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茶碱经微处理机程序输注迅速达到期望兔血浆稳态水平

R965.2

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摘要 自制二室模型药物程序输液泵．向控制器输入兔茶碱药物动力学参数，期望血浆稳态浓度(C_{pss})，体重及时间．输注速率(K_r)＝$C_{pss}K_{10}V_c$ wt$\{1+[(K_{21}-\beta)/\beta]EXP(-K_{21}t)\}$，血药浓度预报式为$C_{(t)}=C_{pss}(1-e^{-\alpha t})$，依据显示配药液，自动输注．比色法测定血药浓度．96 %的执行百分误小于±30 %，其绝对值的中位数为8.3 %．虽然$T_{\frac{1}{2}\beta}$＝6.08 h，但输注后30 min ($5T_{\frac{1}{2}\alpha}$)，血药浓度达期望C_{pss}．

关键词 茶碱；药物动力学；计算机辅助药物治疗；用药计划表

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Protective effect of tetramethylpyrazine against damages of aortic endothelial cells elicited by low-density lipoproteins[1]

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ABSTRACT Effects of tetramethylpyrazine (TMP) on endothelial cells damaged by low-density lipoproteins (LDL) were investigated. When endothelial cells were incubated with LDL (1.5 mg protein·ml^{-1}) the level of malondialdehyde (MDA) was increased and the activity of superoxide dismutase (SOD) was inhibited, and levels of cGMP and epoprostenol were decreased. TMP at concentrations of both 20 and 150 mg·L^{-1} nullified the inhibition of SOD activity and the reduction of cGMP and epoprostenol content elicited by LDL. However, the elevation of MDA content induced by LDL was negated by TMP only at 150 mg·L^{-1}. TMP also caused a reduction in MDA content and an increase of epoprostenol level in normal endothelial cells. This study suggests that TMP protects endothelial cells against damages elicited by LDL

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and that the protection of TMP might be due to reduction in lipid peroxidation through stimulation of production and / or release of epoprostenol.

KEY WORDS vascular endothelium; LDL lipoproteins; pyrazines; cyclic GMP; epoprostenol; malondialdehyde; superoxide dismutase

Low-density lipoproteins (LDL) damage endothelial cells and stimulate proliferation of vascular smooth muscle, suggesting that LDL may play an important role in the development of atherosclerosis (AS). Injury of endothelial cells is an initial event in the development of AS. Tetramethylpyrazine (TMP), a major component of *Ligusticum wallichii* Franch alleviates histomorphological changes of AS in the high fat-fed rabbit[1], inhibits proliferation of vascular smooth muscle cells[2], and stimulates epoprostenol production[3]. In the present study, the protective effect of TMP against the damage of endothelial cells due to LDL was evaluated.

MATERIALS AND METHODS

Cell culture[4] Bovine aortic endothelial cells were isolated and cultured in Minimum Essential Medium Eagle (MEM) with 20 % calf serum and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) 25 mmol·L^{-1}. Experiments were performed on 5 — 8 passage endothelial cells. The endothelial cell was identified by electron microscopy.

Preparation of LDL[5] Plasma was obtained from fresh normal human blood, LDL was isolated by sequential ultracentrifugation at 119 000 × *g* in the presence of edetic acid 0.2 mmol·L^{-1}. Then LDL was filtered aseptically (0.2 μm) into dialysis tubing and dialyzed in phosphate-buffer solution (PBS) at 4 ℃ for 24 h. Dialyzed LDL was concentrated and stored at 4 ℃ in dark.

Experimental design Three days before the experiment, endothelial cells were cultured with serum-free medium. Experiments were performed in 6 groups: control, TMP_1(20 mg·L^{-1}), TMP_2(150 mg·L^{-1}), LDL (1.5 mg protein·ml^{-1}), LDL + TMP_1, and LDL + TMP_2. TMP and LDL were given at the same time. After incubation for 19 h, the medium was collected and stored at −20 ℃ for assaying epoprostenol (6-keto-prostaglandin $F_{1\alpha}$, 6-keto-$PGF_{1\alpha}$), a stable hydrolytic product of epoprostenol. After addition of PBS (1 ml) the cells were scraped off with a rubber policeman, sonicated for 20 s, then stored at −70 ℃ until assay for SOD, MDA, and cGMP.

Statistics ANOVA and Tukey's test were used.

RESULTS

Morphology of endothelial cells After incubation with LDL for 19 h, the endothelial cells exhibited a contraction and an irregular arrangement. After pretreatment with TMP, they maintained their normal cobblestone pattern in the presence of LDL.

SOD TMP increased slightly the SOD activity of endothelial cells, but there was no significant difference in SOD activity between normal endothelial cells and endothelial cells treated with TMP ($P > 0.05$). However, TMP 20 and 150 mg·L^{-1} negated the reduction of SOD activity elicited by LDL (Tab 1).

