SHORT COMMUNICATIONS

INFLUENCE OF PEPTIDE COMPLEXES FROM KIDNEYS AND THYMUS ON DEXAMETHASONE– AND IONOPHORE–INDUCED APOPTOSIS IN THYMOCYTES AND PERIPHERAL BLOOD LYMPHOCYTES

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ВЛИЯНИЕ ПЕПТИДНЫХ КОМПЛЕКСОВ, ВЫДЕЛЕННЫХ ИЗ ПОЧЕК И ТИМУСА, НА АПОПТОЗ, ИНИЦИИРОВАННЫЙ ДЕКСАМЕТАЗОНОМ И ИОНОФОРОМ, В ТИМОЦИТАХ И ЛИМФОЦИТАХ ПЕРИФЕРИЧЕСКОЙ КРОВИ

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Óêðàèíñêàÿ ìåäèöèíñêàÿ ñòîìàòîëîãè÷åñêàÿ àêàäåìèÿ, Ïîëòàâà, Óêðàèíà

On the basis of combined morphological, immunocytochemical and electrophoretic study the patterns of dexamethasone- and ionophore A23187-induced apoptosis in thymocytes and peripheral blood lymphocytes (PBL) were analyzed. PBL have been shown to be less susceptible to those agents than thymocytes. Incubation of dexamethasone- and ionophore-treated cells with peptide complexes from kidney or thymus resulted in decrease of their susceptibility to the apoptosis-inducing agents.
Key Words: **apoptosis, thymocytes, peripheral blood lymphocytes, peptide complexes from kidney and thymus.**

Ñ ïîìîùüþ ìîðôîëîãè÷åñêîãî, èìóíîöèòîõèìè÷åñêîãî è ýëåêòðîôîðåòè÷åñêîãî ìåòîäîâ ïðîàíàëèçèðîâàíû îñîáåííîñòè èíäóöèðîâàííîãî àïîïòîçà äâóõ àíàëîãîâ ãëþêîêîðòèêîèäîâ è êàëüöèåâîãî èîíîôîðà À23187 â òèìîöèòàõ è ëèìôîöèòàõ ïåðèôåðè÷åñêîé êðîâè. Óñòàíîâëåíî, ÷òî ëèìôîöèòû ïåðèôåðè÷åñêîé êðîâè ìåíåå ÷óâñòâèòåëüíû ê äåéñòâèþ ýòèõ àïîïòîç-èíäóöèðóþùèõ àãåíòîâ, ÷åì òèìîöèòû. Îáðàáîòêà êëåòîê ïåïòèäíûìè êîìïëåêñàìè âèëî÷êîâîé æåëåçû è ïî÷åê ñíèæàåò èõ ÷óâñòâèòåëüíîñòü ê àïîïòîç-èíäóöèðóþùèì àãåíòàì.
Êëþ÷åâûå ñëîâà: **àïîïòîç, òèìîöèòû, ëèìôîöèòû ïåðèôåðè÷åñêîé êðîâè, ïåïòèäíûå êîìïëåêñû ïî÷åê è âèëî÷êîâîé æåëåçû.**

Apoptosis is described as an active form of cell death defined on the basis of special morphological changes in the cells; it plays an important role in embryogenesis and the maintenance of multicellular organisms [1]. Particularly, apoptosis is involved in the negative selection of self–reactive lymphocytes to establish normal T cell repertoire within the thymus [2]. At the same time, the blocking of apoptotic death in lymphoid cells seems to represent the integral component of lymphomogenesis [3, 4]. Apoptosis of thymocytes may be induced *in vitro* by phorbol esters, calcium ionophores (ionomycin or A23187), dibutyrylcyclic AMP and glucocorticoids [5]. The data suggesting the role of disturbances in functions of genes regulating apoptosis in the development of cancer have been obtained [6, 7].

However, some physiologic factors (hormones, metabolites, cytokines, low molecular weight peptides, etc) may influence apoptotic processes in the thymocytes and the peripheral blood lymphocytes (PBL). The role of the peptides bound to major histocompatibility complex (MHC) molecules and the influence of these peptides on the apoptosis pathway in thymocytes and lymphocytes were investigated [8–10, 11].

The mature T cell receptor repertoire is shaped by positive and negative selection events taking place during T cell development. These events are regulated by interactions between TCR and MHC molecules presenting self–peptides. It has been shown that many antagonist peptides are efficient at mediating positive selection. Recently it was found that transgenic peptides inhibit maturation of $CD8^+$ single positive thymocytes [11].

Earlier we had isolated the kidney peptide complex (KPC) [12]. KPC was shown to increase an expression of TCR–CD3 complex and HLA–DR [13] and modulate the interleukin–2–induced lymphocyte response [14]. KPC preparation blocked an antigen specific response to kidney tissue antigens in Heymann's nephritis and kidney allografts [15]. The synthetic analogs of some kidney peptides have similar activities [16, 17].

In this investigation, we examined the effects of KPC and thymic extract (as a control) on apoptotic processes in PBL and thymocytes.

