4], which has been used in the treatment of occlusive cerebral vascular diseases. Our previous studies demonstrated that NB exerted a remarkable activity against platelets *in vitro* and against thrombus formation *in vivo*[5,6]. The present study was to compare the effects of NB on cyclooxygenase and / or lipoxygenase pathways of arachidonic acid metabolism in platelets, leukocytes, and endothelial cells and to investigate its pharmacologic significance.

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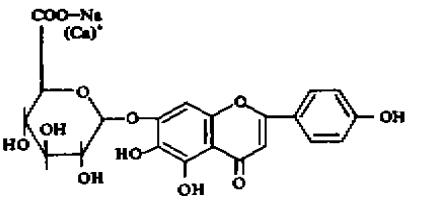

New breviscapine

MATERIALS AND METHODS

NB was extracted by Dr LIN Yin-Yue of Yunnan Institute of Materia Medica. Its purity was 95%; the other 5% is apigenin-7-*o*-glucuronide. Arachidonic acid (Fluka), dextran T500 (Pharmacia), calcimycin and collagenase (Sigma), thrombin (Tianjing Biochemical Factory). The radioimmunoassay kits for thromboxane B_2 (TXB_2) and 6-ketoprostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) were prepared in our laboratory[7,8]. The enzyme immunoassay kit for leukotriene B_4 (LTB_4) was a gift from Dr J Maclouf, Unité 150 INSERM, France.

Preparation and stimulation of washed platelets Blood was drawn into 2% Na_2EDTA (vol : vol, 9 : 1). The platelet-rich plasma (PRP) was taken after centrifugation for 10 min at 120× *g*. After washed twice, the platelets were adjusted to 2×10^{11} platelets · L^{-1}, preincubated with NB 1 mg · ml^{-1} at 37℃ for 10 min, then incubated with calcimycin 20 μmol · L^{-1} for 20 min. Finally an equal volume of methanol / acetonitrile containing PGB_2 was added and the mixture was centrifuged to collect the supernatant for measurement of TXB_2 and hydroxyeicosatetraenoic acid (HETE).

Preparation and stimulation of leukocytes Blood was collected into 3.8% trisodium citrate. After removing the PRP, the remaining blood was mixed

with 2% dextran T500 to precipitate the red cells. The leukocytes suspended at 1×10^{10} cells · L^{-1} were incubated with $CaCl_2$ 2 μmol · L^{-1} at 37℃ for 5 min, and with calcimycin 2 μmol · L^{-1} for another 5 min. The reaction was terminated by the addition of the equal volume of methanol / acetonitrile. The supernatant was collected after centrifugation for 20 min at 500× g.

Endothelial cells culture and treatment Human umbilical vein endothelial cells were isolated and cultured according to the method previously described with slight modification[9]. The cell monolayer was incubated with NB 1 mg · ml^{-1} at 37℃ for 30 min, then with bovine thrombin 0.1 IU · ml^{-1} for another 5 min. The solution was collected for analysis.

Radioimmunoassay for TXB_2 and 6-keto-$PGF_{1\alpha}$ The samples (or standard), ^{125}I-labeled TXB_2 or 6-keto-$PGF_{1\alpha}$ tracer and the corresponding antibody were mixed and incubated overnight at 4℃. Separation of the bound from free ligand was performed by adding polyethylene glycol 4000. After centrifugation at 500× *g* for 30 min, the supernatant was removed and the pellet of ^{125}I-labeled TXB_2 or 6-keto-$PGF_{1\alpha}$ tracer combined with antibody was counted in a gamma counter[7,8].

Enzymeimmunoassay for LTB_4 Samples (or standard), LTB_4 acetylcholinesterase tracer and LTB_4 antiserum were added to the plates which had been coated with the mouse monoclonal antibody against rabbit IgG and incubated for 18 h at 4℃, then the plates were washed. After Ellman's reagent was added, the plates were read at 412 nm wavelength in the enzyme immunoassay kit.

