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Dauricine and anisodamine inhibited leukotrienes- and platelet activating factor-induced DNA synthesis and proliferation of bovine cerebral microvascular smooth muscle cells in culture

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ABSTRACT The effects of leukotrienes (LT) and platelet activating factor (PAF) on DNA synthesis and proliferation of bovine cerebral microvascular smooth muscle cells (BCMSMC) were studied. At 100 pmol·L^{-1}, LTB_4, LTC_4, LTD_4, and PAF promoted the DNA synthesis by 44%, 50%, 48%, and 57%, and enhanced the cell proliferation by 33%, 47%, 27%, and 40%, respectively. Dauricine and anisodamine inhibited the DNA synthesis of the cells induced by LT and PAF (0.1 — 100 μmol·L^{-1}). These results indicate the bright future of the 2 drugs in the prevention and treatment of cerebral vascular diseases.

KEY WORDS isoquinolines; atropine derivatives; DNA; vascular smooth muscle; cerebral arteries; leukotrienes; platelet activating factor

Leukotrienes (LT) is generated from arachidonic acid (AA) through the action of 5-lipooxygenase, and elicits a number of biological effects. In particular, LTC_4 promoted proliferation of human glomerular epithelial cells[1]. LT stimulate DNA synthesis in human epidermal keratinocytes[2] and stimulate the initiation of DNA synthesis in rat aortic smooth muscle cells[3]. LTC_4 induces the proliferation of bovine aortic endothelial cells[4]. Platelet activating factor (PAF) is another potent phospholipid mediator involved in many disorders. The present investigation deals with the effects of LT and PAF on the DNA synthesis and proliferation of bovine cerebral microvascular smooth muscle cells (BCMSMC) as well as the inhibitory effects of dauricine (Dau) and anisodamine (Ani) on LT and PAF-induced DNA synthesis were studied.

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MATERIALS AND METHODS

Reagents Minimum Eagle Medium (MEM) was purchased from Gibco Laboratories, and trypsin was purchased from Sigma, USA. [^{3}H] TdR (814 TBq·mol^{-1}) was purchased from Shanghai Institute of Nuclear Research, Chinese Academy of Sciences. LT was kindly provided by Dr J ROKACH (Merck Frosst Co, Canada). PAF was a gift from Dr P HADVARY (F Hoffmann-La Roche Co, Switzerlsnd). Dau was supplied by Department of Medicinal Chemistry, China Pharmaceutical University. Ani was from School of Pharmacy, Second Military Medical University. Fetal calf serum (FCS) was purchased from Department of Pathophysiology, Second Military Medical University.

Cultivation of BCMSMC BCMSMC were derived from bovine cerebral microvasculature according to the filtration method[5]. In brief, the gray matter of the cattle were homogenized in MEM. The homogenate was filtered through nylon mesh with pore size of 149 μm. The microvessels on the mesh were collected and washed by centrifugation (800×g, 8 min) with 10 ml MEM. The pellet was digested with 0.1% collagenase (II) for 30 min at 37℃. At the end of incubation, the suspension was recentrifuged and the pellet was suspended in MEM with 20% FCS. The incubation was carried out at 37℃ with 5% CO_2. The cells were subcultured after 1 wk when the flask was full of cells.

Determination of number of BCMSMC The proliferation of BCMSMC was determined by a modified method reported previously[4]. Cells were seeded in 24-well culture plate (3×10^5 cells/well) and incubated for 3 d. Then the drugs were added into the wells. After 24 h of incubation, the cells were stained with 0.5% crystal violet for 20 min and then washed with distilled water to remove the unstained color. Extraction

solution (2 ml) containing trinatrium citrate (0.9%) and ethanol (47.5%) was added to each well. The absorbance of the crystal violet fixed in the cells was determined on a 752-C spectrophotometer at 595 nm.

Measurement of DNA synthesis When the cells grew to confluence, they were digested with trypsin and suspended in fresh MEM with 10% FCS, and seeded in a 96-well plate (1×10^4 cells/well). After 3 d of incubation, the medium was discarded and MEM with 0.4% FCS was added into each well for 3 d of incubation. Then, [^{3}H]TdR 18 500 Bq and the compound to be tested, were added into each well. At the end of another 24 h of incubation, the incorporation of [^{3}H]TdR was stopped by washing with D-Hanks solution. The cells were harvested through filtration on Model 49 glass fiber filters. The filters were air dried and placed into scintillation vials with 0.2 ml scintillation liquid. The radioactivity of [^{3}H]TdR was determined on a FJ-2107 scintillation counter.

Statistical analysis was performed by using t test.

