

and protein phosphorylation during platelet shape change. *Blood* 1985; **65**: 1141 - 8.

2 Halushka PV, Mais DE, Mayeux PR, Mornelli TA. Thromboxane, prostaglandin and leukotriene receptor. *Annu Rev Pharmacol Toxicol* 1989; **29**: 213 - 39.

3 Narumiya S, Okuma M, Ushikubi F. Binding of a radioiodinated 13-azapinane thromboxane antagonist to platelets: correlation with antiaggregatory activity in different species. *Br J Pharmacol* 1986; **88**: 323 - 31.

4 Takahara K, Murray R, Fitzgerald GA, Fitzgerald DJ. The response to thromboxane A₂ analogues in human platelets. *J Biol Chem* 1990; **265**: 6836 - 44.

5 Ushikubi F, Nakajima M, Hirata M, Okuma M, Fujiwara M, Narumiya S. Purification of the thromboxane A₂/prostaglandin H₂ receptor from human blood platelets. *J Biol Chem* 1989; **264**: 16496 - 501.

6 Li BY, Bai Y, Li GZ, Li WH, Katori M. Effects of MK-447 on thrombin-induced aggregation, secretion of ATP, and [Ca²⁺]_i mobilization in rabbit platelets. *Acta Pharmacol Sin* 1995; **16**: 108 - 13.

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

8 Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *J Biol Chem* 1985; **260**: 3440 - 50.

9 Furci L, Fitzgerald DJ, Fitzgerald GA. Heterogeneity of prostaglandin H₂/thromboxane A₂ receptors: Distinct subtypes mediate vascular smooth muscle contraction and platelet aggregation. *J Pharmacol Exp Ther* 1991; **258**: 74 - 81.

10 Hirata M, Hayashi Y, Ushikubi F, Yokota Y, Kageyama R,

Nakanishi S, *et al.* Cloning and expression of cDNA for a human thromboxane A₂ receptor. *Nature* 1991; **349**: 617 - 20.

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ONO-3708 和 S-145 对 STA₂ 介导的家兔血小板变形和聚集反应的抑制作用

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关键词 血小板; 血小板聚集; 血小板聚集抑制剂; 血栓素 A₂; 钙; STA₂; ONO-3708; S-145

目的: 评价 ONO-3708 和 S-145 对血小板变形和聚集反应的不同抑制模式。方法: 以透光度法测量血小板变形和聚集反应, 荧光图像分析法测量单细胞内游离钙的变化。结果: (1) STA₂ 的聚集反应可被依他酸, ONO-3708 和 S-145 抑制 (P < 0.01), 血小板变形仅被 S-145 抑制。 (2) S-145 的抑制作用随孵育时间延长而增强, ONO-3708 不变。 (3) 洗脱后 ONO-3708 的作用消失, 而 S-145 抑制作用依然存在。 (4) STA₂ 的细胞内游离钙动员部分被 ONO-3708 和依他酸取消 (P < 0.01), 但可被 S-145 完全抑制。结论: S-145 和 ONO-3708 分别作用于血小板 TXA₂ 受体的不同结合位点。

R 965.1 R 963

Protective effects of tetrandrine on CCl₄-injured hepatocytes

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KEY WORDS tetrandrine; liver; cultured cells; carbon tetrachloride poisoning; malondialdehyde; calcium; membrane fluidity; lactate dehydrogenase

AIM: To study the protective effects of tetrandrine (Tet) on CCl₄-injured hepatocytes. METHODS: The cultured rat liver cells were poisoned by CCl₄ (10 mmol · L⁻¹). The membrane fluidity was detected by 1,6-diphenyl-1,3,5-hexatriene (DPH), a

lipid probe. The Ca²⁺ concentration was assayed with Fura 2-AM, a sensitive calcium indicator. RESULTS: Tet (1 - 1000 nmol · L⁻¹) increased viability of liver cell (from 71 % to 72 % - 89 %), reduced lactate dehydrogenase (LDH) release, and malondialdehyde (MDA) formation. Tet prevented the heightening of the intracellular Ca²⁺ concentration and the attenuation of the membrane fluidity of liver cells (P < 0.05). CONCLUSION: Tet had a protective effect on CCl₄-injured hepatocytes by inhibiting the lipid peroxidation,

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improving the membrane fluidity, and lessening the Ca^{2+} concentration.

Tetrandrine (Tet) is a bisbenzylisoquinoline alkaloid from *Stephania tetrandra*. The anti-arrhythmic effects of Tet were reported⁽¹⁾. Its electrophysiologic mechanism was found to be related to Ca^{2+} channel blockade. Many Ca^{2+} channel blockers have a protective effect on hepatocytes⁽²⁾. The present paper is to examine the protective action of Tet against hepatotoxicity induced by CCl_4 .