HETE measurement by HPLC HETE in the platelet supernatant was separated and quantified by reverse phase HPLC with a C18 silica gel column and a solvent of acetonitrile-methanol-water (5 : 3 : 2). PGB_2 was monitored with an uv photometer at 280 nm and HETE was monitored at 237 nm. The HETE concentration was calculated in comparison with the standard of HETE.

Statistics Statistical analysis for significance was calculated using *t* test.

RESULTS

Effect of NB on platelet arachidonic acid metabolism The concentrations of TXB_2 and HETE in untreated platelet suspension were 0.33 ± 0.07 and 0.10± 0.08 ng per 2×10^7 platelets, respectively. They were remarkably altered in the presence of NB. When platelets were stimulated by calcimycin, the levels of TXB_2 and HETE were increased to 4.7± 1.1 and 1.1± 0.4 ng per 2×10^7 platelets, respectively. The increase of platelet TXB_2 due to calcimycin was significantly suppressed to 1.6± 1.3 ng per 2×10^7 platelets by NB with IC_{50} being 0.5± 0.21 mg · ml^{-1}, but that of HETE was not remarkably affected (Tab 1).

Tab 1. Effect of new breviscapine (NB) on platelet arachidonic acid metabolism. $\bar{x} \pm s$. $^{*}P > 0.05$ *vs* **control;** $^{+}P > 0.05$, $^{++}P < 0.01$ *vs* **calcimycin 2** μmol · L^{-1}.

NB, mg · ml^{-1}	Calcimycin, μmol · L^{-1}	TXB_2, ng / 2×10^7 platelets $n=7$	HETE, $n=6$
0	0	0.33± 0.07	0.10± 0.08
1	0	0.50± 0.27 *	0.09± 0.04 *
0	2	4.7± 1.1	1.1± 0.4
1	2	1.6± 1.3 ++	0.8± 0.3 +

Effect of NB on epoprostenol formation by endothelial cells NB blocked the cyclooxygenase of endothelial cells. When the endothelial cells were stimulated by thrombin 0.5 IU · ml^{-1}, the amount of 6-keto-$PGF_{1\alpha}$ (a stable derivative of epoprostenol) secreted into the medium was 5.2± 1.2 ng · ml^{-1} ($n=6$). If the monolayer of endothelial cells was pretreated with NB 1 mg · ml^{-1} before adding thrombin, the amount of 6-keto-$PGF_{1\alpha}$ was decreased to 4.0± 0.6 ng · ml^{-1} ($n=6$, $P<0.05$).

Effect of NB on leukocyte arachidonic acid metabolism The amount of TXB_2 produced by calcimycin-stimulated leukocytes was 0.57± 0.28 ng per 10^6 cells ($n=6$). If leukocytes were pretreated with NB 1 mg · ml^{-1} before adding calcimycin, the TXB_2 concentration was 0.8± 0.3 ng per 10^6 cells ($n=6$) without significant alteration. On the other

hand, the production of LTB_4 by leukocytes which were stimulated by calcimycin was 0.7± 0.5 ng per 10^6 cells (n=6). NB alone did not stimulate leukocyte LTB_4 production. However, when leukocytes were incubated with 2 μmol· L^{-1} calcimycin and NB 1 mg· ml^{-1}, the LTB_4 production was increased to as high as 6.0± 1.1 ng per 10^6 cells (n=6, $P<0.01$). This enhancing effect was positively related to the NB concentration (Fig 1).

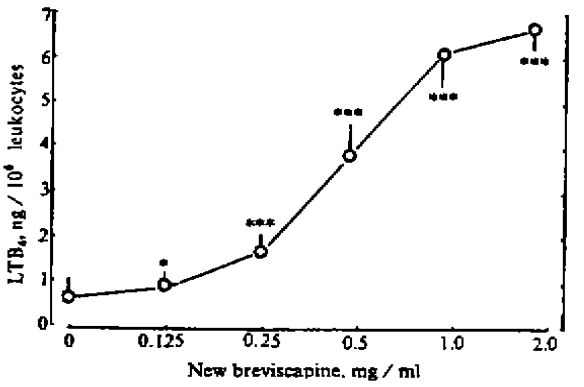

Fig 1. Effect of new breviscapine on the production of leukotriene B_4 (LTB_4) by leukocytes stimulated by 2 μmol· L^{-1} calcimycin. n= 6, $\bar{x} \pm s$. $^{*}P>0.05$, $^{***}P<0.01$ *vs* **control.**

DISCUSSION

In the present study we also measured the leukocyte LTC_4 value and found that NB had a similar effect on LTC_4 production to that on LTB_4.