MDA TMP caused a reduction of MDA content in normal endothelial cells only at 150 mg·L^{-1}. Incubation of endothelial cells with LDL increased the content of MDA, an effect which was nullified in the presence of TMP 150 mg·L^{-1} (Tab 1).

cGMP TMP had no effect on cGMP content in normal endothelial cells, but reversed the reduction of cGMP content elicited by LDL (Tab 1).

Epoprostenol TMP 20 and 150 mg·L^{-1} not only increased the content of epoprostenol in normal endothelial cells, but also reversed the reduction of epoprostenol in endothelial cells treated with LDL. The effect of TMP

Tab 1. Effect of TMP (20 and 150 mg·L^{-1}) on MDA, cGMP, 6-keto-PGF$_{1\alpha}$ content and SOD activity of aortic endothelial cells in presence of LDL (1.5 mg protein·ml^{-1}). $n=5-7$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control, $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* LDL.

TMP μg/ml	LDL 1.5 mg protein/ml	MDA nmol/mg protein	SOD μg/mg protein	6-Keto-PGF$_{1\alpha}$ ng/mg protein	cGMP pmol/mg protein
0	0	0.12±0.01	0.19±0.02	0.94±0.46	32.8±10.5
20	0	0.09±0.05^e	0.21±0.04^a	1.52±0.14^b	29.7±2.3^a
150	0	0.08±0.01^b	0.23±0.06^a	2.55±0.48^c	40.5±5.5^a
0	1.5	0.16±0.04^b	0.15±0.01^b	0.36±0.05^b	4.9±1.0^c
20	1.5	0.17±0.06^d	0.23±0.04^f	1.35±0.10^f	22.1±4.6^f
150	1.5	0.10±0.04^e	0.25±0.04^f	2.26±0.68^f	20.2±10.7^f

at 150 mg·L^{-1} was more pronounced both in untreated endothelial cells and endothelial cells in the presence of LDL (Tab 1).

DISCUSSION

In the present study, we demonstrate for the first time that TMP protects endothelial cells from histomorphological and functional changes, such as epoprostenol and cGMP contents. These results suggest that TMP possesses a protective effect of the endothelial cells against LDL-induced damages.

LDL is modified to oxidized low-density lipoproteins (ox-LDL) by oxidation or autoxidation, and ox-LDL can damage the endothelial cells[6]. In the present study, incubation with LDL caused histomorphological and functional damages in the cultured endothelial cells while at the same time increased the level of MDA and decreased the activity of SOD, in further support of the conclusion that damages of endothelial cells elicited by LDL is due to cytotoxicity of ox-LDL.

In order to explore whether protection of TMP against damages of endothelial cells due to LDL is secondary to its anti-lipid peroxidation, we measured the content of MDA reflecting the level of lipid peroxidation and the activity of SOD which scavenges O_2^-. In the present study, TMP significantly inhibited the increase of MDA content and the reduction of SOD activity elicited by LDL. The effects of TMP have also been observed in myocardial reperfusion injuries[7]. Therefore, our results together with findings from others suggest that the protective effect of TMP on endothelial cells is likely to be associated with anti-lipid peroxidation.

Epoprostenol, besides relaxing vascular smooth muscle tone, is also capable of protecting the cells. Epoprostenol as well as angiotensin converting enzyme inhibitors or calcium channel blocking agents which stimulate the production and/or release of epoprostenol protect the cells via anti-oxygen free radical and anti-lipid peroxidation[8,9,10]. Recently, it has been reported that in normal endothelial cell cultures TMP can stimulate epoprostenol production. To investigate whether epoprostenol is involved in the ability of TMP to protect the endothelial cells against damages induced by LDL, we examined the effect of TMP on the level of epoprostenol. In this study TMP not only increased the content of epoprostenol in normal endothelial cells, but also abrogated the reduction of epoprostenol in the cultured endothelial cells incubated with LDL. These results support the proposal that the protective effects of TMP may be related

to promoting synthesis and / or release of epoprostenol.

In summary, the present results suggest that TMP presents a protective effect of the endothelial cells against damage due to LDL. The mechanism of TMP action may be due to anti-lipid peroxidation via facilitating synthesis and/or release of epoprostenol.

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四甲基吡嗪对低密度脂蛋白损伤内皮细胞的保护作用

R365.2

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A 摘要 观察了四甲基吡嗪(TMP)对 LDL 损伤内皮细胞(EC)的保护作用. LDL (1.5 mg protein·ml^{-1})显著增高 EC 的 MDA 含量，抑制 SOD 活性，降低 cGMP 和 epoprostenol 含量. LDL 的毒性作用可被 TMP (20 mg 和150 mg·L^{-1}所消除. 结果提示，TMP 可通过促进 epoprostenol 的生成和/或释放保护 EC.

关键词 血管内皮；低密度脂蛋白；吡嗪类；环鸟苷一磷酸；依前列醇；丙二醛；超氧化物歧化酶

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