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Influence of nicardipine on the morphology, calcium and cAMP level of immune organs in mice [1]

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ABSTRACT Nicardipine, 10 mg/kg sc qd×4d, caused morphological changes of immune organs in mice. The germinal centers of lymph nodes markedly reduced. The white pulps of spleens and the cortex of thymuses atrophied slightly. In these lymphoid tissues, the small lymphocytes markedly increased and the heterochromatin of nuclei increased. In the subcellular level, nicardipine damaged the structure of mitochondria in various cells. The lymphocytes in peripheral blood and the calcium content and cAMP level in immune organs markedly decreased. These results suggest that nicardipine inhibited proliferation of lymphocytes *in vivo*, and this effect was probably associated with the decreasing calcium content in immune organs. Influence on the structure and functions of mitochondria may be one of the mechanisms of nicardipine inhibiting functions of immune cells.

KEY WORDS nicardipine; immunity; electron microscopy; lymphocytes; calcium; adenosine cyclic monophosphate

In our previous research, the calcium channel blocker nicardipine (Nic) 5-15 mg/kg daily was found to inhibit the immune functions of mice. (1) Decrease in weight of thymus and spleen; (2) inhibition of phagocytosis of macrophages; (3) reduction of hemolysin concentration; and (4) suppression on delayed type hypersensitivity and lymphocyte transformation[1,2]. In this study we selected the dose of 10 mg/kg and observed the influence of Nic on the morphology, calcium content and cAMP level of immune organs in mice.

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MATERIALS AND METHODS

Nicardipine was obtained from Nanjing Medical Industrial Institute. Standard cAMP and [^{3}H]cAMP (102.3 GBq/mmol) were obtained from Pharmacological Department of Institute of Basic Medical Sciences, Beijing. Protein kinase was obtained from Institute of Nuclear Research, Chinese Academy of Sciences. All the reagents were AR.

Kunming mice, body weight 20±SD 3 g, 5-6 wk old, both ♀, ♂ were used. Nic was injected sc 10 mg/kg qd×4 d. The mice were killed 24 h after the last dose.

Morphological observation For light microscopy, the spleens, thymuses, groin lymphnodes and livers were fixed in 10% formol. HE stain. For electron microscopy, the spleen, thymus, liver and heart were fixed in 2.5% glutaraldehyde and 1% osmic acid, dehydrated in acetone, embedded in Epon-812, sectioned with LKB-V model ultra-microtome, stained with uranium acetate and photoed with JEOL 100 CX.

Lymphocytes in peripheral blood Before the mice were killed, peripheral blood was taken behind the orbit. Wright's stain. Lymphocytes were counted among 200 leucocytes under oil immersion light microscope.

Tissue calcium content The organs were dried at 140℃, and digested in nitric acid and perchloric acid for 24-48 h. The mediator of test liquid was 5% HCl, and the liberator was 10% strontium choloride. The

calcium contet was measured with Shimadzu atomic absorption spectrophotometer.

Competitive protein combination assay of cAMP The thymuses and spleens were dropped into liquid nitrogen. The cAMP was measured as reference (3).

RESULTS

Influence on morphology

1 *Light microscopc* In lymph nodes, the boundary between cortex and medulla was blurred, the germinal centers decreased or even disappeared, and the lymphocytes were all smaller than control (Fig 1 B). In spleens, the red pulps dilated slightly, while the white pulps became atrophied, and the number of lymphocytes in the red pulps was diminished. In thymuses, the cortex became somewhat thinner, the lymphocytes became smaller(Fig 1 D), and the number of lymphocytes in medulla increased slightly. In liver, the intercellular spaces and sinusoids reduced in width. The livers cells and Kupffer cells atrophied slightly. The nulei of Kupffer cells showed some pyknosis.

2 *Electron microscope* In spleen, the intercellular spaces became smaller. The microvilli of lymphocytic surface disappeared and the heterochromatin of nuclei increased (Fig 1 F). The mitochondria of lymphocytes and macrophages were damaged markedly. The electron-density of matrix of mitochondria decreased, some cristae of mitochondria became fractured and formed some plaques (Fig 1 G). The lysosomes in the macrophages increased. In the plasma cells, the rough endoplasmic reticulum dilated slightly and their lumina were filled with electron dense materials. In thymus, the intercellular spaces were smaller. The projections of lymphocytic surface became less. The mitochondria in macrophages and in epithelial-reticular cells were damaged markedly. Some mitochondria were condensed, and others formed plaques. The secondary lysosomes and autophagic vacuoles in macrophages increased. The myelin figures in the epithelial-reticular cells were often seen (Fig 1 H, I). In liver, some mitochondria in liver cells were damaged and the myeline figures frequently appeared in damaged mitochondria, and the lysosomes increased (Fig 1 J). The microvilli of liver cells surface increased and became thicker. In Kupffer cells, mitochondria was damaged and lysosomes increased. In addition, the mitochondria of myocardium cells were damaged, but the extent was slighter.

Influence on the number of lymphocytes in peripheral blood The lymphocytes amounted to $77\pm10\%$ in the control mice ($n=9$), and $61\pm10\%$ in the treated mice ($n=9$, $P<0.01$).

Influence on the calcium content of tissues The calcium content was markedly reduced in lymph nodes and thymuses, but slightly in spleens ($P>0.05$). The calcium content in livers was not markedly influenced (Tab 1). The tissue with higher calcium content was more sensitive to the action of Nic.

Influence on the cAMP level Nic markedly reduced the cAMP levels in thymus and spleens (Tab 1).

