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short communication

Effects of host defense peptides B2RP, Brevinin-2GU, D-Lys-Temporin, Lys-XT-7 and D-Lys-Ascaphin-8 on peripheral blood mononuclear cells: Preliminary study

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Abstract

***Background and purpose**: Host defense peptides have considerable therapeutic potential. One of the limitations for their therapeutic use is insufficient selectivity of some peptides, i.e. toxicity for eukaryotic cells. In this study, we have investigated effect of two naturally occurring and three analogs of frog skin-derived peptides on viability/proliferation of resting peripheral blood mononuclear cells and activated lymphocytes.*

***Materials and Methods**: Effect of tested peptides was assessed using MTT colorimetric assay. Concanavalin A was used as lymphocyte mitogen.*

***Results**: Brevinin-2GU induced cell death only in the highest tested concentration, whereas other peptides were not cytotoxic to resting peripheral blood mononuclear cells. Moreover, high concentrations of B2RP, D-Lys-Ascaphin-8 and Lys-XT-7 induced cell proliferation and this effect was more prominent in lymphocytes ($p<0.05$). Tested peptides had opposite effect on activated lymphocytes inhibiting proliferative response to Concanavalin A (Brevinin-2GU, B2RP and D-Lys-Temporin $p<0.05$).*

***Conclusions**: Tested peptides (with exception of Brevinin-2GU) didn't show cytotoxicity toward peripheral blood mononuclear cells. Moreover, they have potential to modulate immune response by inducing proliferation of resting peripheral blood mononuclear cells and limiting proliferative response to the activation stimulus. Regarding their potent antimicrobial and low hemolytic activity this makes them good candidates for therapeutic use.*

INTRODUCTION

Host defense peptides (HDPs) represent a heterogeneous group of positively charged, amphipathic molecules composed of 12 to 50 amino acids, with diversity of properties. They are found in almost all living organisms, from bacteria to humans *(1)*. HDPs have multiplicity of biological functions. They exert a broad-spectrum antimicrobial activity and therefore are often referred to as "natural antibiotics" *(2)*. The structure and positive net charge of HDPs enable strong binding to negatively charged membranes of microorganisms, as well as cancer cells, that bear more negative net charge than healthy cell *(3)*. Electrostatic binding to outer surface of cell is followed by disruption of membrane structure, binding to vital intracellular molecules and interference with synthesis of macromolecules, resulting in cell death.

Although the most studied feature of HDPs is their antimicrobial activity, there are opinions that primary task of these peptides is immunomodulation. HDPs promote immune response to inflammation and at the same time restrain it, protecting organism from excessive reaction that can be detrimental for the host *(4)*. They have impact on both innate and adaptive immunity and coordinate their action, influencing various processes, such as proliferation, chemotaxis, cytokine production and activation of adaptive immune response. Furthermore, HDPs participate in wound healing and neovascularization *(5)*.

Currently, potential of HDPs as new therapeutic agents in infectious diseases, cancer and immune-related disorders is objective of increasing number of studies *(6)*. However, although recognized as promising new drugs, there are some limitations for their use. These referre to their sensitivity to enzymatic degradation and inactivation in serum by albumins and low-density lipoproteins, inadequate tissue distribution and potential systemic toxicity owning to their inefficient specificity. To overcome these problems, modifications of peptide structure have been under study *(7)*.

In the present study we have tested effects of five peptides, selected on the basis of their high antimicrobial potency and low hemolytic activity. Naturally occurring peptides Brevinin-2GU *(8)* and brevinin-2-related peptide-ERa (B2RP-ERa) *(9)* were isolated from the skin of the Asian frog *Hylarana*. Analogs [D4k]ascaphin-8, [G4K]XT-7 *(10–12)* and [T5k]temporin-DRa *(13)*, showing potent antimicrobial and low hemolytic activity, were derived from frog skin peptides that have shown broad-spectrum antibacterial activity, but high hemolytic activity.

