8 Joseph J, Grun JL, Lublin FD, Knober RL. Interleukin-6 induction *in vitro* in mouse brain endothelial cells and astrocytes by exposure to mouse hepatitis virus (MHV-4, JHM). J Neuroimmunol 1993; **42**: 47—52.

9 Libermann TA, Baltimore D. Activation of interleukin-6 gene expression through the NF-κB transcription factor. Mol Cell Biol 1990; **10**: 2327—34.

10 Natsuka S, Akira S, Nishio Y, Hashimoto S, Sugita T, Isshiki H, *et al*. Macrophage differentiation-specific expression of NF-IL-6, a transcription factor for interleukin-6. Blood 1992; **79**: 460—6.

11 Le PT, Lazorick S, Whichard LP, Haynes BF, Singer KH. Regulation of cytokine production in the human thymus: epidermal growth factor and transforming growth factor α regulate mRNA levels of interleukin-1α (IL-1α), IL-1β and IL- 6 in human thymic epithelial cell at a post-transcriptional level. J Exp Med 1991; **174**: 1147—57.

甲硫氨酸脑啡肽增强白细胞介素-6的产生及其基因表达[1]

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目的: 研究甲硫氨酸脑啡肽对白细胞介素-6的产生及其基因表达的影响. **方法**: 用依赖株 MH60·BSF2和 MTT 法测定 IL-6、分离 RNA 和 IL-6 cDNA 杂交后测定其基因表达. **结果**: 甲啡肽体外诱导小鼠 IL-6 mRNA 的表达并提高其稳定性. 腹腔注射甲啡肽0.1和1 mg·kg^{-1} 也能明显提高 IL-6水平并促进脾细胞 IL-6 mRNA 的表达. **结论**: 甲啡肽能通过提高转录活力并增加其 mRNA 稳定性上调 IL-6.

关键词 甲硫氨酸脑啡肽; 白细胞介素-6; 基因表达; 信使 RNA; 培养的细胞

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Effects of tetrandrine on rabbit platelet aggregation and platelet activating factor generation

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AIM: To study the effects of tetrandrine (Tet) on platelet aggregation and platelet activating factor (PAF) generation in rabbit platelet-rich plasma (PRP). **METHODS**: The aggregation rate of platelets induced by calcimycin (Cal) and PAF and the inhibition rate of Tet on platelet aggregation were measured. The amount of PAF in PRP stimulated with Cal and treated with Tet was also measured. **RESULTS**: Cal 1—8 μmol·L^{-1} and PAF 9.5—190.5 pmol·L^{-1} induced platelet aggregation. At the final concentrations of 4—64 μmol·L^{-1}, Tet inhibited the aggregation induced by Cal 4 μmol·L^{-1} and PAF 142.9 pmol·L^{-1}. The IC_{50}(95 % confidence limits) were 8.6 (6.0—12.2) μmol·L^{-1} for Cal and 14.0 (6.4—30.4) μmol·L^{-1} for PAF. In the PRP aggregation by Cal, there was a marked increase in PAF content. Tet dependented the release of PAF from platelets by Cal in a concentration-dependent manner, with IC_{50} of 21 (8—54) μmol·L^{-1}. **CONCLUSION**: The inhibition effect of Tet on platelet aggregation might be concerned with the reduction of endogenous PAF generation.

KEY WORDS tetrandrine; platelet aggregation; platelet activating factor; calcimycin

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Tetrandrine (Tet), as an effective component of *Stephania tetrandra* S Moore[1], have been applied to the treatment of hypertension and angina pectoris caused by coronary heart disease. Recent researches showed that Tet inhibited platelet aggregation, reduced the activity of calmodulin and the generation of thromboxane A_2 (TXA_2). Calcium-calmodulin system was considered to be responsible for these results[2]. Platelet activating factor (PAF) can active inflammatory cells (such as platelets, leukocytes etc), increase vascular permeability and constrict smooth muscle. It was considered that PAF mediates the third pathway of aggregation[3]. Calcimycin (Cal, calcium ionophore A_{23187}) induces platelet aggregation[4]. This study is to explore the action of Tet on platelet aggregation induced by Cal.

MATERIALS AND METHODS

Tet was produced by Jin Hua Pharmaceuticals, Zhejiang. Calcimycin and platelet activating factor (PAF) were made by Sigma. All other reagents were of AR grade.

Platelet aggregation and PAF generation The anticoagulant blood were taken from the auricular artery of rabbits, and the platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared with centrifugation. The number of platelets in PRP was modified to about 4×10^8 cell · mL^{-1} with PPP. Tet 0.05 mL at different concentrations and normal saline (NS) were added to 0.45 mL PRP, respectively. After 5 min, Cal (final concentration 4 $\mu mol \cdot L^{-1}$) and PAF (final concentration 142.9 $pmol \cdot L^{-1}$) were given separately. The aggregation rate of platelets and the inhibition rate of Tet on platelet aggregation were recorded according to Born's method[5].

