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Galactosylated liposomes with proton sponge capacity: a novel hepatocyte-specific gene transfer system

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Hepatocyte-directed liposomal gene delivery has received much attention due to the lack of suitable treatment for several liver-associated disorders. While targeting of liposomes to the asialoglycoprotein receptor (ASGP-R), nearly-exclusive to hepatocytes, is a well-documented means of achieving cell-specificity, endo/lysosomal degradation of the internalised DNA is one of several factors which hinder successful gene transfer. This study has attempted to address this concern by modifying hepatotropic liposomes with an endosomal escape-inducing proton sponge moiety.

Novel galactosylated (SH02) and imidazolylated (SH04) cholesterol derivatives were successfully synthesised with the aim of conferring the respective functions of ASGP-R-specificity and proton sponge capability upon cationic liposome formulations. These formed unilamellar vesicles with the cytofectin, 3β[*N*-(*N*′,*N*′-dimethylaminopropane)-carbamoyl] cholesterol (Chol-T) and co-lipid, dioleoylphosphatidylethanolamine (DOPE), when incorporated at 10 mol%. Liposomes effectively bound pCMV-*luc* plasmid DNA, provided protection against serum nucleases; and were well tolerated by both hepatocytes and kidney cells in culture. Competitive inhibition assays showed that liposomes containing SH02 were internalised predominantly via the ASGP-R. Acid titration experiments highlighted the endosomal pH-buffering capacity of SH04. SH04 improved the transfection activity of the Chol-T/DOPE system, but not that of its targeted counterpart, in kidney cells only. Both SH02 and SH04 individually exhibited transfection-enhancing properties and the transgene expression levels using both novel lipids were promising. With further optimisation of the proton sponge and targeting abilities, the liposomes may achieve desired transgene expression levels for use *in vivo*.

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Evaluation of skin angiogenesis stimulated by ointment preparations containing angiogenic genes

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From a point of view of classic pharmacotherapy genes should be treated as active substances that condition the biological activity of a medicinal product that is used. In the case of angiogenic genes, a gene therapy product exerts angiogenic properties - and after having been introduced into appropriate cells it stimulates processes leading to the formation of new blood vessels. In this work we performed a series of experiments aimed to select a group of vehicles, ointment ingredients that could be useful in the systems that could introduce genes into the skin of laboratory animals. Experiments were conducted on plasmids encoding VEGF, FGF, SDF proteins. Appropriate ointment formulas were prepared for experiments, and they were applied on the skin of laboratory mice; after pre-determined time mice were sacrificed, transfected skin specimens were collected and the presence of a pDNA sequence in samples was analysed with qPCR. The analysis of angiogenesis stimulation was also performed. The sequences of apllied pDNAs were found in the mouse skin. Selected vehicles make it possible to introduce pDNA into skin cells; however, the *in vivo* transfection capacity is not high. Based on estimations 10–30% of pDNA molecules applied in ointment pass into the animal skin cells. Experiments also indicate that plasmid pVEGF, pSHH, pSDF stimulate angiogenesis in animal skin and proangiogenic properties depend on a plasmid dose which is used. This work was supported by a grant from Polish Ministry of Science and Higher Education (N N 405 456039).

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Muscle spontaneous regeneration in dwarf mice treated with a bicistronic vector followed by electrotransfer

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Gene therapy combines the correction of defective or missing gene with low risk to the patient. Our group has developed an *in vivo* gene therapy model for the treatment of growth hormone (GH) deficiency based on injection of naked DNA followed by electrotransfer. This strategy provided the presence of human growth hormone (hGH) for at least 60 days in the circulation of immunodeficient/dwarf (lit/scid) mice, that presented a weight gain of up to 33%. The aim of the present work is to verify the safety of our method, evaluating the presence of inflammatory infiltrate and the pattern of muscle regeneration at the electroporation site. A bicistronic vector containing the murine GH (mGH) and the GFP genes under the control of the CMV promoter was utilized. Lit/lit mice were treated with 50 μg of DNA or saline (control group), injected into the quadriceps muscle, followed by electrotransfer using eight 50-V pulses of 20 ms at a 0.5s interval. Histological analysis was performed on day 0, 1, 3, 6 and 12. Muscle damage was verified on the initial days after treatment, but appeared regenerated on the 12th day. GFP maximum expression was observed on the third day. Since increased circulating mGH levels were not observed, GH mediator, i.e. mouse insulin-like growth factor-I (mIGF-I), will be determined to evaluate electroporation efficiency. The results indicate that muscle spontaneously regenerates after this treatment.

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Novel ultrasound-responsive gene carrier with ternary structure.

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