MATERIALS AND METHODS

Peptides

Brevinin-2GU (GVIIDTLKGAAKT VAAELLRKAHCKLTNSC), B2RP-ERa (GVIKSVLKGVAKTVALGML.NH2), [D4k]ascaphin-8 (GFKkLLKGAAKALVKTVLF.NH2), [G4K]XT-7 (GLLKPLLKIAAKVGSNLL.NH2) and [T5k]temporin-DRa (HFLGkLVNLAKKILNH2), supplied in crude form by GL Biochem Ltd. (Shanghai, China) and purified to near homogeneity by reverse-phase HPLC (>98%), were a gift from Dr. M. Conlon (United Arab Emirates University, Dubai, UAE).

Cell isolation and culture

Cells were isolated from peripheral blood of healthy volunteers. Our institutional Ethics Committee approved the study and prior to initiation written informed consent was obtained from all subjects according to the Declaration of Helsinki. Peripheral blood mononuclear cells (PBMNC) were isolated by density gradient centrifugation (Histopaque 1077, Sigma, Germany), washed three times and finally suspended in the supplemented culture medium RPMI 1640 (Sigma, Germany). Lymphocyte population (PBL) was isolated from PBMNC by adherence method *(14)*. Cell number and viability were determined using Acridine orange/Ethidium bromide staining (all from Sigma, Germany).

Cell viability and proliferation assay

The effect of cationic peptides on peripheral blood mononuclear cells was determined by MTT assay that is widely used for assessing cell proliferation, cell viability, and/or cytotoxicity *(15)*. Mononuclear cells or lymphocytes were grown in 96-well plates at a starting density of $0.2x10^6$ cells/well in presence of increasing doses (1, 5, 10 and 20 μg/ml) of peptides or in medium alone (control). In some experiments Concanavalin A (ConA) was used (5μg/ml). Cells were cultured for 24h at 37°C in 5% $C0_2$. Cultured cells viability was determined by assaying the reduction of MTT to formazan.

Statistics

The data were expressed as the mean ± SD. The distributions of data were evaluated for normality using the Kolmogorov-Smirnov test. One-way ANOVA was performed to compare parametric data between more than two groups. When ANOVA indicated significant differences, the Bonferroni test was used to identify intergroup differences. All statistical analyses were carried out with commercial statistical software (SPSS version 13.0; SPSS Inc., Chicago, IL).

RESULTS

The effect of peptides on cell viability

As shown in Figure 1., naturally occurring peptide Brevinin-2GU was slightly cytotoxic for PBMNC and PBL, but only the highest concentration (20μg/ml) decreased PBMNC viability with statistical significance ($p<0.05$), whereas effect on PBL was similar, but with no statistical significance. The other naturally occurring peptide, B2RP-ERa, didn't show significant cytotoxicity toward PBMNC. However, it demonstrated opposite effect on PBL, inducing cell proliferation reaching statistical significance with the highest concentrations of 10 and 20μg/ml ($p<0.05$). Similarly, analogs [D4k]ascaphin-8, [G4K] XT-7 and [T5k] temporin-DRa did not show cytotoxic effect to PBMNC and PBL in either concentration tested, while, in contrast, promoted proliferation of both PBMNC and PBL. However, this effect was noticeable only with the highest concentration (20μg/ml) of [D4k] ascaphin-8, reaching statistical significance for PBL ($p<0.05$), and [G4K] XT-7, achieving significance for both PBL ($p<0.001$) and PBMNC ($p<0.05$). Importantly, this mitogenic action of both [D4k]ascaphin-8 and [G4K] XT-7 was more prominent in PBL ($p<0.05$).

