并不被钙或哇巴因取消. 但体内应用 Tet 未改变酶对哇巴因的反应性并使其增高的钙敏性趋于加重. Tet 对 WKY 大鼠心肌 Na^+, K^+-ATPase 具有类似但较弱的体外激活作用.

关键词 粉防己碱; 钠, 钾-转运腺苷三磷酸酶; 钙; 哇巴因; 心脏; 近交 WKY 大鼠; 近交 SHR 大鼠; 微粒体

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Effects of tetrandrine on production of leukotriene B_4 and thromboxane B_2 in rabbit blood

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ABSTRACT The effects of tetrandrine (Tet) on the production of 2 major metabolites of arachidonic acid (AA), the leukotriene B_4 (LTB_4) and the thromboxane B_2 (TXB_2) in rabbit whole blood were investigated by reversed phase high pressure liquid chromatography (HPLC) and radioimmunoassay (RIA), respectively. After incubation with different doses of Tet for 15 min *in vitro*, the production of LTB_4 and TXB_2 by rabbit whole blood stimulated with calcimycin (20 $\mu mol \cdot L^{-1}$) was inhibited in a dose-dependent manner, with IC_{50} value of 17.8 ± 8.6 and 17.7 ± 9.2 $\mu mol \cdot L^{-1}$, respectively. In the presence of exogenous AA, the inhibitory effects of Tet were markedly lessened. The effects of Tet were much like those of calmodulin (CaM) antagonist fluphenazine (Flu). Dexamethasone (Dex) also inhibited the production of LTB_4 and TXB_2 when incubated with rabbit whole blood for 60 min. Tet iv 10 $mg \cdot kg^{-1}$ also inhibited the production of LTB_4 and TXB_2 in rabbit whole blood stimulated with calcimycin. These results suggest that Tet may be an antagonist of CaM, thus suppressing the release of AA which was catalyzed by CaM dependent phospholipase A_2 (PLA_2) from the membrane phospholipids of blood cells.

KEY WORDS tetrandrine; leukotrienes B; thromboxane B_2; calcimycin; high pressure liquid chromatography; radioimmunoassay

Tetrandrine (Tet) is the active principle of *Stephania tetrandra* S Moore. It has analgesic, anti-inflammatory, antianaphylactic, antihypertensive, and anti-arrhythmic effects[1]. Our laboratory found that Tet inhibited the ADP- and collagen-induced rabbit platelet aggregation and thromboxane A_2 (TXA_2) biosynthesis, but did not inhibit the TXA_2 generation induced by exogenous AA, and suppressed the calmodulin (CaM) activity[2]. Tet inhibited the synthesis of LTB_4 and TXB_2 inrabbit neutrophils (Neu) stimulated by calcimycin[3]. In the present investigation, the effects of Tet on production of LTB_4 and TXB_2 by calcimycin-stimulated rabbit whole blood were studied.

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MATERIALS AND METHODS

Tet (Zhejiang Jinhua Pharmaceutical Factory); fluphenazine (FPZ, Shanghai Huanghe Pharmaceutical Factory); dexamethasone (Dex, Tianjin Huajin Pharmaceutical Factory); LTB_4, prostaglandin B_2 (PGB_2), sodium arachidonate, and calcimycin (free acid) (Sigma, USA); [^{3}H]TXB_2 RIA kit (General Hospital of PLA, Beijing, China, radioactivity 3.7 $MBq \cdot ml^{-1}$) and C_{18} Bond-Elut extraction column (3 ml vol, Analytichem International Co, USA).

