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Nasal administration of gentamicin using a novel microsphere delivery system

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Summary

The concept of improving the bioavailability of nasally administered drugs by means of a gelling microsphere delivery system has been investigated in rats and sheep using gentamicin as a model drug. It was found that the combination of gentamicin with the microsphere delivery system increased the uptake of the drug across the nasal membrane. This uptake was further enhanced by incorporation of an absorption enhancer in the form of lysolecithin into the microspheres. In this way the bioavailability for nasally administered gentamicin was increased to about 50% of the i.v. dose as compared to less than 1% for a simple nasal gentamicin solution.

Introduction

Nasal administration of drugs is becoming increasingly important, largely because of the advent of molecules that are poorly absorbed from the gastrointestinal tract. Such molecules are often in the form of polypeptides or even small proteins. A detailed review of the literature on nasal delivery of drugs has recently been provided by Chien and Chang (1987). Commercially available products that are given via the nasal route include LHRH, vasopressin and analogues, and calcitonin. Furthermore, reports in the literature suggest that insulin and growth hormone may be possible candidates for this mode of administration (Ponteroli et al., 1986; Moore et al., 1986).

The nasal route for the administration of so-called difficult molecules exploits the fact that the nasal mucosa has far higher permeability than other mucosal surfaces to include the various regions of the gastrointestinal tract, buccal and vaginal cavities. Fisher et al. (1987) have studied the relationship between the molecular weight of hydrophilic compounds and their systemic absorption via the nasal route and found that although the uptake of drugs decreased with increasing molecular weight, polar molecules of quite high molecular weight could be taken up to a significant extent. Thus, about 4% of dextran 70 (mol.wt. 70,000 Da) was found to cross the nasal membrane in in situ experiments in rats. Similarly, MacMartin et al. (1987) have studied literature values of bioavailability for a variety of compounds and found that the transport across the nasal membrane falls off sharply at molecular weights higher than 1000.

Low molecular weight drugs such as propranolol and steroids were shown to be well absorbed

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after administration using nasal spray and indeed, in some cases, the resultant blood level vs time profiles are almost indistinguishable from i.v. administered drugs (Hussain et al., 1979, 1980a and b, 1981; David et al., 1981). However, generally speaking, polar species including peptides and proteins are not sufficiently well absorbed after nasal administration to reach clinically relevant plasma levels. As a consequence, various means of enhancing the absorption of such compounds have been investigated, including the use of so-called membrane modifiers and flux enhancers in the form of bile salts (and their analogues) and surfactants that modify the properties of the nasal mucosa thereby enhancing uptake. Thus, Hanson et al. (1986) reported that the nasal absorption of the peptide salmon calcitonin was increased 10-fold using an enhancer system (as expressed by the area under the blood level–time profile (AUC)) as compared to a non-enhanced system. Similarly, the striking effect of increasing amounts of bile salt (sodium deoxycholate) on the absorption of insulin has been well described by Gordon et al. (1985) and by Longenecker et al. (1987), the latter group using a fusidic acid derivative.

Generally, absorption enhancers are believed to function by changing the permeability of the nasal membrane, by reducing the viscosity of the mucus or by inhibiting the enzymes present in the nasal mucosa (e.g. peptidases and proteases) or by a combination of these effects. Thus, enhancer systems could well have an impact on the nasal membrane by disturbing the integrity of the membrane and hence produce possible permanent damage to the membrane, including the cilia, especially when a delivery system is used to treat a chronic disease such as diabetes (Hersey and Jackson, 1987).

We would argue that too much attention has been focussed on drug absorption per se and not enough on other physicochemical and biological factors that may well limit the bioavailability of administered compounds. Some compounds could well have satisfactory absorption characteristics if sufficient time was given for contact with the absorptive regions of the nasal mucosa. That is, poor absorption may not necessarily be related to insufficient permeability. Rapid clearance by the mucociliary clearance mechanism in the nasal cavity may be an equally important factor (Illum, 1986, 1987).