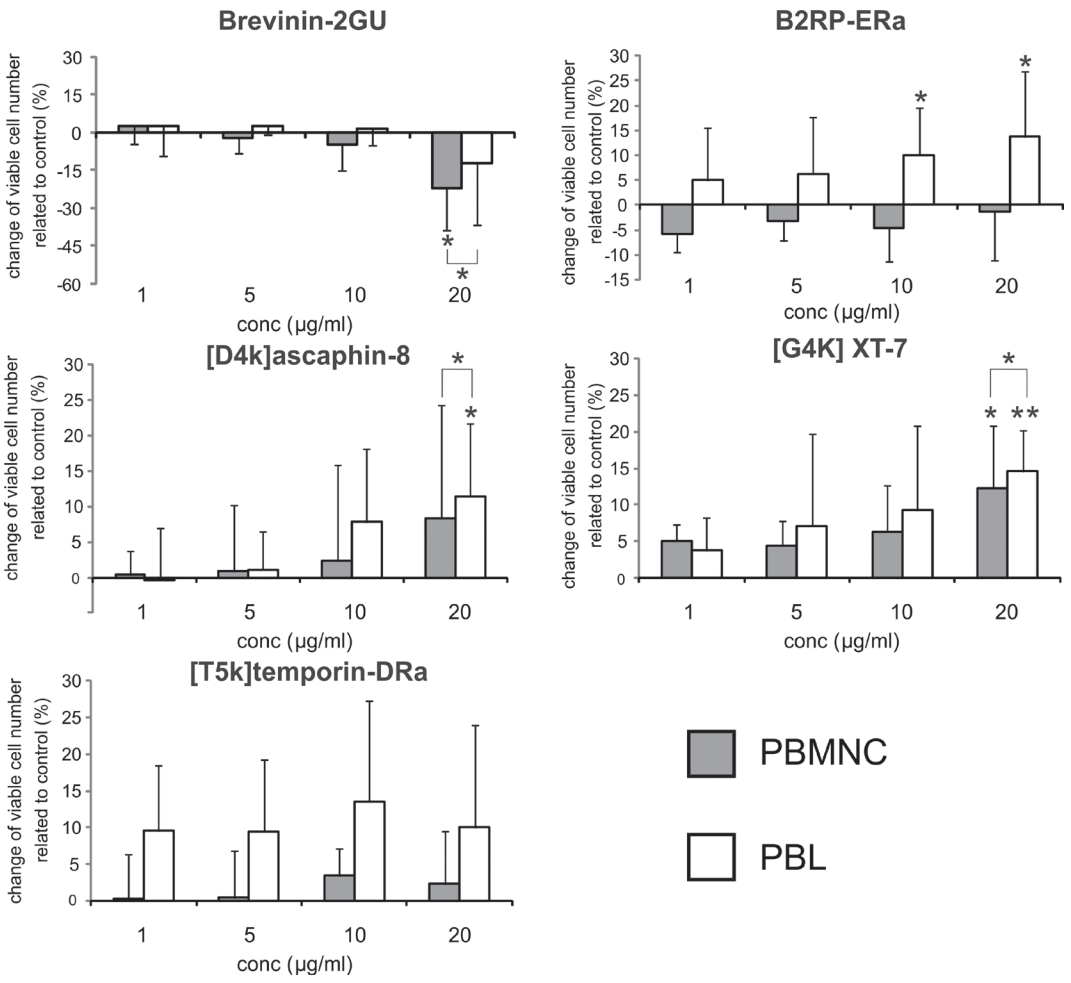

Figure 1. *The effect of peptides on PBMNC and PBL.*

Peripheral blood mononuclear cells (PBMNC) and purified lymphocytes (PBL) were incubated for 24h in media alone (control) and with increasing doses (1, 5, 10 and 20 μg/ml) of peptides. Cytotoxic or proliferative effect of peptides is presented as percent of change in cell number related to control. The values represent the mean (±SD) of five different experiments. *p<0.05; **p<0.001

Peptides diminished mitogenic effect of ConA

To determine whether tested peptides have influence on proliferative response induced by Concanavalin A, PBLs were incubated in media alone (control), or treated with 5μg/ml ConA, either in absence or presence of different concentration of peptides. Our results showed that peptides decrease the mitogenic action of ConA (Figure 2.), and this effect was dose-dependent in that way that increase in concentration of peptides resulted in enhanced inhibition of ConA induced PBL proliferation.

Peripheral blood lymphocytes (PBL) were incubated for 24h in media alone (control) and with Concanavalin A (Con A) (5μg/ml) either in presence or absence of peptides (1, 5, 10 and 20 μg/ml). Proliferative response induced by ConA and influence of tested peptides on the response are presented as percent of change in cell number related to control. The values represent the mean (±SD) of five different experiments. *p<0.05

The most potent immunomodulator of all was Brevinin-2GU, that at the highest concentrations (10 and 20μg/ml) significantly inhibited ConA-induced proliferation of PBL, and this effect was most pronounced at a concentration of 20μg/ml which almost completely nullifies the effect of ConA (p<0.05). Other peptides showed a similar effect, but statistically significant inhibition of the mitogenic action of Con A, although somewhat less than the Brevinin-2GU, showed only the highest concentrations (10 and 20μg/ml) of naturally occurring peptide B2RP-ERa and analog [T5k]temporin-DRa (p<0.05).