***In vitro* experiments**

1 Incubation protocol of drugs Blood was collected from Japanese white rabbits of either sex, weighing $2.1 \pm s$ 0.4 kg by carotid artery intubation, and anticoagulsted with heparin (20 IU/ml blood). Aliquots of whole blood 1.5 ml were transferred into siliconized glass tubes and incubated for 15 or 60 min

(Dex only) at 37℃ with 0.5 ml test drugs or their vehicle (Hanks' balance salt solution, HBSS, pH 7.4). Calcimycin (final concentration 20 $\mu mol \cdot L^{-1}$) was added. The samples were mixed by vortexing, and further incubated for 30 min[4-6]. The reaction mixture was centrifuged at $1800 \times g$ for 5 min. Supernatants were collected immediately for further processing.

2 Extraction of LTB_4 in plasma[7] An aliquot of the supernatant 1.0 ml was acidified to pH 3.0 with phosphate buffered saline (PBS 0.01 $mol \cdot L^{-1}$, pH 2.0) and mixed with PGB_2 standard 15 ng in methanol 50 μl. After centrifugation at $800 \times g$ for 3 min, the samples were poured into C_{18} Bond-Elut extraction columns which were washed with distilled water 3 ml twice followed by benzene 3 ml; then eluted with ethyl acetate 3 ml. The ethyl acetate eluates were collected and dried under N_2 stream. The residues were reconstituted with 30% methanol 110 μl and 100 μl were used for HPLC assay. The recoveries of PGB_2 and LTB_4 were 97.8 ± 3.9% ($n = 6$ injections) and 87.4 ± 2.6% ($n = 6$), respectively.

3 LTB_4 assay[6,8] HPLC was carried out with a Waters system with Model 510 pump, Lambda-Max Model 481 variable wavelength uv/visible detector, Hitachi 056 recorder and CDMC-2A integrator (*Shanghai Institute of Computing Technology*) were used. A Nucleacil 7 μm ODS column (250 × 4 mm, Dalian Institute of Chemical Physics, Academia Sinica) was eluted with mobile phase (methanol/distilled water/acetic acid = 66/33/0.08 by volume, adjusted to pH 6.20 with NH_4OH) at a flow rate of 1.5 $ml \cdot min^{-1}$. The pressure of the column was 15.8-16.5 MPa. Samples were injected in 100 μl aliquots. The effluent was monitored at 280 nm (generally set at 0.001 aufs). The retention times and areas of peaks of PGB_2 and LTB_4 were recorded and integrated. As an internal marker, PGB_2 was used to identify LTB_4 in the samples. The values of LTB_4 in the samples were calculated from the regression equation of LTB_4 standard curve. The detecting limit was 1.6 $ng \cdot ml^{-1}$.

4 Extraction and RIA of TXB_2 TXB_2 was a stable degradation product of TXA_2. TXB_2 was extracted and assayed following the TXB_2 RIA kit instruction. The values of TXB_2 were calculated from the regression equation of TXB_2 standard curve.

***In vivo* experiments**

The rabbits were randomly divided into either Tet (iv 10 $mg \cdot kg^{-1}$) group or HBSS group. Tet and HBSS solutions were injected iv. Blood samples were collected prior to and 15, 60, 120, and 240 min after the iv. The subsequent procedures of assaying the LTB_4 and TXB_2 were carried out in the same way as those in the *in vitro* experiments.

Statistical analysis The t test and 2-factor variance analysis were used for data in the *in vitro* and *ex vivo* experiments, respectively.

RESULTS

LTB_4 formation in rabbit whole blood stimulated by calcimycin The HPLC chromatograms of LTB_4 and other metabolites of 5-lipoxygenase pathway of AA in rabbit whole blood showed that the retention time of the peak of LTB_4 in samples was the same as that of LTB_4 standard, and the ratios of LTB_4/PGB_2 retention times were identical in various samples (Fig 1). The contents of LTB_4 in controls were 32.8 ± 8.7 $ng \cdot ml^{-1}$ ($n = 12$).

