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Cyclodextrins in nasal drug delivery

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Abstract

Nasal drug delivery is an attractive approach for the systemic delivery of high potency drugs with a low oral bioavailability due to extensive gastrointestinal breakdown and high hepatic first-pass effect. For lipophilic drugs nasal delivery is possible if they can be dissolved in the dosage form. Peptide and protein drugs often have a low nasal bioavailability because of their large size and hydrophilicity, resulting in poor transport properties across the nasal mucosa. Cyclodextrins are used to improve the nasal absorption of these drugs by increasing their aqueous solubility and/or by enhancing their nasal absorption. With several cyclodextrins very efficient nasal drug absorption has been reported, but also large interspecies differences have been found. Studies concerning the safety of cyclodextrins in nasal drug formulations demonstrate the non-toxicity of the cyclodextrins and also clinical data show no adverse effects. Therefore, some cyclodextrins can be expected to become effective and safe excipients in nasal drug delivery. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Nasal drug absorption; Nasal mucociliary clearance; Methylated β-cyclodextrins; Interspecies differences; Cyclodextrin toxicity; Insulin

Contents

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1. Introduction

The intranasal application of tobacco snuff, cocaine, various psychotropic and hallucinogenic agents has been known for a long time. It is therefore surprising that only in the past two decades intranasal administration of systemic drugs has attracted much attention. The nasal route circumvents the first-pass elimination associated with oral drug delivery. Furthermore, nasal drug delivery is an attractive alternative to the injection therapy, it is easily accessible and suitable for self administration. Despite numerous references in the recent literature on nasal drug delivery, the list of compounds that are currently on the market or investigated in patients or volunteers is limited. Examples are desmopressin, vasopressin, oxytocin, buserelin, nafarelin, calcitonin, insulin, glucagon, human growth hormone, butorphanol, dihydroergotamine, vit B12, metoclopramide, midazolam, nicotine, steroid hormones, scopolamine, sumatriptan.

The majority of the investigations published so far demonstrate, mainly in animal experiments, the large potential of nasal drug delivery, but only a few authors realize that large interspecies differences exist in the nasal absorption of drugs. In human subjects the potential for nasal drug formulations is limited to drugs which are active in a low dose and possess a sufficient aqueous solubility. Many lipophilic drugs are poorly soluble in water and large hydrophilic drugs like peptides and proteins show an insufficient nasal absorption. Cyclodextrins, especially methylated β-cyclodextrins, have proven to be excellent solubilizers and absorption enhancers in nasal drug delivery.

2. Cyclodextrins as excipients in nasal drug formulations

A pharmaceutical excipient used as solubilizer and absorption promoter in nasal drug delivery should be potent in a very low concentration, but inert from a pharmacological-toxicological point of view. This means that the selected excipient should (a) have no local or systemic effect, (b) exert no damage to the mucosal integrity, (c) show no severe ciliostatic effect, (d) enhance the drug permeation through the nasal epithelium in a transient and reversible way and (e) be non-irritating and non-allergenic. Also, the chemical and pharmaceutical quality of the selected cyclodextrin are important issues. For instance, methylated β-cyclodextrins are available in various qualities. It is possible to prepare a pure 2,6-dimethyl-β-cyclodextrin (DM-β-CD; degree of substitution 2.0), but selective methylation of the 2- and 4-OH group requires expensive solvents and a production process causing environmental pollution. Commercially available products consist of about 75% dimethylated β-cyclodextrin (of which 65% is 2,6 dimethylated). Randomly methylated β-cyclodextrin (RAMEB; degree of substitution 1.8) consists of about 50% dimethylated β-cyclodextrin (of which about 25% is 2,6 dimethylated), but the production is much cheaper, whereas the chemical and pharmaceutical properties are similar. An additional advantage is that the randomly methylated product is amorphous and avoids the risk of an in vivo crystallization. The (di)methylated β-cyclodextrins are extremely water soluble.