Scintigraphic studies have shown previously that a nasally administered solution or powder is cleared extremely rapidly from the nasal cavity of man with a half-life of clearance of approximately 15–20 minutes (Hardy et al., 1985; Illum et al., 1987). In contrast, the so-called gelling microspheres made from biocompatible materials such as starch, albumin, gelatin and dextran were retained in the nasal cavity with half-life of clearance of 3 h or longer. These types of bioadhesive microsphere delivery systems have now been investigated for their ability to alter the bioavailability of nasally administered drugs. The polar antibiotic material gentamicin was selected as a model compound in order to investigate the concept of improving the bioavailability of drugs through the use of gelling microspheres administered intranasally. It has been reported by Duchateau et al. (1986) that gentamicin given via the nasal route is only negligibly absorbed, probably due to its high polarity. The absorption was, however, improved considerably by the coadministration of bile salts.

In the present studies an alternative absorption enhancer, in the form of lysophosphatidylcholine, has been investigated. This material is known to be membrane-active in low concentrations. Furthermore, it may be a more acceptable material from a regulatory standpoint in that it is a natural material that is converted within the membrane to intact phosphatides which are normal cell components and after administration are able to enter the metabolic pool (De Vries et al. 1985; Christiansen and Carlsen, 1983).

The experiments in this study have been performed using the in situ rat model modified from Hirai et al. (1981) and an in vivo animal model using sheep.

Materials and Methods

Materials

Gentamicin sulphate and L-α-lysophosphatidylcholine were purchased from the Sigma Chemical Company Ltd. (Dorset, U.K.). The degradable

starch microspheres (mean size 48 μm) (DSM), were a gift from Pharmacia A/B (Uppsala, Sweden). All other chemicals were of reagent grade.

For the sheep studies the gentamicin was prepared as a solution in isotonic phosphate buffer pH = 7.4 at a concentration of 386 mg/ml and for the rat studies at 20 mg/ml. For some experiments the gentamicin solution contained lysophosphatidylcholine at a concentration of 0.2% w/v.

Preparation of drug loaded microspheres

The microspheres loaded with gentamicin and used for the sheep experiments were prepared by adding the gentamicin solution to the freeze-dried starch microspheres, leaving the preparation for 24 h and then freeze drying until a light powder was obtained. A typical concentration of gentamicin in the microsphere preparation was 1.9 mg gentamicin/mg starch microspheres. In some experiments, L-α-lysophosphatidylcholine (LPC) was added to the preparation before freeze drying in a concentration of 0.075 mg LPC/mg starch microspheres.

Animal experiments

Rat studies

The rat in situ experimental model described by Hirai et al. (1981) and modified by Fisher et al. (1987) was used to study the effect of LPC on the absorption of gentamicin in solution. Male Wistar rats (JABU, Sutton Bonington, U.K.) (3 in each group) of about 200 g were anaesthesized by i.p. injection of 60 mg/kg of pentobarbitone (60 mg/ml, Sagatal, May and Baker). The rats were tracheotomized, the oesophagus sealed and the carotid artery cannulated. 50 μl of the gentamicin solution containing 2% of the drug with and without added LPC (0.2%) was instilled into the nasal cavity. Blood samples (150 μl) were withdrawn from the carotid artery at 0, 5, 10, 15, 30, 45, 60 and 120 min after drug administration. The serum was separated by centrifugation and the samples were stored at −20 °C awaiting analysis. The gentamicin level was determined by the EMIT technique as described by O'Connell et al. (1984) with a lower level of sensitivity of 0.2 μg/ml.

Sheep studies

Gentamicin was also administered to sheep in order to study nasal absorption in a model where a normal mucociliary clearance mechanism was in operation.

Fourteen cross-bred (Suffolk and Texel) sheep were divided into 4 groups of 3 and 1 group of 2. The mean weight of the sheep was about 40 kg.