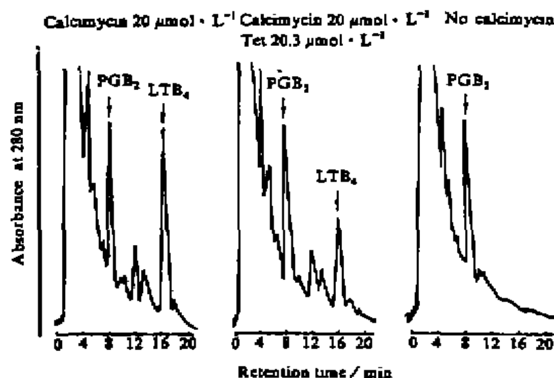

Fig 1. RP-HPLC chromatograms of LTB_4 production stimulated by calcimycin and inhibited by tetrandrine in rabbit whole blood.

Inhibitory effects of Tet on formation of LTB_4 and TXB_2 in rabbit whole blood The productions of LTB_4 and TXB_2 were inhibited by Tet in a dose-dependent manner in the absence of exogenous AA. The IC_{50} values and its 95% fiducial limits were 17.8 (28.9-11.0) and 17.7 (27.2-11.5) $\mu mol \cdot L^{-1}$,

respectively (Fig 2). Tet did not inhibit the formation of LTB_4 and TXB_2 in the presence of exogenous AA even when its concentration was elevated to 50 $\mu mol \cdot L^{-1}$ (Tab 1).

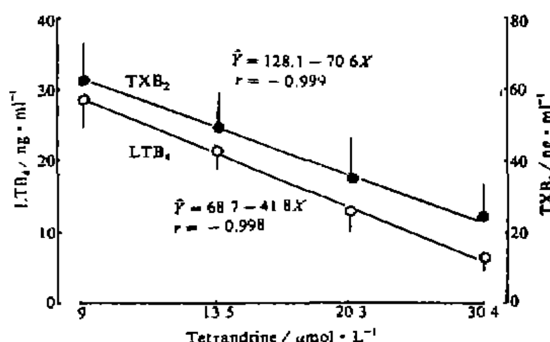

Fig 2. Effects of tetrandrine on productions of LTB_4 and TXB_2 of calcimycin-stimulated rabbit whole blood *in vitro* ($n=3$).

Tab 1. Effects of tetrandrine (Tet) or fluphenazine (FPZ) on production of LTB_4 and TXB_2 by calcimycin stimulated rabbit whold blood with (+) or without (−) exogenous AA. $n=3-6$ (number of observations), $\bar{x} \pm s$. ** $P<0.05$, *** $P<0.01$ vs without (−) exogenous AA.

Drugs/ $\mu mol \cdot L^{-1}$	AA	$LTB_4/ng \cdot ml^{-1}$	$TXB_2/ng \cdot ml^{-1}$
Tet (30.4)	−	7±2	23±5
Tet (30.4)	+	33±6***	77±14***
Tet (50)	−	0±0	0±0
Tet (50)	+	33±7***	71±20***
FPZ (100)	−	0±0	0±0
FPZ (100)	+	15±6**	25±9**

Effects of FPZ on synthesis of LTB_4 and TXB_2 in rabbit whole blood FPZ 100 $\mu mol \cdot L^{-1}$ inhibited the synthesis of LTB_4 and TXB_2 significantly in the absence of exogenous AA, but the effects were markedly lessened in its presence (Tab 1).

Effects of Dex on formation of LTB_4 and TXB_2 in rabbit whole blood There was no inhibitory effect on LTB_4 and TXB_2 synthesis when Dex was incubated with rabbit whole blood for 15 min. But there was an inhibitory effect in the case for 60 min (Tab 2).

Tab 2. Effects of dexamethasone (Dex, 10 $\mu mol \cdot L^{-1}$ on production of LTB_4 and TXB_2 of calcimycin stimulated rabbit whole blood. $n=3-6$ (number of observations), $\bar{x} \pm s$. * $P>0.05$, *** $P<0.01$ vs control.