The PRP after aggregation by Cal was put into 1 mL pre-cooled acetone, shook 30 s, and kept at 4 ℃ for 10 min, then centrifuged $1200 \times g$ for 5 min at 4 ℃. The supernate was taken and put into 2 mL pre-cooled chloroform, shook 30 s again, centrifuged $1200 \times g$ for 5 min at 4 ℃. The organic layer was abstracted and dried under N_2 flow, stored at −20 ℃. The sample was redissolved in chloroform and spotted on silica gel plate for thin-layer chromatography (TLC). The spot area was recorded and calculated with a TLC scanner. The PAF content was calculated according to the standard curve of PAF[6]. Fig 1 showed the relation of the PAF content and its area under curve.

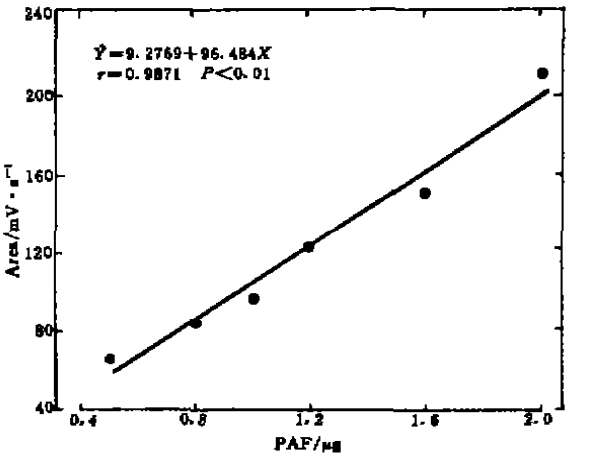

Fig 1. PAF standard and its area under curve with thin-layer chromatography.

RESULTS

Platelet aggregation induced by Cal and PAF Both Cal (1 − 8 $\mu mol \cdot L^{-1}$) and PAF (9.5 − 190.5 $pmol \cdot L^{-1}$) induced platelet aggregation. Their effects were concentration-dependent. There was a long latent time (0.125 − 8.6 min) for aggregation in Cal, while PAF induced an immediate aggregation with no significant difference in latent time in all concentration used. Tet inhibited the aggregation of platelets induced by Cal and PAF (Tab 1). At the final concentrations of 4 − 64 $\mu mol \cdot L^{-1}$, Tet inhibited the aggregation of platelets induced by Cal 4 $\mu mol \cdot L^{-1}$ and PAF 142.9 $pmol \cdot L^{-1}$. The IC_{50} (95 % confidence limits) were 8.6 (6.0 − 12.2) $\mu mol \cdot L^{-1}$ for Cal and 14.0 (6.4 − 30.4) $\mu mol \cdot L^{-1}$ for PAF.

PAF generation in platelets induced by Cal Cal induced platelet aggregation as well as PAF release in a concentration-dependent

Tab 1. Effects of tetrandrine on aggregation of rabbit platelets induced by Cal and PAF *in vitro*. $n=3$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* 4 μmol·L^{-1} Cal treated group; $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* PAF 142.9 pmol·L^{-1} treated group.

Cal/μmol·L^{-1}	PAF/pmol·L^{-1}	Tet/μmol·L^{-1}	Latent period/min	Aggregation rate/%	Inhibition rate/%
1	—	—	7.5±1.5	6.7±4.7	—
2	—	—	3.5±0.6	36.3±6.3	—
4	—	—	1.6±0.3	48.3±4.5	—
8	—	—	0.2±0.1	70.3±5.6	—
—	9.5	—	—	13.0±2.2	—
—	19.0	—	—	25.3±1.7	—
—	47.6	—	—	34.3±2.1	—
—	95.2	—	—	44.7±3.3	—
—	142.9	—	—	52.7±3.3	—
—	190.5	—	—	74.0±5.1	—
4	—	0	1.6±0.3	—	0
4	—	4	2.6±1.2^a	—	27.2±1.8^a
4	—	8	3.5±1.2^a	—	45.4±1.1^b
4	—	16	5.3±1.1^b	—	66.8±7.9^b
4	—	32	9.5±0.7^c	—	98.6±2.0^c
4	—	64	>10^c	—	100^c
—	142.9	0	—	—	0
—	142.9	4	—	—	15.9±11.3^d
—	142.9	8	—	—	24.6±11.6^e
—	142.9	16	—	—	53.0±12.1^f
—	142.9	32	—	—	77.4±7.7^f
—	142.9	64	—	—	95.1±4.2^f

manner. There was a markedly positive correlation between the rate of platelet aggregation induced by Cal and PAF release from aggregated platelets ($r=0.9507$, $P<0.01$). Tet concentration-dependently inhibited PAF generation (Tab 2). The IC_{50} (95 % confidence limit) was 21 (8—54) μmol·L^{-1}.