The animals were not fasted prior to the administration of gentamicin. An in-dwelling Viggo secalon universal central venous catheter of 1.2 mm, i.d. with a secalon universal flow switch was placed in the right jugular vein of each animal on the first day of the study and whenever necessary was kept patent by flushing with heparinised normal saline (50 IU/ml). The catheter was removed upon completion of the study. For intranasal administration, the sheep were sedated by an i.v. dose of ketamine hydrochloride at 2 mg/kg to prevent sneezing during administration. The sedation lasted about 3 minutes. The animals that received gentamicin by the i.v. route were also sedated.

For the intranasal administration of solutions a blueline umbilical cannula of 35 cm length (size 6 FG, Portex Ltd. Hythe, Kent, U.K.) was inserted into the nostril of the sheep to a preset depth of 10 cm before the delivery of the solution from a 1 ml syringe. For intranasal administration of powdered formulations a BOC endotracheal tube (red rubber, cuffed) of 6.5 mm was loaded with the powder formulation and then inserted into the nostril of the sheep to a preset depth of 6 cm before blowing the powder into the nasal cavity.

The first group of sheep ($n = 2$) was given 0.25 ml of gentamicin solution (386 mg/ml) (5.0 mg/kg) into each nostril. The second group ($n = 3$) received 0.25 ml of gentamicin solution (386 mg/ml) (5.0 mg/kg) containing 0.2% w/v LPC into each nostril. The third group ($n = 3$) received 5.0 mg/kg gentamicin in combination with starch microspheres (1.9 mg gentamicin/mg starch microspheres). The fourth group of sheep ($n = 3$) received 5.0 mg/kg gentamicin in combination with 0.2 mg/kg LPC and starch microspheres (1.9

mg gentamicin/mg starch microspheres). The last group of sheep ($n = 3$) was given 2 mg/kg gentamicin administered intravenously as a solution (40 mg/ml) through the jugular vein.

Blood samples (2 ml) were collected through the jugular vein at 0, 8, 16, 24, 32, 45, 60, 90, 120, 180 and 240 min after drug administration. The serum was separated by centrifugation and the samples were stored at −20 °C awaiting analysis. No heparin was added to any of the samples. The gentamicin levels were determined by the EMIT technique as described for the rat studies. To minimise any possible contamination with heparin from the catheter the first 0.5 ml of blood was always discarded.

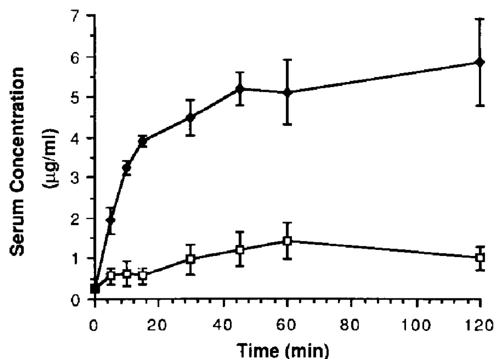

Fig. 1. Intranasal administration of gentamicin (5 mg/kg) to rats (in situ model) in a phosphate buffer solution (20 mg/ml) (□) and in a phosphate buffer solution with added lysophosphatidylcholine (2 mg/ml) (◆); mean ± S.E.M.

Results and Discussion

Rat studies

Studies were performed in rats to investigate whether the gentamicin could be absorbed when the mucociliary clearance mechanism was impaired and whether the biological enhancer system would increase the absorption. The effect of the LPC enhancer is demonstrated in Fig. 1. The administration of gentamicin solution alone resulted in a poor bioavailability whereas adding the enhancer system gave rise to a 5-fold greater peak level. The AUC's (from $t = 0$ to $t = 120$ min) were 128 and 577 $\mu g \cdot min/ml$ respectively.

Sheep studies

Having demonstrated the success of the absorption enhancer in rat it was important to test the concept of bioadhesion and enhancer systems in a model such as sheep where clearance was unimpeded.

Gentamicin was administered intranasally to the sheep as a simple solution and together with LPC (Fig. 2). Neither system gave rise to a significant level of gentamicin in the plasma. These data demonstrate clearly the difference between an in situ rat model, where the drug is allowed to remain in contact with the absorptive surface for a long period of time together with any enhancing agent, and the far more physiologically relevant situation where clearance can take place.