2.1. Lipophilic drugs

The nasal administration of the female steroid hormones estradiol and progesterone has been studied in animals and humans [1–4]. Nasal administration of estradiol makes it possible to decrease the dose administered compared to oral administration, circumventing high blood levels of the metabolite estrone and thus providing a physiological estrone/estradiol ratio [3,4]. Estradiol was administered with dimethyl-β-cyclodextrin to rats and rabbits, resulting in mean absolute bioavailabilities of 94.6% and 67.2%, respectively [1]. Also in oophorectomized women estradiol and dimethyl-β-cyclodextrin were administered nasally, giving a rapid absorption of

estradiol [3]. During a 6-month trial estradiol replacement therapy was achieved in oophorectomized postmenopausal women without side effects [3]. A combination of progesterone and estradiol with dimethyl-β-cyclodextrin was administered in rats and humans, resulting in nasal absorption comparable to the separate administration of both steroids [2,4].

The lipophilic antiviral drug pirodavir was given intranasally to humans, with 10% hydroxypropyl-β-cyclodextrin as solubilizer [5]. Frequent intranasal sprays (six times daily) were effective in preventing the development of clinical colds following experimentally induced rhinovirus infection. However, irritating effects of the formulation on the nasal mucosa were observed, such as nasal dryness and blood in the mucus. These side effects were attributed to the viscosity of the administration vehicle, and the high frequency of administration [5].

Dimethyl-β-cyclodextrin enhanced the nasal absorption rate of morphine and its entry into the cerebrospinal fluid, while 2-hydroxypropyl-γ-cyclodextrin sustained the plasma and cerebrospinal fluid levels of morphine. These experiments were carried out in rats [6]. Obviously there are species differences in the nasal absorption process, because in men other results were obtained. Merkus et al. studied in six male volunteers the bioavailability of two morphine HCl nasal formulations, containing 25 mg morphine base/ml = 2.5 mg/100 μl [7]. One of the formulations contained also 5% dimethyl-β-cyclodextrin. A single dose of 5 mg was administered by spraying 100 μl into each nostril, containing 2.5 mg morphine. Plasma samples for the determination of morphine were taken just prior to dosing and over 12 h after dosing. After a rapid nasal absorption of morphine (T_{max} about 30 min), levels declined after about 3 h below the limit of quantification of the HPLC method. The two nasal formulations revealed a similar morphine absorption (Fig. 1). The 5-mg dose resulted in peak plasma morphine levels of about 7 ng/ml, which are comparable to those published after a 10 mg dose of an immediate release tablet. The maximal levels of morphine 6-glucuronide were reached after about 2 h and were ~10 ng/ml, which is much lower than after oral administration, indicating the circumvention of the first-pass metabolism by nasal absorption.

An interesting example is the antimigraine drug

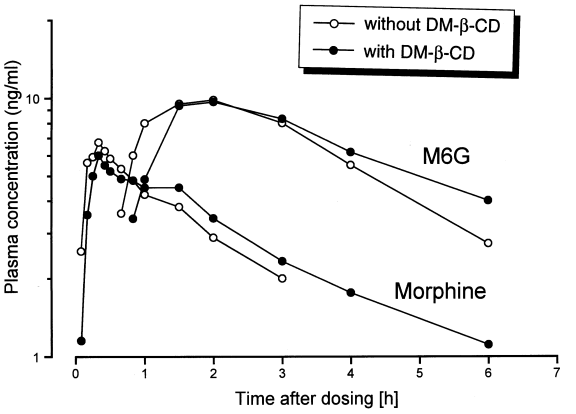

Fig. 1. Mean plasma levels of morphine and morphine 6-glucuronide (M6G) in six male volunteers after administration of 5 mg morphine in a nasal formulation with 5% dimethyl-β-cyclodextrin (●) and without dimethyl-β-cyclodextrin (○). Obviously the absorption profile of morphine is not influenced by the presence of the cyclodextrin [7].

dihydroergotamine (DHE). In a number of countries DHE is on the market as a nasal preparation (e.g. Migranal, Diergo). This nasal formulation contains 4 mg/ml DHE, glucose (5%) and caffeine (1%). The spray is available in an ampoule which has to be opened when the migraine attack occurs, then provided with a spraying device, and subsequently four puffs (two puffs in each nostril) of 0.125 ml, can be administered to achieve a dose of 2 mg DHE. This large volume of 0.500 ml that has to be administered and the fact that the open ampoule is only stable for 24 h are serious disadvantages.