Thymocytes were isolated from 10–12–month–old pigs (Poltava White breed). PBL were isolated from human whole blood stabilized by heparin and collected by ficoll–verographin technique [18]. Dexamethasone and calcium ionophore A23187 (Sigma, USA) were added

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Abbreviations used: KPC – kidney peptide complex; MHC – major histocompatibility complex; PBL – peripheral blood lymphocytes.

to the medium to the final concentration 10^{-7} M and 200 nM respectively, and the cells were incubated for 24 h at 37 °C [19]. Peptide complex from pork kidneys was extracted according to [12]. The commercial peptide complex thymalyn was used as a control. Both peptide complexes were added to the medium to the final concentration 0.12 μg/ml for 24 h at 37 °C. Each series consisted of 6 samples. The significance of the differences between the series was assessed using *t*–test.

Cell morphology was assessed in cytospin preparations upon May – Grunwald – Giesa (MGG) staining. Apoptotic changes in cells were studied by staining cell suspension with Hoechst 33342 (Sigma, USA). DNA was isolated from the cells by modified pronase–phenol–chlorophorm method [20]. Cytospin preparations of cultured cells were examined immunohistochemically using primary monoclonal antibodies to Bcl–2 (IgG_1, K, DAKO), to CD95 (LT95, IgG_1, Sorbent) and to p53 protein (clone DO–7, subclass IgG_{2b}, K, DAKO) labelled by avidin–biotin immunoperoxidase technique [21]. Nuclei were counterstained with methyl green solution.

The effects of dexamethasone, thymalyn and KPC have been studied in PBL and thymocytes. While in PBL dexamethasone induced in a number of cells (around 15% above the control level) typical apoptotic features revealed upon microscopy and Hoechst staining, DNA fragmentation was not evident (Fig. 1, 2, Table). The effects of thymalyn and KPC as single agents were essentially the same with fewer number of Hoechst–positive cells (see Fig. 1, Table). Like dexamethasone, thymalyn and KPC increased CD95 expression in PBL. Bcl–2 expression increased only in PBL treated with thymalyn or KPC.

To further analyze the possibility of apoptosis modulation in PBL the cells treated with dexamethasone were incubated with thymalyn or KPC. KPC as well as thymalyn were shown to inhibit significantly dexamethasone–induced apoptosis in PBL judging by the number of Hoechst–positive cells. While dexamethasone alone did not increase Bcl–2 expression, treatment with thymalyn or KPC as single agents or in combination with dexamethasone resulted in slight, although significant increase in Bcl–2 expression level. Nevertheless the percentage of Bcl–2–positive cells in case of combined treatment with dexamethasone and peptide complexes did not exceed the percentage of Bcl–2–positive cells in case of the treatment with dexamethasone as the single agent (see Table). Therefore, peptide complexes from thymus and kidney seemed to inhibit dexamethasone–induced apoptosis in PBL with accompanying increase in percentage of Bcl–2–positive cells.

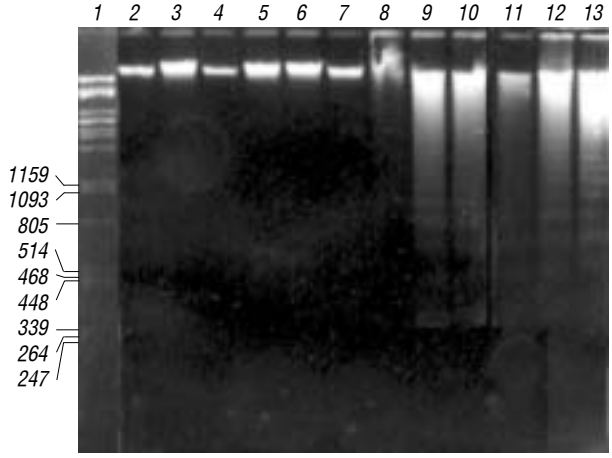

Fig. 1. Electrophoretic analysis of DNA degradation in PBL and thymocytes. Lane 1, λ DNA digested by *Pst* 1; lane 2, control (PBL); lane 3, thymalyn–treated PBL; lane 4, KPC–treated PBL; lane 5, dexamethasone–treated PBL; lane 6, dexamethasone–treated PBL incubated with thymalyn; lane 7, dexamethasone–treated PBL incubated with KPC; lane 8, control (thymocytes); lane 9, thymalyn–treated thymocytes; lane 10, KPC–treated thymocytes; lane 11, dexamethasone–treated thymocytes; lane 12, dexamethasone–treated thymocytes incubated with thymalyn; lane 13, dexamethasone–treated thymocytes incubated with KPC