NB itself did not stimulate leukocyte LTC_4 formation. In the presence of 0, 0.25, and 2 mg· ml^{-1} of NB, stimulation of leukocyte suspension with calcimycin 2 μmol· L^{-1} resulted in LTC_4 formation of 1.2, 4.3, and 6.5 ng per 10^7 cells, respectively. But the data were insufficient for statistical analysis. The mechanism by which NB enhance LT formation by leukocytes stimulated by calcimycin was unlikely due to a simple re-direction of substrate arachidonic acid from the cyclooxygenase pathway to the lipoxygenase pathway, because NB alone could not augment leukotriene formation. It has been reported that protein kinase C alone was not capable of inducing LTB_4 formation in human leutrophil, it appeared necessary to couple the increased intracellular Ca^{2+} to LTB_4 formation[10]. Whether NB potentiated 5-lipoxygenase via activation of protein kinase C remains to be confirmed. In our previous studies, we have found that NB could markedly inhibit the platelet activation induced by many agonists[5]. However, the inhibition of epoprostenol and the augmentation of leukotrienes might offset the benefits of anti-thrombosis of NB.

In contrast to many of other enzymes in the arachidonic acid cascade which did not require activation and their activity was dependent solely on the generation of arachidonic acid, there was such a requirement for the activation of lipoxygenase. LTB_4 could be formed in *in vitro* experiments only when leukocytes were provided with relatively large amount of arachidonic acid extracellularly and / or calcimycin[11,12]. A few other stimuli (eg, opsoninized zymosan or fMLP) induced only a low and quite variable level of lipoxygenase products in leukocytes. The differential effects of NB on different types of blood cells and endothelial cells, especially its strongly enhancing action on LTB_4 formation by calcimycin-stimulated leukocytes, would be of some importance in biochemical and pharmacological researches of arachidonic acid metabolism.

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新灯盏素对血细胞与内皮细胞花生四烯酸代谢影响的差异

R973.2

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摘要 新灯盏素是从灯盏花中提取的 4′–羟基–7–*o*–葡萄糖醛酸甙的可溶性钠盐与钙盐. 该药抑制血小板 TXB_2 生成而不影响羟廿碳四烯酸的产量; 抑制内皮细胞 6–酮 $PGF_{1\alpha}$ 的生成; 对白细胞 TXB_2 生成无影响, 但明显加强钙离子载体刺激 LTB_4 生成的作用. 结果表明新灯盏素对血细胞与内皮细胞的花生四烯酸代谢有不同的影响.

关键词 黄酮类; 新灯盏素; 血小板; 白细胞; 血管内皮; 血栓素 B_2; 前列腺素 F 类; 羟廿碳四烯酸; 白细胞三烯 B 类

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Effects of mebendazole, albendazole, and praziquantel on succinate dehydrogenase, fumarate reductase, and malate dehydrogenase in *Echinococcus granulosus* cysts harbored in mice

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ABSTRACT *Echinococcus granulosus* cyst wall possesses high biochemical activities of malate dehydrogenase (MD) and fumarate reductase (FR), but low activity of succinate dehydrogenase (SD), suggesting that the cyst wall may utilize a partial reverse tricarboxylic acid cycle. When infected mice were given intragastrically with mebendazole, 25–50 mg·kg^{-1}·d^{-1}, albendazole 300 mg·kg^{-1}·d^{-1} or praziquantel 500 mg·kg^{-1}·d^{-1} for 7–14 d, no apparent effects on SD and FR activities of the cyst wall were found, while the MD activity was suppressed by all the 3 drugs, the inhibition rates being 34.6–61.6%, 59.8%, and 50.6%, respectively. The results suggested that MD may not be an important target for the antihydatidosis drugs.

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