Lactoferrin-Conjugated Dendritic Nanoconstructs for Lung Targeting of Methotrexate

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ABSTRACT: The present investigation was aimed at developing and exploring the potential of lactoferrin (Lf)-conjugated dendritic nanocomposite for lung targeting of methotrexate (MTX). The 5.0G poly(propylene imine) (PPI) dendrimer and Lf-conjugated 5.0G PPI dendrimer were synthesized and characterized by Fourier-transform infrared spectroscopy, nuclear magnetic resonance, and transmission electron microscopy . The entrapment efficiency, *in vitro* release, and hemolytic toxicity were assessed. Pharmacokinetic and organ distribution studies were carried out to evaluate *in vivo* targeting potential of developed system. The pharmacokinetic studies showed that elimination half-life of MTX-loaded plain PPI dendrimer (10.41 ± 2.12 h, $p < 0.05$) and MTX-loaded Lf-conjugated PPI dendrimer (12.23 ± 1.53 h, $p < 0.01$) was significantly higher than the free drug (5.85 ± 1.19 h). Organ distribution assessment of different formulations displayed significant ($p < 0.05$) higher accumulation of drug in lungs by MTX–Lf–PPI (1329 ± 26.7 ng/g of tissue) as compared with MTX–PPI (721 ± 23.4 ng/g of tissue) and free MTX (575 ± 19.7 ng/g of tissue) after 6 h of administration. The result suggested that Lf-conjugated 5.0G PPI dendrimer-based formulations to be approximately 1.5 times and 2.5 times superior to plain 5.0G PPI dendrimer as well as pure MTX, respectively, for lung targeting of anticancer drugs.
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Keywords: dendrimers; methotrexate; lactoferrin; lung targeting; nanoconstructs; pulmonary drug delivery; cancer chemotherapy; drug targeting; macromolecular drug delivery

INTRODUCTION

Lung cancer is the major cause of cancer mortality worldwide. It is still a serious malignancy in many cases due to the lack of major advancements in treatment strategy.[1] Current treatments for lung cancer have shown little success because they cannot cure disseminated tumors with an acceptable level of toxicity. Thus, one alternative strategy that has shown promise in the treatment of lung cancer is targeted therapy.[2] Targeted therapy was conceptualized as a means of exploiting specific molecular alterations associated with cancers in order to selectively kill transformed cells and spare normal healthy tissues. Targeted therapies are anticipated to have fewer associated toxicities than standard chemotherapies, which rely predominately on increased rates of cell division to enhance killing of the tumor cells compared with healthy tissues. For tumors that are treated with radiation and/or surgery, systemically delivered targeted therapies also have the potential to eliminate micrometastases that might not be eliminated with radiation therapy (RT) and/or surgery.[3]

Drug delivery to lungs appears to be an attractive proposition on account of large surface area of alveolar region. It provides tremendous opportunities to improve drug therapies using novel drug delivery systems. Lower thickness of the epithelial barrier, extensive vascularization, relatively low proteolytic activity in the alveolar space as compared with other routes of administration, and absence of the first-pass metabolism facilitate enhanced systemic availability of the active medicament.[4] Ranney[5] demonstrated the importance of the targeted drug delivery to the lungs and pointed out that diseases located in this region lend themselves to a drug-targeting approach because of ready access to these organs by both the intravenous (i.v.) and intratracheal routes. Carrier systems play an important role in the targeting of drug(s), proteins, and peptides. Carriers could supplement sustained drug delivery to the lungs, extend duration of action, reduce therapeutic dose, improve

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patient compliance, and reduce adverse effects of highly toxic drugs.[6] The targeting of drugs to the lungs can deliver therapeutic agents to the diseased regions by reducing their distribution to the nontarget organs.[7]

Lactoferrin (Lf) is a nonheme iron-binding protein that is part of the transferrin protein family, along with serum transferrin, ovatransferrin, melanotransferrin and the inhibitor of carbonic anhydrase,[8] and cationic in nature.[9] Lf was investigated as a targeting ligand for receptor-mediated gene delivery to human bronchial epithelial cells. A high number of Lf receptors (LfRs) were detected on bronchial epithelial cells (BEAS-2B) but not on alveolar epithelial (A549) cells by fluorescence microscopy and FACS measurements, suggesting potential targeting selectivity for bronchial epithelial cells. Previous studies have demonstrated that LfRs are expressed on the apical surface of bronchial epithelial cells.[10] Thus, Lf may serve as a suitable targeting ligand for receptor-mediated delivery to the lung for treating specific lung cancers originating from bronchioles (Fig. 1), for example, nonsmall cell lung carcinoma, which usually starts near a central bronchus or in peripheral lung tissue.