Tab 1. Influence of sc nicardipine 10 mg/(kg·d) ×4 d on calcium content and cAMP levels of immune organs and livers in mice. $\bar{x}\pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$.

	n	Saline	Nicardipine
		Calcium content (mg/g dry weight)	
Lymphaden	7	4.57 ± 1.50	$2.69\pm0.61^{**}$
Thymus	7	1.54 ± 0.82	$0.72\pm0.14^{**}$
Spleen	7	0.23 ± 0.08	$0.18\pm0.05^{*}$
Liver	7	0.07 ± 0.01	$0.06\pm0.01^{*}$
		cAMP (pmol/mg wet weight)	
Spleen	10	0.73 ± 0.26	$0.36\pm0.15^{***}$
Thymus	9	0.82 ± 0.17	$0.62\pm0.19^{**}$

DISCUSSION

Following the administration of Nic, the immune tissues and cells underwent involution of morphological structure, which was slight and reversible. These morphological changes suggested that *in vivo*, Nic made

lymphocytes be in the state of the quiescent and not active, and inhibited the proliferation of lymphocytes. These actions were a base of producing immune inhibition of Nic.

At the subcellular level, Nic produced an influence on structure of mitochondria in various cells. This action suggested that the mitochondria probably were target of Nic action. Nic inhibited the oxidative phosphorylation, and diminished calcium storage of mitochondria of liver cells[4]. There was a causal relation between structure and function of mitochondria. Our observation provided a morphological demonstration for Nic action on the mitochondria. We consider that the influence of Nic on the structure and function of mitochondria can not be ignored, and the influence probably is in close relationship with some pharmacological effects of Nic.

Probably there were two causes by which Nic decreased calcium content of immune organs: (1) by depressing Ca^{2+} influx, for example, in the cardiac and smooth muscles, and another calcium antagonists: verapamil, nifedipine, diltiazem, which could also inhibit Ca^{2+} influx in lymphocyte[5-7]. (2) Because the mitochondria are one of calcium storages in cells[8], the damage of structure and functions may result in reduction of capability to store calcium in mitochondria, and thus decreased the calcium content in the cells.

In vitro, some calcium antagonists inhibited lymphocyte proliferation by decreasing calcium influx[6,7]. *In vivo* the lymphocyte proliferation was stimulated during hypercalcaemia but depressed when the serum Ca^{2+} concentration was lower than normal[9]. According to these data we think that Nic influenced on the morphological structure of immune organs, which reflected on the inhibition of proliferation of lymphocytes by Nic, and that might associate with the reduction of calcium content of immune organs by Nic.

In general, the cell proliferation is inhibited during increase of cAMP level but stimulated during decrease of cAMP level [10,11]. However, under our experimentary conditions, simultaneously, Nic inhibited proliferation of lymphocytes, and depressed the cAMP level of immune organs. Because Nic damaged the structure of mitochondria, resulting in reduction of ATP which is precursor of cAMP, it, in turn, decreased cAMP production. On the other hand, since the adenylate cyclase activity is activated by calmodulin which is a Ca-dependent regulatory protein[12,13], thus decrease of cAMP could result from Nic decreasing calcium content. In terms of our experiments, it was possible that calcium plays an essential role on the lymphocytic proliferation, and during decrease of calcium content of the cells the proliferation was depressed.

Taken together, in vivo condition: (1) Nic inhibited proliferation of lymphocytes, and the action might be due to a decrease of calcium content in the immune organs by Nic: (2) Mitochondria probably were a target of Nic action, and influence on the structure and functions of mitochondria may be one of the mechanisms of Nic inhibiting the functions of immune cells.

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尼卡地平对小鼠免疫器官的形态、钙及腺苷环一磷酸水平的影响

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提要 尼卡地平 10 mg/kg sc qd×4 d，引起小鼠免疫器官形态结构的改变。淋巴结的生发中心明显减少，脾脏白髓和胸腺的皮质轻度萎缩。在这些淋巴组织里，小淋巴细胞明显增多，淋巴细胞核的异染色质增加。在亚细胞水平，尼卡地平损害各种细胞线粒体的结构，除了上述形态学的改变，小鼠外周血淋巴细胞的%，免疫器官的钙含量及 cAMP 水平也明显降低。这些结果提示，在整体条件下，尼卡地平抑制淋巴细胞的分裂增殖，这一作用可能和其减少免疫器官钙含量有关。尼卡地平对线粒体结构及功能的影响可能是其抑制免疫细胞功能的机理之一。

关键词 尼卡地平；免疫性；电子显微镜检查；淋巴细胞；钙；腺苷环--磷酸

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细粒棘球蚴囊对[^{3}H]吡喹酮的摄入与分布

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Uptake and distribution of [^{3}H]praziquantel in *Echinococcus granulosus* cysts

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ABSTRACT [^{3}H] Praziquantel was absorbed rapidly by the cysts in mice infected with metacestodes of *Echinococcus granulosus* and treated with ig [^{3}H]praziquantel 400 mg/kg (containing ^{3}H 1.48 MBq). Within 24 h after treatment, ^{3}H content in the ectocyst was 27.7-54.9% higher than that in cyst wall. The peak concentration of ^{3}H in cyst wall and cyst fluid were 11.7-15.3% of those found in plasma. However, when the cysts were exposed to [^{3}H]praziquantel 20 μg/ml (containing ^{3}H 3.7 kBq), the ^{3}H radioactivity in the cyst wall and cyst fluid reached equilibrium within 30 min-48 h after medication, corresponding to 45.5-63.3% of ^{3}H radioactivity in the medium. Autoradiography showed that