RESULTS

Stimulation of DNA synthesis and proliferation of BCMSMC by LT and PAF After 3 d of incubation in MEM with 0.4% FCS, the cells were arrested and LT or PAF was added for another 24 h of incubation in MEM with 0.4% FCS. Both LT and PAF resulted in an increase of the number of the cells and a stimulation of [^{3}H]TdR incorporation (Tab 1). The maximal inducing effects were seen at 100 pmol·L^{-1}.

Tab 1. Effects of leukotrienes and platelet-activating factor (PAF) on DNA synthesis and proliferation of bovine cerebral microvascular smooth muscle cells incubated for 24 h. $n=5$, $\bar{x}\pm s$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

	pmol·L^{-1}	Absorbance	[^{3}H]TdR incorporation/dpm
Control		0.30±0.03	1 870±250
LTB_4	0.1	0.33±0.02*	2 440±260***
	100.0	0.40±0.02***	2 700±396***
	10 000.0	0.41±0.03***	2 850±32***
LTC_4	0.1	0.37±0.03***	2 670±252***
	100.0	0.44±0.02***	2 800±414***
	10 000.0	0.46±0.03***	2 800±332***
LTD_4	0.1	0.37±0.02**	2 700±368***
	100.0	0.38±0.01**	2 760±164***
	10 000.0	0.44±0.02***	2 900±532***
PAF	0.1	0.38±0.03***	2 780±212***
	100.0	0.42±0.03***	2 940±126***
	10 000.0	0.33±0.03*	2 000±128*
10% FCS		0.53±0.06***	4 600±440***

Effects of Dau and Ani on LT and PAF induced DNA synthesis of BCMSMC Both Dau and Ani (0.1—100 μmol·L^{-1}) exihibited inhibitory effects on LTB_4, LTC_4, LTD_4, and PAF (100 pmol·L^{-1})-induced DNA synthesis. The maximal effects were at 10 μmol·L^{-1} for Dau and 100 μmol·L^{-1} for Ani (Tab 2).

Tab 2. Inhibitory effects of daurieine (Dau) and anisodamine (Ani) on leukotrienes and platelet-activating factor (PAF) (0.1 nmol·L^{-1}) induced DNA synthesis of bovine cerebral microvascular smooth muscle cells. 10^4 cells/well were incubated with drugs for 24 h. $n=6$, $\bar{x}\pm s$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

	μmol·L^{-1}	[^{3}H]TdR incorporation/dpm LTB_4	LTC_4	LTD_4	PAF
Control		4 900±1 672	5 400±1 042	5 600±214	6 500±1 222
Dau	0.1	3 200±562**	4 800±902*	5 230±214***	5 500±1 204*
	1.0	2 800±330**	3 900±882**	4 200±544***	4 900±808**
	10.0	1 900±300***	3 400±402***	3 600±326***	3 300±410***
Ani	1.0	4 100±1 024*	5 200±1 016*	5 800±508*	6 500±738*
	10.0	3 300±588***	4 400±352**	4 400±796**	5 300±566**
	100.0	3 000±516***	3 400±957***	3 700±982***	3 900±870***

DISCUSSION

In the present study, we found LT and PAF promoted DNA synthesis and proliferation of BCMSMC. This indicated that LT and PAF might take part in the pathogenesis of cerebral vascular disorders. Our previous investigation[6] found that LTC_4 receptors were present in the above-specified smooth muscle cells. Thus the effects of LT and PAF on the DNA synthesis and proliferation of the smooth muscle cells might be specific receptor-mediated.

Dau and Ani are 2 kinds of active compounds extracted from Chinese herbs. The former is used clinically as an antiarrhythmic and the later as an antiendotoxic shock remedy. Both Dau and Ani inhibited the metabolism of arachidonic acid[7]. We found in the present study that Dau and Ani exerted strong inhibitory effects on DNA synthesis of BCMSMC induced by LT and PAF. This implied that Dau and Ani might have a bright prospect in the prevention and treatment of cerebral vascular diseases.

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蝙蝠葛碱和山莨菪碱抑制白三烯和血小板活化因子刺激的牛脑微血管平滑肌细胞的增殖及脱氧核糖核酸的合成

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摘要 LTB_4, LTC_4, LTD_4和 PAF 在100 pmol·L^{-1}时对培养的牛脑微血平滑肌细胞脱氧核糖核酸(DNA)合成的刺激率分别为44%，50%，48%，57%. 在同样浓度下四种物质对该细胞增殖的刺激率分别为33%，47%，27%，40%. 蝙蝠葛碱和山莨菪碱在0.1—100 μmol·L^{-1}范围内均显著抑制LT和PAF诱导的该细胞的脱氧核糖核酸的合成.

关键词 异喹宁类；阿托品衍生物；脱氧核糖核酸；血管平滑肌；脑血管；白三烯；血小板活化因子

合成