Incubation time/min	$LTB_4/ng \cdot ml^{-1}$ Control	Dex	$TXB_2/ng \cdot ml^{-1}$ Control	Dex
15	33±9	30±8*	79±22	75±22*
60	31±10	18±8***	76±22	41±15***

Inhibitory effects of Tet on biosynthesis of LTB_4 and TXB_2 of rabbit whole blood in *in vivo* experiments The generations of LTB_4 and TXB_2 in rabbit whole blood were inhibited markedly by iv Tet 10 $mg \cdot kg^{-1}$, and sustained for 4 h (Tab 3).

Tab 3. Effects of tetrandrine (Tet 10 $mg \cdot kg^{-1}$ on LTB_4 and TXB_2 productions in rabbit whole blood (*in vivo*). $n=9$, $\bar{x} \pm s$. *** $P<0.01$ vs HBSS group.

Time/min	$LTB_4/ng \cdot ml^{-1}$ HBSS	Tet	$TXB_2/ng \cdot ml^{-1}$ HBSS	Tet
0	33±9	32±9	78±22	78±22
15	31±8	21±8***	74±15	51±10***
60	31±7	13±4***	71±11	27±9***
120	32±8	14±4***	74±18	28±9***
240	32±8	16±5***	76±20	38±8***

DISCUSSION

We found that Tet inhibited the formation of LTB_4 and TXB_2 in rabbit whole blood with similar IC_{50} values, and the inhibitory effects of Tet would be completely reversed after exogenous AA was added. These results indicated that Tet preferred to inhibit the release rather than the utilization of AA, suggesting that Tet may act as an inhibitor of PLA_2. The pattern of the effects of Flu was similar to that of Tet. It had been demonstrated in our laboratory that Tet could inhibit the CaM

activity of rabbit platelets[2]. Since CaM has no species, tissue or cell specificity, we suggested that Tet inhibited the production of LTB_4 and TXB_2 in rabbit whole blood by reducing the activity of CaM and then suppressing the Ca^{2+}-CaM dependent PLA_2 activity. These were similar to those in our previous report[3], although the IC_{50} values in whole blood were greater than those in isolated Neu. The characteristics of Tet on AA metabolism was the same in isolated Neu and whole blood.

Being a steroid anti-inflammatory agent, Dex induced the synthesis of lipocortin which was capable of inhibiting the activity of PLA_2[11]. The pattern that Tet showed was not the same as that of Dex which had a lag time. Thus, Tet most probably inhibited the activity of PLA_2 via suppressing the activity of CaM directly.

Tet iv also showed inhibitory effects on LTB_4 and TXB_2 production in rabbit whole blood. The effects weakened along with the metabolism and elimination of the drug *in vivo*. It was reported that $T\frac{1}{2}$ of Tet was 66.7 −112 min in dogs[11]. However Tet still presented significant inhibitory effects at 240 min in our experiments. This phenomenon may be attributable to the high Tet dosage.

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粉防己碱对兔血 LTB_4和 TXB_2产生的影响

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R965.2

摘要 用 HPLC 和 RIA 分别研究粉防己碱(Tet)对 calcimycin 刺激兔全血产生 LTB_4和 TXB_2的影响. 在体外 Tet 明显抑制兔全血 LTB_4和 TXB_2的产生，其效应呈剂量依赖性，IC_{50}值分别为17.8±8.6和17.7±9.2 $\mu mol \cdot L^{-1}$. 外源性 AA 使 Tet 作用明显减弱. Tet 的作用特征与 CaM 拮抗剂 Flu 相似. Tet (10 $mg \cdot kg^{-1}$) iv 也能抑制兔全血产生 LTB_4和 TXB_2，提示 Tet 可能通过拮抗钙调素而抑制细胞膜 AA 释放.

关键词 粉防己碱；白细胞三烯 B 类；血栓素 B_2；卡西霉素；高压液相色谱法；放射免疫测定法