Summarized results are presented in Figure 3.

The effects of host defense peptides on peripheral blood mononuclear cells.

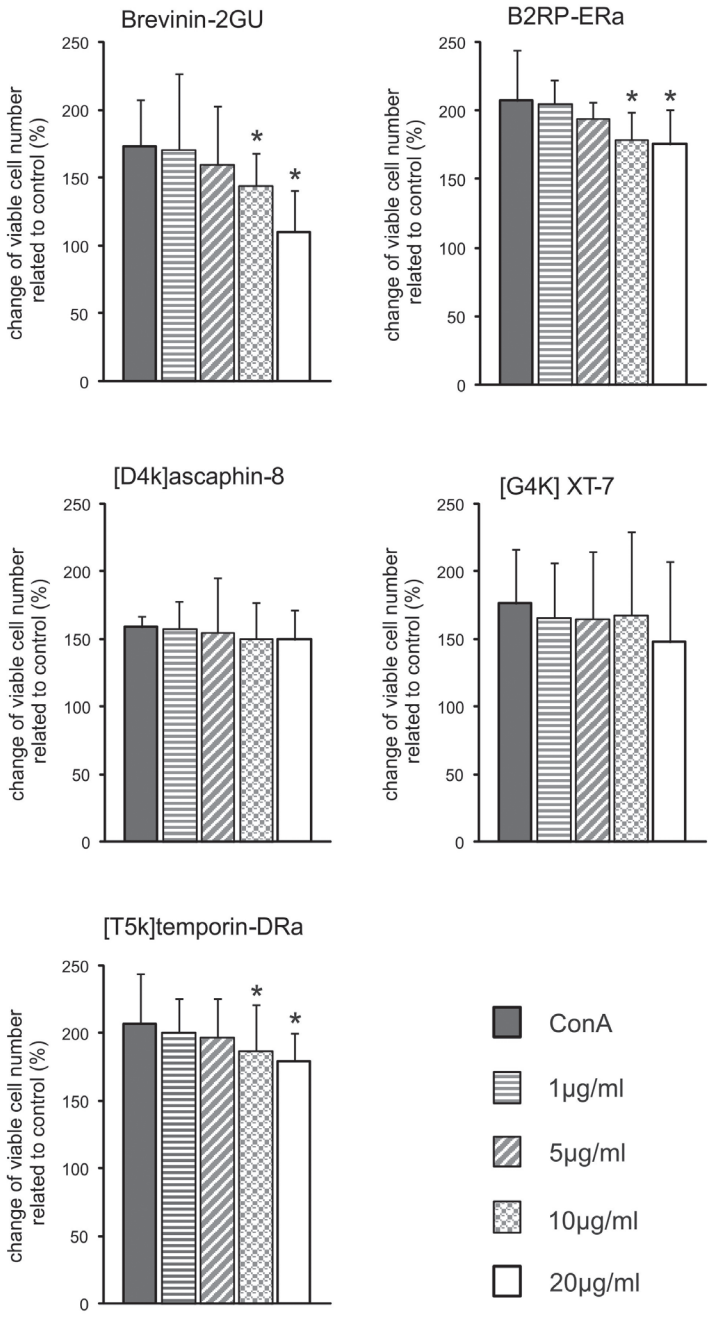

Figure 2. *Peptides decreased mitogenic effect of ConA.*

DISCUSSION

In spite of great potential of HDPs as new therapeutics, development of HDP-based drugs is limited by several shortcomings, such as instability and insufficient specificity. Therefore, a number of investigations are directed toward designing analogs of naturally occurring peptides that will outpass these disadvantages. In this study we have investigated effect of two naturally occurring peptides and three analogs on healthy peripheral blood mononuclear cells.