MATERIALS AND METHODS

Wistar rats of either sex weighing $190 \pm s 20$ g (Animal Center of The Third Military Medical College) were used. Tet, (Jinhua Pharmaceutical Co, China) was dissolved in 0.9 % NaCl after acidification with HCl (pH 3) and then neutralized with 5 % NaHCO_3 to obtain a $10 \text{ mmol} \cdot \text{L}^{-1}$ stock solution. CCl_4 $10 \text{ mmol} \cdot \text{L}^{-1}$ was dissolved in 0.4 % Me_2SO . Collagenase (Type I), and 1, 6-diphenyl-1, 3, 5-hexatriene (DPH) were from Sigma. DPH was prepared with tetrahydrofuran to the concentration of $2 \text{ mmol} \cdot \text{L}^{-1}$ and stored at 4°C , and diluted to $2 \mu\text{mol} \cdot \text{L}^{-1}$ with phosphate-buffered saline (PBS) $10 \text{ mmol} \cdot \text{L}^{-1}$ before use. Fura 2 acetoxymethyl ester $1 \text{ mmol} \cdot \text{L}^{-1}$ (Fura 2-AM, 1 mg/ampul) (from Shanghai Institute of Physiology, Chinese Academy of Sciences) was dissolved in Me_2SO and stored at -20°C , and diluted to $2.5 \mu\text{mol} \cdot \text{L}^{-1}$ with Krebs-Henseleit solution. All other reagents were of AR.

Culture of hepatocytes Hepatocytes were isolated from rats by the 2-step collagenase perfusion method⁽³⁾. The cells were diluted with MEM medium supplemented with 10 % fetal calf serum, penicillin $50 \text{ kU} \cdot \text{L}^{-1}$, streptomycin $50 \text{ mg} \cdot \text{L}^{-1}$, insulin $24 \text{ U} \cdot \text{L}^{-1}$, 0.2 % bovine serum albumin. After the cells were cultured with MEM medium in 35 mm diameter plates in 5 % CO_2 incubator at 37°C for 4 h, the medium was changed. Tet ($1 - 1000 \text{ nmol} \cdot \text{L}^{-1}$) was added to the culture medium for 1 h prior to CCl_4 ($10 \text{ mmol} \cdot \text{L}^{-1}$).

Hepatocyte viability and release of lactate dehydrogenase (LDH) In each plate, 4×10^5 cells were added. After 4-h incubation in the presence of CCl_4 , liver cell viability was ascertained by trypan blue exclusion method. The activity of LDH in medium was assayed with lactic acid reaction and determined by a spectrophotometer⁽⁴⁾.

Malondialdehyde (MDA) Cells (2×10^6 /plate) were incubated with CCl_4 for 1 h. The amount of MDA product in the cultured cells was determined⁽⁵⁾.

Membrane fluidity Membrane fluidity was determined with the lipid probe DPH. 1×10^7 cells were suspended in 2

mL phosphate buffer ($10 \text{ mmol} \cdot \text{L}^{-1}$, pH 7.4). CCl_4 was added. The fluorescence intensity was measured by a Hitachi MPF-4 spectrofluorometer ($\lambda_{\text{ex}} 362 \text{ nm}$, $\lambda_{\text{em}} 432 \text{ nm}$). The fluorescence polarization (ρ) and viscosity (η) of hepatocytes were calculated⁽⁶⁾. Larger was the η or ρ , smaller was the lipid membrane fluidity.

Ca^{2+} concentration Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) was determined by Ca^{2+} indicator Fura 2⁽⁷⁾. Cells (2×10^6) were incubated in 2 mL Krebs-Henseleit solution (supplemented with 0.12 % BSA). The degree of fluorescence was assayed using a Hitachi model MPF-4 spectrofluorometer ($\lambda_{\text{ex}} 350 \text{ nm}$, $\lambda_{\text{em}} 500 \text{ nm}$) at 37°C . The slit width was 10 nm.

Statistical methods All data were analyzed with *t* test.

RESULTS

Liver cell viability, LDH release, and MDA formation CCl_4 induced a weakening of cell viability, an increase of LDH release, and an increase of MDA formation. These changes were prevented by Tet (Tab 1), indicating that Tet protected the hepatocytes against CCl_4 toxicity *in vitro* and inhibited the lipid peroxidation of liver cells.

Membrane fluidity and Ca^{2+} concentration of hepatocytes When the liver cells were incubated with CCl_4 at 25°C , the fluorescence polarization and viscosity of cells were increased, indicating that the fluidity of the plasma membrane was attenuated. Tet alleviated the reduction of membrane fluidity.

CCl_4 ($10 \text{ mmol} \cdot \text{L}^{-1}$) caused a heightening of $[\text{Ca}^{2+}]_i$. Tet inhibited this elevation in a dose-dependent manner (Tab 1).