If the gentamicin is then administered in combination with the starch microspheres, a significant increase in bioavailability is obtained (Fig. 2). An even more dramatic effect is seen when the gentamicin plus enhancer are administered in the form of the starch microsphere formulation, the blood level peaking at 6.3 $\mu g/ml$ as compared to 0.4 $\mu g/ml$ for gentamicin solution. The combination of microspheres with LPC enhancer provides a blood level–time profile that is very similar to that obtained when gentamicin is given i.v. (Fig. 2). Accounting for the doses administered the bioavailabilities for the intranasally administered

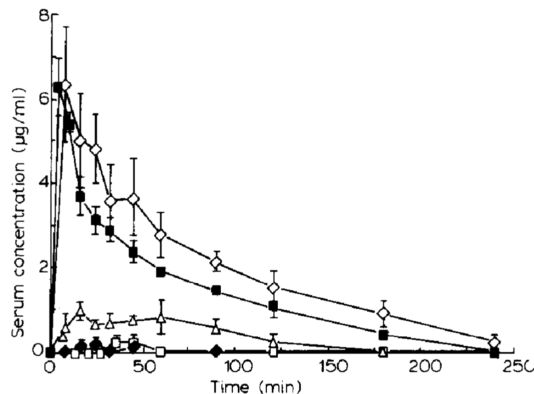

Fig. 2. Intranasal and i.v. administration of gentamicin to sheep (mean ± S.E.M.). □, intranasal gentamicin solution (5 mg/kg) ($n = 2$); ◆, intranasal gentamicin solution (5 mg/kg), containing 2 mg/ml lysophosphatidylcholine (0.026 mg/kg) ($n = 3$); △, intranasal gentamicin (5 mg/kg) in combination with starch microspheres ($n = 3$); ◇, intranasal gentamicin (5 mg/kg) in combination with starch microspheres and lysophosphatidylcholine (0.2 mg/kg) ($n = 3$); ■, i.v. gentamicin solution (2 mg/kg) ($n = 3$).

gelling microsphere system and for the microspheres in combination with LPC were 9.7% and 57.3%, respectively, as compared to the i.v.-dose.

Thus, bioadhesive microspheres such as those made from starch are indeed able to significantly enhance the nasal absorption of poorly absorbed polar molecules and should have great potential as a nasal delivery system for administration of drugs such as peptides and proteins. The gelling microsphere concept should be applicable to a whole range of the so-called difficult molecules. For those of low molecular weight, it should be possible to enhance bioavailability considerably without the use of additional additives, while for larger molecular weight drugs, absorption enhancers such as surfactants, enzymatic inhibitors or mucolytic agents may be required. Preliminary results have shown that by means of the novel bioadhesive starch microsphere system it is indeed possible to significantly enhance the absorption of large molecular weight peptides such as insulin across the nasal membrane in different animal models (Illum et al., to be published).

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References

Chien, Y.W. and Chang, S.-F., Intranasal drug delivery for systemic medications. *CRC Crit. Rev. Ther. Drug Carrier Syst.*, 4 (1987) 66–194.

Christiansen, K. and Carlsen, J., Reconstitution of a protein into lipid vesicles using natural detergents. *Biochim. Biophys. Acta*, 735 (1983) 225–233.

David, G.F.X., Puri, C.P. and Anand Kumar, T.C., Bioavailability of progesterone enhanced by intranasal spraying. *Experientia*, 37 (1981) 533–534.

De Vries, A.C.J., Batenburg, J.J. and Van Golde, L.M.G., Lysophosphatidylcholine acyltransferase and lysophosphatidylcholine: lysophosphatidylcholine acyltransferase in alveolar type II cells from fetal rat lung. *Biochim. Biophys. Acta*, 833 (1985) 93–99.

Duchateau, G.S.M.J.E., Zuidema, J. and Merkus, W.H.M., Bile salts and intranasal drug absorption. *Int. J. Pharm.*, 31 (1986) 193–199.

