METHODS AND COMPOSITIONS FOR LIPIDIZATION OF HYDROPHILIC MOLECULES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a division of appl. Ser. No. 08/524,362, filed Sep. 5, 1995 now U.S. Pat. No. 4,907,030, which is a continuation-in-part of appl. Ser. No. 08/349,717, filed Jan. 25, 1995, abandoned.

Statement Regarding Federally Sponsored Research or Development

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BACKGROUND OF THE INVENTION

The present invention relates generally to the fields of biology and medicine. More particularly, the present invention is directed to methods and compositions useful in increasing in mammals the absorption and retention of hydrophilic mole cues, in particular peptides and proteins.

Advances in biotechnology have made possible the production of large amounts of therapeutically active and pure proteins and peptides. Currently, the therapeutic effects of most of these agents can be achieved only when they administered via invasive routes, such as by injection. Since most proteins have very short half lives, effective concentrations of these agents can be maintained only when administered by frequent injections.

Although the administration of proteins by injection is the most effective means of their delivery in vivo, patient tolerance of multiple injections is very poor. In addition, the administration of drugs via the injection routes is a skilled job and requires training; this skill and training may not always be transferable to patients. In cases where protein drugs have a life-saving role, the administration by the injection route can be accepted by the patients. However, in cases where protein drugs are just one of several possible therapies, injections of proteins and peptides are unlikely to be accepted by the patients. Therefore, alternative routes of protein and peptide delivery need to be developed.

Alternative routes of protein and peptide delivery may include the buccal, nasal, oral, pulmonary, rectal and ocular routes. Without exception, these routes are less effective than the parenteral routes of administration. However, these routes of protein and peptide delivery are still far more attractive than the parenteral routes because they offer convenience and control to the patients. The oral route is particularly attractive because it is the most convenient and patient-compliant.

Mucosal barriers, which separate the inside of the body from the outside (e.g. GI, ocular, pulmonary, rectal and nasal mucosa), comprise a layer of tightly joined cell monolayers which strictly regulates the transport of molecules. Individual cells in barriers are joined by tight junctions which regulate entry into the intercellular space. Hence, the mucosa is at the first level a physical barrier, transport through which depends on either the transcellular or the paracellular pathways [Lee, V. H. L. (1988) *CRC Critical Rev. Ther. Drug Delivery Sys.* 5, 69–97].

Paracellular transport through water filled tight junctions is restricted to small molecules (MW<1kDa) and is essentially a diffusion process driven by a concentration gradient across the mucosa [Lee (1988), supra; Artursson, P., and Magnusson, C. (1990) *J. Pharm. Sci.* 79, 595–600]. The tight junctions comprise less than 0.5% of the total surface area of the mucosa [Gonzalez-Mariscal, L. M. et al. (1985) *J. Membrane. Biol.* 86, 113–125; Vetvicka, V., and Lubor, F. (1988) *CRC Critical Rev. Ther. Drug Deliv. Sys.* 5, 141–170]; therefore, they play only a minor role in the transport of protein drugs across the mucosa.

The transcellular transport of small drugs occurs efficiently provided the physiochemical properties of the drug are suited to transport across hydrophobic cell barriers. However, the transcellular transport of proteins and peptides is restricted to the process of transcytosis [Shen, W. C. et al. (1992) *Adv. Drug Delivery Rev.* 8, 93–113]. Transcytosis is a complex process in which proteins and peptides are taken up into vesicles from one side of a cell, and are subsequently shuttled through the cell to other side of the cell, where they are discharged from the endocytic vesicles [Mostov, K. E., and Semister, N. E. (1985) Cell 43, 389–390]. The cell membrane of mucosal barriers is a hydrophobic lipid bilayer which has no affinity for hydrophilic, charged macromolecules like proteins and peptides. In addition, mucosal cells may secrete mucin which can act as a barrier to the transport of many macromolecules [Edwards, P. (1978) *British Med. Bull.* 34, 55–56]. Therefore, unless specific transport mechanisms exist for protein and peptide, their inherent transport across mucosal barriers is almost negligible.

In addition to providing a tight physical barrier to the transport of proteins and peptides, mucosal barriers possess enzymes which can degrade proteins and peptides before, after, and during their passage across the mucosa. This barrier is referred to as the enzymatic barrier. The enzymatic barrier consists of endo- and exopeptidase enzymes which cleave proteins and peptides at their terminals or within their structure. Enzymatic activity of several mucosa have been studied and the results demonstrated that substantial protease activity exists in the homogenates of buccal, nasal, rectal and vaginal mucosa of albino rabbits and that these activities are comparable to those present in the ilium [Lee et al. (1988), supra]. Therefore, regardless of the mucosa being considered, the enzymatic barrier present will feature strongly in the degradation of the protein and peptide molecules.

The N and the C termini of peptides are charged and the presence of charged side chains impart highly hydrophilic characteristics on these macromolecules. In addition, the presence of charged side chains means that proteins and peptides have strong hydrogen binding capacities; this H-binding capacity has been demonstrated to play a major role in inhibiting the transport of even small peptides across cell membranes [Conradi, R. A. et al. (1991) *Pharm. Res.* 8, 1453–1460]. Therefore, the size and the hydrophilic nature of proteins and peptides combine to severely restrict their transport across mucosal barriers.

One approach that has been used to alter the physical nature of the mucosal barriers is the use of penetration enhancers. The use of penetration enhancers is based on the disruption of the cell barriers by the use of low molecular weight agents which can fluidize cell membranes [Kaji, H. et al. (1985) *Life Sci.* 37, 523–530], open tight junctions [Inagaki, M. et al. (1985) *Rhinology* 23, 213–221], and create pores in the cell membrane [Gordon, S. et al. (1985) *Proc. Natl. Acad. Sci U.S.A.* 82, 7419–7423; Lee, V. H. L. et al. (1991) *Critical Reviews in Therapeutic Drug Carrier Systems, CRC Press* 8, 91–192]. The use of these agents leads to a non-specific loss of barrier integrity and can lead