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Synthesis and biological activity of tuftsin, its analogue and conjugates containing muramyl dipeptides or nor-muramyl dipeptides

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Abstract: Several conjugates of muramyl dipeptide (MDP) or nor-muramyl dipeptide (nor-MDP) with tuftsin were synthesized. Conjugates **8a–f** were prepared by acylation of protected tuftsin with the isoglutamine carboxyl group of MDP or nor-MDP **2a–f**. Also tuftsin analogue **6** (H-Thr-Lys-Pro-Arg(NO_2)-OH) was obtained. All synthesized compounds were investigated at the Medical University of Gdansk. The biological activity of the examined compounds was estimated using *in vitro* cultures of human monocytes and lymphocytes. The substances displayed cytotoxic effects, as was revealed in the viability tests performed. The effects were most probably mediated by the induction of an oxidative burst in monocytes and the stimulation of redox enzymes in lymphocytes. In addition, the analogues turned out to be efficient stimulators of TNFα and IL6 secretion by monocytes and lymphocytes. Nevertheless, the secretion of cytokines did not affect the viability of the leukocyte population used in the experiments.

The beneficial properties of the compounds examined (mainly **6**, **3**, **8a** and **8c**), which implies their usefulness as potential therapeutic agents, are connected with their rapid start of action and more efficient effects compared with tuftsin alone. An *in vivo* assay on animal models will be performed.

Keywords: muramyl dipeptide conjugates; tuftsin analogues; synthesis; biological activity

INTRODUCTION

Tuftsin, a natural peptide (H-Thr-Lys-Pro-Arg-OH) occurring in the blood of humans and other mammals, is capable of stimulating certain white blood cells (monocytes, macrophages and neutrophils), and was isolated at Tufts University in 1970 [1]. Since then, a good deal of research has been conducted to discover its properties and to detect the mechanism of its tetrapeptide action. Tuftsin has been found to display several types of potentially beneficial activity [2,3]: activation of macrophages toward fighting bacterial infections; improvement of communication among the immune systems (macrophages, T-cells and antibody-producing B-cells) resulting in increased antibody production; enhancement of the immune response against tumours and retardation of the growth of tumours (in animal experiments). Tuftsin is useful in the control of yeast infections and is able to stimulate the production of blood cells by the bone marrow. The susceptibility of tuftsin to biodegradation and the antagonistic properties of its decomposition products inspired scientists to make efforts to find analogues that were more stable, more selective, easier to purify and of longer lasting activity. The other component of the conjugates — muramyl dipeptide (MDP) a minimal structure of bacterial cell wall peptidoglycan — possesses immune potentiating activity [4–6]. Similar to tuftsin (a bioactive peptide of animal origin), MDP (a synthetic bioactive glycopeptide of microbiological origin) stimulates various functions of macrophages (such as phagocytosis, pinocytosis, motility, chemotaxis, bactericidal and antitumour activity) and increases the non-specific resistance of the host against numerous microorganisms. However, its action does not last long and is not very effective. Numerous derivatives of MDP were designed and synthesized with the aim of obtaining molecules of improved and more defined immunological profiles. The efforts gave good results and some of the compounds were introduced to therapy or are under clinical trials [7–9]. A synergistic effect of MDP on many therapeutic agents is well known and its synergy with tuftsin was also checked. Preliminary information on the activity of a mixture of MDP and tuftsin was published in 1993 [10]. Tests performed on macrophage bovine teat revealed that the application of a mixture of tuftsin and MDP intensified the macrophage inhibition effect on intracellular replication of *Brucella abortus type 2308*. Titov *et al.* [11] synthesized conjugates of tuftsin with *N*-acetyl-glucosaminyl muramyl dipeptide (GMDP) in which tuftsin (H-Thr-Lys-Pro-Arg-OH) was attached to the γ-carboxylic group of D-Gln either through the

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