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Chitin Nanogels as an Effective Nanocarrier for the Treatment of Melanoma via the Transdermal Route

Abstract

The use of nanocarriers is one among the many strategies used for enhancing skin penetration of drugs in transdermal delivery. The nanogels have the advantage of environment responsive property too. The main hypothesis of this study was that the chitin nanogels (CNGs) based on its size and surface properties can be a good carrier for enhanced permeation and retention of drugs through the skin. The chitin nanogels prepared by regeneration was characterized by various techniques like DLS, SEM, FTIR, TG/DTA etc and the hemocompatibility as well as cytocompatibility were assessed. The potential for skin penetration was assessed by FTIR analysis of the acceptor fluid collected as well as by UV imaging of the acceptor fluid from the study using Rhodamine123 conjugated chitin nanogels (Rhod-CNGs), the results of which clearly indicated the skin penetration capability of CNGs.

In the second part of the study, we prepared drug loaded chitin nanogels with one lipophilic drug curcumin and one hydrophilic drug 5-Fluorouracil. These two nanogels were also characterized in the same way as chitin nanogels. Both these nanogels showed cationic charge, desirable size in the nanoregimen and enhanced thermal stability. The hemolysis assay and PT-APTT test were carried out to ensure hemocompatibility. The control as well the drug loaded nanogels showed pH responsive swelling at acidic pH leading to enhanced drug release in the acidic environment. This is desirable as pH in the tumor environment is acidic. The cytotoxicity assay results of curcumin loaded chitin nanogels (CCNGs) showed specific toxicity towards human melanoma (A375) cells compared to the normal human dermal fibroblast (HDF) cells. The CCNGs at the higher concentration selected showed almost 80% cell death in case of A 375 in the MTT assay whereas it was only around 30% in case of HDF. The FCNGs on the other hand killed only 50% of cells in case of A375 and 40% in case of HDF. The cell uptake studies carried out by fluorescent microscopy indicated good uptake of CNGs, CCNGs and FCNGs by A 375 as well as HDF cells. DAPI/actin staining showed cellular localization of Rhod-CNGs with no signs of change in morphology in either of these cells. The same with
Rhod-CCNGs and Rhod-FCNGs were also studied. Both the cells showed cellular localization and in case of Rhod-CCNGs an obvious change in morphology was visible in case of A375 but not in HDF indicating the specificity towards cancer cells. Confocal images were taken to confirm the cellular internalization of Rhod-CCNGs and Rhod-FCNGs. In case of Rhod-CCNGs there was significant difference in fluorescent intensity as we go deeper into the cells and deeper penetration was shown in A 375 compared to HDF whereas such a difference in internalization was not found for Rhod-FCNGs. The apoptotic effect of CCNGs was analyzed by a flow-cytometric assay in which CCNGs at the higher concentration of the cytotoxic range showed comparable apoptosis as the control curcumin, and there was negligible apoptosis induced by the control chitin nanogels. The FCNGs as well as control 5-FU even at higher concentrations showed very less apoptotic effect on melanoma cells. The apoptotic effect of both these drugs on HDF cells were found to decrease after conjugation with chitin in the nanogel formulation. The results of the skin permeation studies showed a 4-fold increase in steady state transdermal flux of curcumin in case of CCNGs as compared to that of control curcumin. But in case of FCNGs, such an enhancement of transdermal penetration was not seen as compared to control 5 FU. The fluorescent images of the skin samples from penetration studies as well concentration depth profile determination showed increased retention of CCNGs as well as Rhod-FCNGs in the deeper skin layers compared to the corresponding control samples. The histopathology studies of the porcine skin samples treated with the prepared materials showed loosening of the horny layer of the epidermis, facilitating penetration with no observed signs of inflammation. These results suggest that the formulated CCNGs offer specific advantage than FCNGs, for the treatment of melanoma, the most common and serious type of skin cancer, by enhanced transdermal permeation and retention.