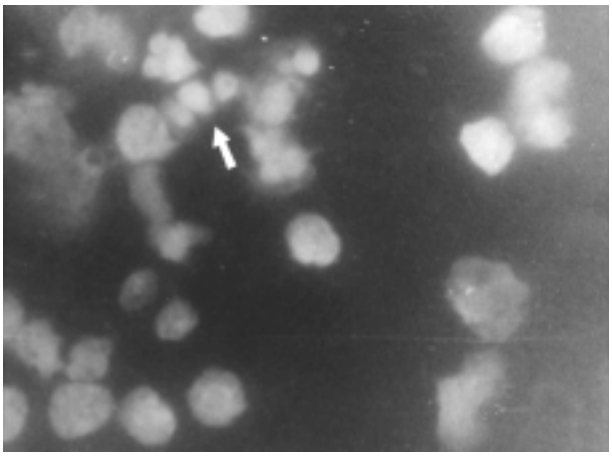

Fig. 2. Hoechst 33342 staining of PBL after the treatment with dexamethasone

In contrast, dexamethasone–treated thymocytes exhibited typical morphological features of apoptosis accompanied by DNA fragmentation revealed as a ladder upon agarose gel electrophoresis and increase in Bcl–2 expression (see Table, Fig. 1). The incubation of

Table. Immunohystochemical analysis of lymphoid cells after dexamethasone or ionophore treatment

Treatment of cells	PBL				Thymocytes			
	CD95 expression,%	p53 expression,%	bcl–2 expression,%	Hoechst staining Fragmentation, %	CD95 expression, %	p53 expression, %	bcl–2 expression,%	Hoechst staining Fragmentation, %
Control	11.6 ± 1.4	3.9 ± 0.7	26.8 ± 1.3	4.6 ± 1.1	20,0 ± 1.5	6.8 ± 0.9	26.0 ± 2.0	10.0 ± 2.8
Thymalyn	16.1 ± 1.1*	4.5 ± 0.5	33.3 ± 1.9*	11.0 ± 2.0*	24.8 ± 1.0*	7.4 ± 0.5	38.0 ± 3.5*	9.2 ± 1.2
KPC	17.0± 1.5*	4.4 ± 0.7	32.4 ± 1.7*	9.0 ± 1.3*	22.3 ± 1.4	7.2 ± 0.6	42.0 ± 5.0*	4.5 ± 1.5*
Dexamethasone	22.8 ± 3.5*	7.3 ± 0.9*	23.2 ± 2.7	19.5 ± 3.2*	24.0 ± 1.0*	7.9 ± 0.5	38.0 ± 4.0*	25.9 ± 4.0*
Dexamethasone + thymalyn	20.2 ± 2.0*	5.8 ± 1.1	31.8 ±1.5 **	12.5 ± 1.2*,**	26.8 ± 1.6*	6.0 ± 0.9	40.0 ± 4.5*	4.9 ± 1.4*,**
Dexamethasone + KPC	19.1 ± 1.3*	5.9 ± 1.0	32.2 ± 1.4**	10.7± 1.2*,**	26.7 ± 1.5*	7.0 ± 0.7	44.0 ± 6.0*	2.0 ± 1.0*,**
Inophore	15.3 ± 1.0	6.3 ± 0.6*	31.0±1.0*	6.0 ± 2.0	21.3± 1.8*	6.1± 0.5	28.5 ± 3.0	12.0 ± 1.0
Ionophore + thymalyn	13.8 ± 1.2	4.4 ± 0.5	33.5 ± 1.3*	4.0 ± 1.0	14.0 ± 1.0	5.2 ± 0.7	31.0 ± 2.5	2.0± 0.9*,**
Ionophore + KPC	10.3 ± 1.1	5.4 ± 0.5	37.5 ± 1,2*	2.0 ± 1.5	15.3 ± 1.4	5.3 ± 0.5	32.0 ± 1.5*	1.0 ± 05*,**

* $P < 0.05$ in comparison with control cells.
** $P < 0.05$ in comparison with ionophore– or dexamethasone–treated cells.

thymocytes with thymalin and KPC had the similar effects except for the absence of overt morphological alterations at the moment when internucleosomal DNA degradation has been already evident. Incubation of dexamethasone–treated thymocytes in the presence of thymalyn or KPC resulted in the pronounced decrease in the number of Hoechst–positive cells.

Calcium ionophore A23187 as a single agent induced neither internucleosomal DNA fragmentation, nor morphologic alterations characteristic of apoptosis (see Table). Apoptotic effects of ionophore have not been evident in PBL as well as in thymocytes. Meanwhile the slight increase in Bcl–2 expression upon treatment with ionophore and peptide complexes, alone or in combination has been shown. It is worth mentioning that incubation of ionophore–treated cells with either thymalyn or KPC resulted in the significant decrease in the number of Hoechst–positive cells comparing to the number of such cells in non–treated control and ionophore–treated samples. Nevertheless the increased level of Bcl–2 expression still persists in the cells treated with ionophore and either thymalyn or KPC.

To sum up, our data suggest that the peptide complexes extracted from thymus or kidney and related to the molecules of major histocompatibility complex could affect some parameters pertaining to apoptosis both in PBL and in thymocytes. The peptide complexes under study also increased the level of Bcl–2 expression in the cells being treated. The exact mechanisms of such effects are to be elucidated. Meanwhile the substances studied could be useful as the modulators of both apoptosis and immune response.

ACKNOWLEDGMENTS

The authors would like to thank for assistance Dr. Harutoshi Kizaki (Department of Biochemistry, School of Medicine, Tokyo Dental College).

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