New nasal DHE formulations have been developed by combining DHE with a cyclodextrin to enhance the concentration and improve the stability. Liquid and powder formulations were prepared, containing dihydroergotamine mesylate (DHEM) in combination with the cyclodextrin derivative RAMEB. In rabbits, liquid and powder formulations were compared with the currently available product and it turned out that it was possible to prepare a stable nasal formulation with a pharmacokinetic profile in rabbits similar to the product on the market [8]. Subsequently, five different preparations of DHEM (with at least 1-week interval) were administered in a randomized cross-over study to nine healthy human subjects [9]. Blood samples were taken at $t = 0$ and during 8 h after drug administra-

tion. The preparations and doses administered were: (A) DHEM i.m. 0.5 mg (Dihydergot, in which the drug is dissolved in an ethanol–glycerol–water solution); (B) DHEM nasal 2 mg as Diergo nasal spray, which means one puff of 0.125 ml in each nostril, repeated after 1 min, thus in total four puffs; (C) DHEM nasal 2 mg as liquid (DHEM 10 mg, RAMEB 20 mg, mannitol 50 mg, water 1 g), which means one puff of 0.100 ml in each nostril; (D) DHEM nasal 2 mg as powder (DHEM 2 mg, RAMEB 4 mg, lactose 4 mg), which means about 5 mg powder in each nostril; and (E) DHEM 2 mg oral as solution. No serious adverse effects were reported by the volunteers. No statistically significant difference in T_{max}, C_{max}, AUC and absorption rate could be found between the three nasal applications, indicating that the two DHEM/RAMEB formulations have pharmacokinetic properties which are comparable to the currently available product. The preference of the volunteers was clearly in favour of the liquid DHEM/RAMEB nasal spray, compared to the Diergo ampoule-spray because (i) a much less complicated handling of the spray and (ii) reduction of the number of puffs from four to two [9]. The better stability of the novel formulations is an additional pharmacoeconomical advantage.

Recently another report appeared on a lipophilic drug administered nasally in combination with a cyclodextrin to human volunteers. In eight volunteers the nasal absorption of melatonin in combination with β-cyclodextrin has been investigated. The nasal absorption of melatonin appeared to be extremely fast and efficient. In contrast, the oral absorption of melatonin is much slower and the oral bioavailability of melatonin is low and variable, due to a high first-pass metabolism (Fig. 2) [10]. In relative terms the peak levels of melatonin after nasal administration appear to be about 50-times higher than after oral administration, demonstrating the efficiency of nasal drug absorption of some drugs.

2.2. Hydrophilic drugs

Nasal absorption in human subjects of hydrophilic drugs, e.g. peptides and proteins, is rather low and decreases with an increasing molecular size. In numerous animal studies, it has been demonstrated that cyclodextrins, particularly α-cyclodextrin and

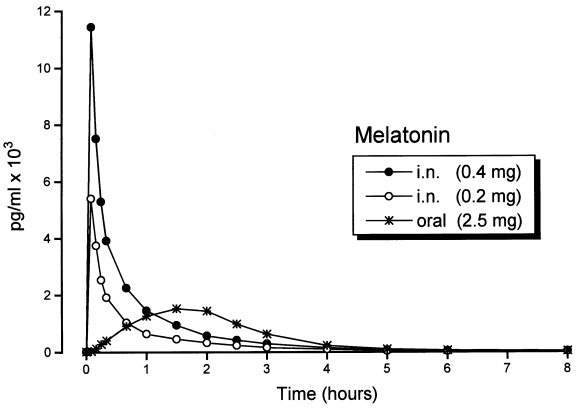

Fig. 2. Mean plasma levels of melatonin in eight male volunteers after administration of a nasal formulation containing melatonin and 0.75% β-cyclodextrin in a dose of 0.2 mg (○) and 0.4 mg (●) and after an oral dose of 2.5 mg (*) melatonin [10].

the methylated cyclodextrins, are efficient absorption enhancers. However, large interspecies differences have been observed between rats, rabbits, other animals and human subjects in nasal absorption of peptides and proteins.