Our results showed that, apart from Brevinin-2GU that was cytotoxic in the highest concentration, tested HDP analogs and naturally occurring peptide B2RP-ERa didn't exhibit cytotoxicity against mononuclear cells isolated from peripheral blood, but rather promoted their proliferation. Our results are consistent with reports certifying that HDPs can enhance proliferation of peripheral blood mononuclear cells *(16)*, which is in accordance with findings about impact of HDPs on immune system response *(17)*.

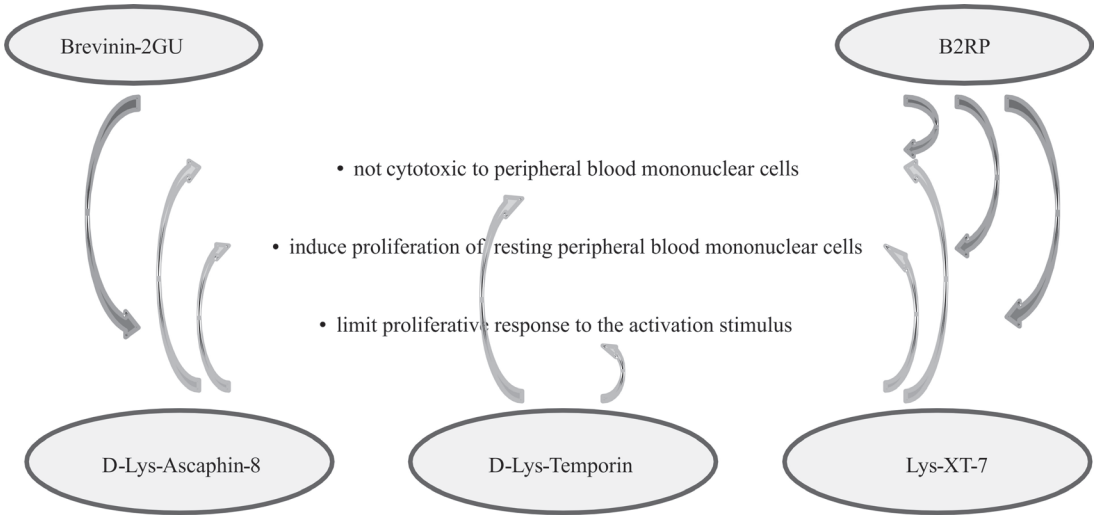

Figure 3. *Summarised results of the study.*

Activated lymphocytes can be, however, more sensitive to HDPs. The foregoing results have shown that tested peptides had more pronounced effect on macrophage depleted peripheral blood mononuclear cells (PBL). Macrophages are necessary for many lymphocyte functions, including activation and mitogenic response, but can also suppress lymphocyte activation. Furthermore, macrophages can uptake culture medium with peptides via endocytosis and thus reduce their concentration beneath the effective doses required for the effect observed on lymphocytes. Therefore, to exclude influence of macrophages and to test effect of peptides on activated lymphocytes, we incubated PBLs with T-cell mitogen ConA, alone or with different concentration of tested peptides.

All tested peptides decreased proliferative effect of ConA in that way that higher concentration of peptides resulted in higher inhibition. This effect was most prominent with naturally occurring peptides Brevinin-2GU and B2RP-ERa and analog [T5k]temporin-DRa ($p<0.05$). This effect of tested peptides can be a result of their cytotoxicity against activated lymphocytes. Nevertheless, in our previous study we have shown that tested peptides inhibited release of proinflammatory cytokines TNFα and IFNγ and stimulated release of antiinflammatory cytokines IL-4, IL-10 and TGFβ in ConA-stimulated PBLs *(18)*. It is well known that IL-4, IL-10 and TGFβ can promote cell-cycle arrest and apoptosis *(19, 20)*. Therefore it is possible that tested peptides exert their antiproliferative activity through stimulation of antiinflammatory cytokines release. In any case, these results point to their capability to influence immune response.

In conclusion, we have shown that tested peptides (except Brevinin-2GU) are not cytotoxic for resting peripheral blood mononuclear cells and even induce cell proliferation, and that both naturally occurring peptides and analog [T5k]temporin-DRa have potential to modulate immune response to the activation stimulus. Concerning their high antimicrobial potency and low hemolytic activity, these peptides can be considered as potential therapeutics.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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