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The effect of PAMAM dendrimers and their bioconjugates with biotin and pyridoxal on the biological properties of different types of cells *in vitro*

Abstract:

Dendrimers are increasingly used in the field of medical science (diagnostics, therapy) as well as in the pharmaceutical industry, mainly as carriers of drugs, genes and other biologically active substances. Cationic poly(amidoamine) dendrimers (PAMAM) belong to the actively investigated group of dendritic polymers, since their use in biology and medicine is associated with relatively low cytotoxicity and the ability to penetrate biological membranes. As the aim for researchers remain the optimization of multifunctional carrier structure for various phenotype of cells and tissues. Such a target requires interdisciplinary investigations with specific cellular models.

Introduction contains information on the properties and synthesis of dendrimers and examples of applications in various fields of biomedical sciences, as well as their interaction with the human immune system. The properties of B vitamins (biotin and pyridoxal) and the structure and function of the skin, with particular emphasis on the three phenotypes of cells examined: fibroblasts, keratinocytes and squamous cell carcinoma, was also discussed.

The aim of the dissertation was to investigate and compare the effect of native cationic generation 3 PAMAM dendrimers terminated with 32 amino groups and their BC-PAMAM bioconjugate modified with vitamins from group B (9 biotin and 10 pyridoxal molecules) on three phenotypes of human skin cell lines: normal fibroblasts BJ, cancer cells squamous SCC-15 and immortalized keratinocytes HaCaT.

The cytotoxicity of dendrimers, after 24 hours incubation (neutral red assay NR and XTT tetrazolium salt assay), their effect on induction of apoptosis (PARP-1 estimation) and cell migration (wound healing assay) were evaluated. The tests has shown that substitution of dendrimers with two biologically active molecules: biotin and pyridoxal reduces their cytotoxicity 5-10 times (5-10 μM against 50 μM) in all investigated cell lines. Normal human fibroblasts are most sensitive against induction of apoptosis, whereas the HaCaT keratinocytes revealed high resistance towards both PAMAM and BC-PAMAM. Both types of dendrimers inhibit cellular motility at cytotoxic concentrations. Interesting is the stimulation of fibroblast motility at a low (1μM) concentration of BC-PAMAM.

The intracellular accumulation and colocalization of both types of dendrimers in cell nuclei and mitochondria was performed by confocal microscopy. Intracellular accumulation of both dendrimers was concentration dependend, with BJ and SCC-15 faster increasing accumulation to non-toxic 2,5 μM concentration. In addition, fibroblast and squamous cell carcinoma showed significantly higher internalization of PAMAM than BC-PAMAM. Bioconjugates were accumulated in a much smaller amount in all cell lines compared to the native dendrimer. The accumulation of dendrimers in the nuclei of cells was differentiated. The highest and concentration dependent signal was observed for PAMAM in fibroblasts. Lower and concentration independent accumulation was demonstrated by SCC-15 cells and the lowest accumulation of HaCaT except for high, cytotoxic of PAMAM concentration. The accumulation of BC-PAMAM in the nuclei was significantly lower than the native dendrimer in all cell lines. The colocalization signal of both types of dendrimers with mitochondria showed interesting quantitative and qualitative differences. The highest signal was from PAMAM was in fibroblasts, depending on concentration, while the lowest was observed in HaCaT. In contrast, an intermediate value of the signal accumulation in cancer cells was observed, but in a concentration-independent manner. An accumulation of BC-PAMAM was significantly lower in all cell lines.

Investigations of PAMAM and BC-PAMAM effects on inflammatory response of HaCaT cells have generally confirmed their anti-inflammatory properties. Inhibition of endogenous level of cytokine IL-1α by both dendrimer types at non-cytotoxic concentrations (1 μM), as well as in HaCaT cells stimulated with bacterial pro-inflammatory factors LPS and GroEL was shown.

In conclusion, marked difference was revealed concerning the response of three lines of various human skin cell line phenotypes towards effects of native PAMAM G3 dendrimer and its bioconjugate with biotin and pyridoxal, with significant lower cytotoxicity of conjugate. The anti-inflammatory action of both dendrimer type in non-cytotoxic concentration in HaCaT cells was confirmed. However, presented results did not confirm other authors observations that biotin increases dendrimers internalization in all cancer cell lines, indicating the importance of precise investigations of individual cell lines.