Dendrimers are unimolecular polymeric systems synthesized in a reiterative manner. At the same time, their synthesis can be so optimized as to control their size, shape, molecular mass, composition, and reactivity.[11] Dendrimers have hyperbranched structure with precisely placed functional groups that bear important role in controlling the properties of therapeutic moieties that are encapsulated or complexed with it.[12,13] Most eminent properties of dendrimer are its monodispersive nature, globular shape, and highly controlled architecture, which also makes them efficient carrier system for drugs.[14,15] In recent times, many advancements have occurred in the utilization of dendrimers to treat cancer, including their use as delivery systems for potent anticancer drugs.[16] The present study was aimed at developing and exploring the Lf-conjugated Poly(propylene imine) (PPI) 5.0G dendrimers for targeting to lungs. Methotrexate (MTX) was taken as a model anticancer drug.

EXPERIMENTS

Materials

Methotrexate hydrate was a benevolent gift from M/s Khandelwal Laboratories, (Mumbai, Maharashtra, India), and Lf was a kind gift from Dr. T.P. Singh, All India Institute of Medical Sciences (New Delhi, India). Ethylene diamine, acrylonitrile, and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from CDH (New Delhi, India). Cellulose dialysis bag (MWCO 6–7 kDa), was purchased from Sigma, whereas Reney Nickel was purchased from Merck India. All other chemicals were of reagent grade and purchased from CDH.

Synthesis of 5.0GPPI Dendrimer

Poly(propylene imine) dendrimers (5.0G) were synthesized by divergent method reported earlier.[17,18] Briefly, double Michael addition reaction method was used to produce half generation (-CN terminated) by

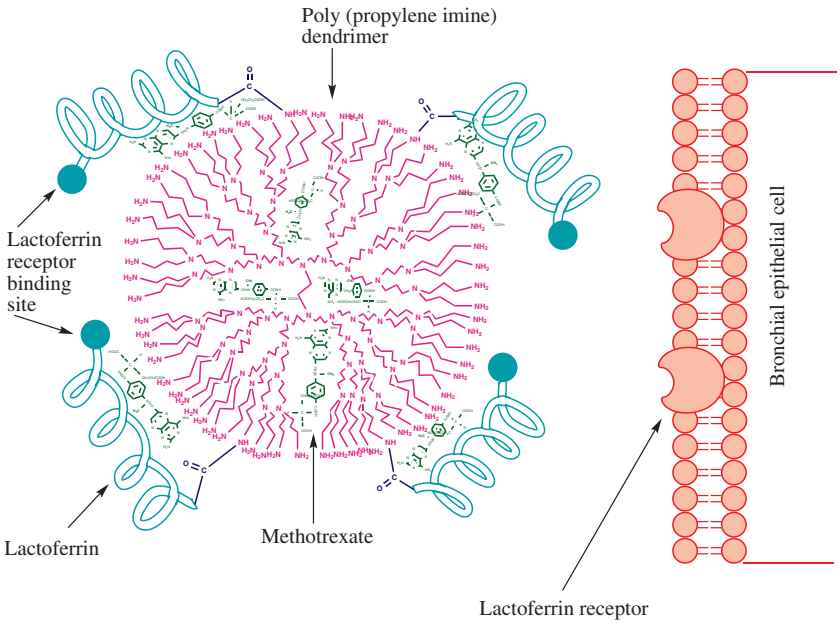

Figure 1. Schematic diagram of methotrexate-loaded Lactoferrin-Conjugated dendrimer for targeting lactoferrin receptors in bronchial epithelial cells.

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