DISCUSSION

Our *in vitro* study demonstrated that Tet had a protective effect on CCl_4 -injured hepatocytes *in rats*. CCl_4 generates methyltrichloride radicals (CCl_3) by the activation of liver cytochrome P-450⁽⁸⁾, initiating lipid peroxidation of biomembranes. MDA, a product of lipid peroxidation, induces a damage of membrane and an attenuation of membrane fluidity. The injured membrane can not maintain a normal Ca^{2+} homeostasis and induces a $[\text{Ca}^{2+}]_i$ elevation. Excessive Ca^{2+} can inappropriately stimulate or inhibit the Ca^{2+} -sensitive metabolic processes and

Tab 1. Effects of tetrandrine on viability, LDH release, MDA production, fluorescence polarization (ρ) viscosity (η) and Ca^{2+} concentration of hepatocytes damaged by CCl_4 $10\text{ mmol}\cdot\text{L}^{-1}$ *in vitro*. $\bar{x} \pm s$. ^b $P < 0.05$, ^c $P < 0.01$ vs CCl_4 group.

CCl_4 , $\text{mmol}\cdot\text{L}^{-1}$	Tet, $\text{nmol}\cdot\text{L}^{-1}$	Via, % (n = 6)	LDH, $\text{U}\cdot\text{L}^{-1}$ (n = 6)	MDA, $\text{nmol}/10^6$ cells (n = 4)	ρ (n = 4)	η (Pa·s) (n = 4)	$[Ca^{2+}]_i$, $\text{nmol}\cdot\text{L}^{-1}$ (n = 4)
0	0	90.6 ± 3.2^c	$3\ 765 \pm 319^c$	0.98 ± 0.11^c	0.186 ± 0.006^c	0.137 ± 0.007^c	248 ± 26^c
10	0	70.7 ± 4.1	$7\ 390 \pm 192$	4.29 ± 1.12	0.233 ± 0.003	0.206 ± 0.006	888 ± 125
10	1	71.9 ± 3.5	$6\ 070 \pm 47^a$	4.04 ± 0.96	0.229 ± 0.007	0.198 ± 0.012	670 ± 132
10	10	76.0 ± 2.3^c	$5\ 453 \pm 192^c$	2.65 ± 0.33^b	0.213 ± 0.007^c	0.172 ± 0.010^c	578 ± 105^c
10	100	78.0 ± 1.0^c	$4\ 827 \pm 305^c$	2.18 ± 0.47^b	0.203 ± 0.005^c	0.158 ± 0.009^c	512 ± 74^c
10	1 000	83.9 ± 2.6^c	$4\ 123 \pm 186^c$	1.42 ± 0.06^c	0.187 ± 0.006^c	0.152 ± 0.009^c	366 ± 152^c

thus initiate or contribute to the lethal cell injury.

Our study indicated that Tet not only produced a marked inhibitory effect on MDA formation induced by CCl_4 , maintained the normal membrane fluidity, but also resisted the increase of Ca^{2+} of hepatocytes. So the protective effect of Tet may be related to the 3 mechanisms.

REFERENCES

- Zeng WZ, Liu TP. Effects of tetrandrine on action potentials and afterhyperpolarization potentials in toad dorsal root ganglia. *Acta Pharmacol Sin* 1992; 13: 420-3.
- Landon EJ, Jaiswal RK, Naukam RJ, Sastry BVR. Effects of calcium channel blocking agents on membrane microviscosity and calcium in the liver of the carbon tetrachloride treated rat. *Biochem Pharmacol* 1984; 33: 3553-60.
- Seglen PO. Preparation of isolated rat liver cells. In: Prescott DM, editor. *Methods Cell Biol*. New York, San Francisco, London: Academic Press 1976; 13: 29-83.
- Xu SK. Lactate dehydrogenase determination. In: Shanghai Medical Laboratory, editor. *Laboratory methods in clinical biochemistry*. Shanghai: Shanghai Science and Technology Press, 1979: 314-21.
- Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem Med* 1976; 15: 212-6.
- Lin KC, Nie SC, Bo HC, Chan YL. Research on membrane

fluidity of ascites cells with fluorescence probe DPH.

Biochem Biophys 1981; 42: 32-5

- Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* 1985; 260: 3440-50.
- Lin L, Xing ST, Zhou JH. Protective effects of gypenosides on rat hepatic lipid peroxidation and membrane fluidity damage: *in vitro* studies. *Chin Pharmacol Bull* 1991; 7: 341-4.

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粉防己碱对四氯化碳损伤的肝细胞的保护作用

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关键词 粉防己碱; 肝; 培养的细胞; 四氯化碳中毒; 丙二醛; 钙; 膜流动性; 乳酸脱氢酶

A目的: 研究粉防己碱(Tet)对四氯化碳损伤的肝细胞的保护作用。 **方法:** 培养细胞中直接加入 CCl_4 $10\text{ mmol}\cdot\text{L}^{-1}$ 造成损伤模型。膜流动性及胞内钙离子浓度分别用DPH, Fura 2-AM进行测定。 **结果:** Tet ($1-1000\text{ nmol}\cdot\text{L}^{-1}$)可以明显抑制 CCl_4 引起的肝细胞存活率降低和LDH的释放。 **结论:** Tet具有保护肝细胞的作用。Tet的这种作用与其抑制肝细胞的脂质过氧化, 稳定钙离子浓度, 维持细胞膜流动性有关。

R 965.1 R 975.5