Fisher, A.N., Brown, K., Davis, S.S., Parr, G.D. and Smith, D.A., The effect of molecular size on the nasal absorption of water soluble compounds by the albino rat, *J. Pharm. Pharmacol.*, 39 (1987) 357–362.

Gordon, G.F., Moses, A.C., Silver, R.D., Flier, G.F. and Carey, E., Nasal absorption of insulin: enhancement by hydrophobic bile salts, *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 7419–7423.

Hanson, M., Gazdick, G., Cahill, J. and Augustine, M., Intranasal delivery of the peptide, salmon calcitonin. In S.S. Davis, L. Illum and E. Tomlinson (Eds.) *Delivery systems for peptide drugs*, Plenum, New York, 1986, pp 233–242.

Hardy, J.G., Lee, S.W. and Wilson, C.G., Intranasal drug delivery by sprays and drops. *J. Pharm. Pharmacol.*, 37 (1985) 294–297.

Hersey, S.J. and Jackson, R.T. Effect of bile salts on nasal permeability in vitro. *J. Pharm. Sci.*, 76 (1987) 876–879.

Hirai, S., Yashiki, T., Matsuzawa, T. and Mima, H., Absorption of drugs from the nasal mucosa of rat. *Int. J. Pharm.*, 7 (1981) 317–325.

Hussain, A., Hirai, S. and Bawarshi, R., Nasal absorption of propranolol in rats. *J. Pharm. Sci.*, 68 (1979) 1196.

Hussain, A., Foster, T., Hirai, S., Kashihara, T., Batenhorst, R. and Jones, M., Nasal absorption of propranolol in humans. *J. Pharm. Sci.*, 69 (1980a) 1240.

Hussain, A., Hirai, S. and Bawarshi, R., Nasal absorption of propranolol from different dosage forms by rats and dogs, *J. Pharm. Sci.*, 69 (1980b) 1411–1413.

Hussain, A., Hirai, S. and Bawarshi, R. Nasal absorption of natural contraceptive steroids in rats — progesterone absorption. *J. Pharm. Sci.*, 70 (1981) 466–467.

Illum, L., Drug delivery systems for nasal application, *S.T.P. Pharma*, 3 (1987) 594–598.

Illum, L. Microspheres as a potential controlled release nasal drug delivery system. In S.S. Davis, L. Illum and E. Tomlinson (Eds.) *Delivery systems for peptide drugs*, Plenum, New York, 1986, pp 205–210.

Illum, L., Jorgensen, H., Bisgaard, H., Krogsgaard, O. and Rossing, N., Bioadhesive microspheres as a potential nasal drug delivery system, *Int. J. Pharm.*, 39 (1987) 189–199.

Longenecker, J.P., Moses, A.C., Flier, J.S., Silver, R.D., Carey, M.C. and Dubovi, E.J., Effects of sodium taurodihydrofusidate on nasal absorption of insulin in sheep. *J. Pharm. Sci.*, 76 (1987) 351–355.

McMartin, C., Hutchinson, L.E.F., Hyde, R. and Peters, G.E., Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity, *J. Pharm. Sci.*, 76 (1987) 535–540.

Moore, J.A., Wilkin, H. and Daugherty, A.L., Delivery systems for recombinant methionyl human growth hormone. In S.S. Davis, L. Illum and E. Tomlinson (Eds.), *Delivery Systems for Peptide Drugs*, Plenum, New York, 1986, pp 317–329.

O'Connell, M.B., Heim, K., Halstenson, C. and Matzke, G.R. Heparin interference with tobramycin, netilmicin and gentamicin concentrations determined by EMIT. *Drug Intell. Clin. Pharm.*, 18 (1984) 503–504.

Ponteroli, A.E., Alberetto, M. Calderara, A., Pajetta, E. and Pozza, G., Metabolic effects of intranasally administered insulin and glucagon in man. In S.S. Davis, L. Illum and E. Tomlinson (Eds.), *Delivery Systems for Peptide Drugs*, Plenum, New York, 1986, pp,. 243–248.