DISCUSSION

In this study, we found that there was a different mechanism in the platelet aggregation by Cal and PAF. It showed different latent periods in platelet aggregation induced by Cal and PAF, and a large amount of PAF release in PRP stimulated with Cal. We know

Tab 2. The release of PAF stimulated by Cal and the effect of Tet on platelets *in vitro*. $n=3$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* Cal 4 μmol·L^{-1} treated group.

Cal/μmol·L^{-1}	Tet/μmol·L^{-1}	PAF/μg
1	—	0.25±0.04
2	—	0.81±0.08
4	—	1.94±0.24
5	—	1.82±0.21
4	4	1.75±0.11^a
4	8	1.57±0.17^a
4	16	1.29±0.04^b
4	32	0.76±0.04^c
4	64	0.27±0.12^c

that phospholipase A_2 is a key enzyme on the synthesis of PAF. Cal could increase the cytoplasmic calcium ions of platelets through accelerating the inward flow of extracellular calcium ion and induce platelet aggregation[7], the action of cytoplasmic calcium ion elevation maybe make phospholipase A_2 activated and result in the synthesis and release of endogenous PAF. Our results implied that the aggregation of platelets by Cal was partly correlated with the release of endogenous PAF, which may be the main reason of the prolongation of latent period and the absence of disintegration within 10 min.

Tet could suppress platelet aggregation induced by adenosine diphosphate (ADP), arachidonic acid and collagen[2] *in vitro*. In this research, we also found that Tet could markedly inhibit the aggregation of platelets caused by Cal and PAF as well as the release of PAF with Cal *in vitro*. These evidences indicated that the inhibitory action of Tet on platelet aggregation included the prohibition of calcium ion inward current and PAF release, so the third pathway of platelet aggregation was ended in a direct or indirect manner[8]. All these also showed that Tet could inhibit the 3 pathways of platelet aggregation.

As a member in the broad spectrum of antiplatelet aggregation agent, Tet would play an important role in the prevention and cure of thrombotic angiocardiopathy.

It should be mentioned that, the content of PAF in unstimulated PRP (without Cal) could not be detected according to our method. We could not compare the significant difference of PAF release between Cal stimulated and unstimulated PRP. Therefore, it is necessary to develop more sensitive methods to detect PAF content in nmol·L^{-1} and pmol·L^{-1} levels.

REFERENCES

1 Zhang HL. The pharmacology and clinic of tetrandrine. Chin J Hosp Pharm 1988; **8**: 25—6.

2 Qian YM, Huang YH. Effects of tetrandrine on rabbit platelet aggregation, thromboxane A_2 generation and calmoduline activity. Acta Pharmacol Sin 1989; **10**: 61—5.

3 Chignard M, Le Couedic JP, Tence M, Vargaftig BB, Benveniste J. The role of platelet-activating factor in platelet aggregation. Nature 1979; **275**: 799—800.

4 Chignard M, Le Couedic JP, Vargaftig BB, Benveniste J. Platelet- activating factor (PAF-acether) secretion from platelets: effect of aggregating agents. Br J Haematol 1980; **46**: 455—64.

5 Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962; **194**: 927—9.

6 Stenton SC, Court EN, Kingston WP, Goodby P, Kelly CA, Duddridge M, *et al*. Platelet-activating factor in bronchoalveolar lavage fluid from asthmatic subjects. Eur Respir J 1990; **3**: 408—13.

7 Colard O, Breton M, Bereziat G. Arachidonate mobilization in diacyl, alkylacyl and alkenylacyl phospholipids on stimulation of rat platelets by thrombin and Ca^{2+} ionophore A_{23187}. Biochem J 1986; **233**: 691—5.

8 Kinlough-Rathbone RL, Packham MA, Reimers HJ, Cazenave JP, Mustard JF. Mechanisms of platelet shape change, aggregation and release induced by collagen, thrombin, or A23, 187. J Lab Clin Med 1977; **90**: 707—19.

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粉防己碱对兔血小板聚集和血小板活化因子生成的影响

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目的: 探讨粉防己碱(Tet)对兔血小板聚集和PAF生成的影响. **方法**: 卡西霉素(Cal)和PAF诱导血小板聚集的聚集率和Tet对血小板聚集的抑制率被测定; 给予或未给予Tet处理之血小板用Cal刺激释放PAF的量也被测定. **结果**: 在4—64 μmol·L^{-1}浓度范围, Tet明显抑制Cal和PAF诱导的血小板聚集. IC_{50}值分别为8.6μmol·L^{-1}和14.0 μmol·L^{-1}. Tet也浓度依赖性的抑制Cal诱导血小板释放PAF, IC_{50}值为21.0 μmol·L^{-1}. **结论**: Tet抑制血小板聚集作用与抑制内源性PAF生成有关.

关键词 粉防己碱; 血小板聚集; 血小板活化因子; 卡西霉素