2.2.1. Oligopeptide drugs

Oligopeptide drugs, such as an ACTH(4-9) analogue and the luteinizing hormone-releasing hormone analogues, buserelin and leuprolide, are absorbed in rats after nasal administration, but their nasal bioavailability is low. With cyclodextrins their nasal absorption can be increased considerably (Table 1).

In rats the mean absolute bioavailability of the ACTH(4-9) analogue, Org2766, appeared to increase to about 70%, using 2 and 5% dimethyl-β-cyclodextrin [11]. With 5% α-cyclodextrin a similar bioavailability could be obtained. Hydroxypropyl-β-cyclodextrin was not effective as an absorption enhancing compound [11]. To determine the duration of the enhancing effect of dimethyl-β-cyclodextrin on the nasal absorption, Org2766 was administered to rats 1 h after 5% dimethyl-β-cyclodextrin instillation in the nasal cavity [11]. The absorption of Org2766 was not enhanced in comparison with simultaneous dimethyl-β-cyclodextrin administration, indicating that the effect of dimethyl-β-cyclodextrin on the nasal mucosa is transient and reversible.

The absolute nasal bioavailability of buserelin in

Table 1
Cyclodextrins in nasal delivery of oligopeptides

Drug	M_w (kDa)	Cyclodextrin (CD)	CD amount/concentration	Species	Bioavailability (%)		Reference
Leuprolide	1.3	–	–	Rat	13		[13]
		α-CD	5%[a]	Rat	15		
		α-CD	5%[a]	Rat	24		
		α-CD	5%[a]	Rat	36		
		α-CD	5%[a]	Rat	21		
		α-CD	5%[a]	Rat	8		
		α-CD	5%	Human	2	4[b]	
ACTH(4-9)	0.9	–	–	Rat	13		[11]
Analogue		α-CD	5%	Rat	76		
(Org2766)		DM-β-CD	2%	Rat	70		
		DM-β-CD	5%	Rat	63	76[b]	
		HP-β-CD	5%	Rat	16		
		–	–	Rabbit	10		
		DM-β-CD	5%	Rabbit	17		
Buserelin	1.3	–	–	Rat	15		[12]
		DM-β-CD	80 mM	Rat	60		
		α-CD	80 mM	Rat	38		
		DM-α-CD	80 mM	Rat	37		
		β-CD	1.85 g/dl	Rat	30		
		CM-β-CD	80 mM	Rat	18		
		γ-CD	80 mM	Rat	17		
		HP-β-CD	80 mM	Rat	13		
		G_2-β-CD	80 mM	Rat	13		
		CM-α-CD	80 mM	Rat	8		
		HP-α-CD	80 mM	Rat	5		
		S-β-CD	80 mM	Rat	4		

All formulations were administered as drops.
[a]Administered in different volumes, ranging from 0.025 to 0.4 ml/kg.
[b]Dependent on the dose of the drug.
α-CD, α-cyclodextrin; DM-β-CD, dimethyl-β-cyclodextrin; HP-β-CD, hydroxypropyl-β-cyclodextrin; DM-α-CD, dimethyl-α-cyclodextrin; β-CD, β-cyclodextrin; CM-β-CD, carboxymethyl-β-cyclodextrin; γ-CD, γ-cyclodextrin; G_2-β-CD, maltosyl-β-cyclodextrin; CM-α-CD, carboxymethyl-α-cyclodextrin; HP-α-CD, hydroxypropyl-α-cyclodextrin; S-β-CD, β-cyclodextrin sulfate.

rats increased to 60% with 10% dimethyl-β-cyclodextrin as absorption enhancer [12]. Less effective were dimethyl-α-cyclodextrin, α-cyclodextrin and β-cyclodextrin, resulting in nasal buserelin bioavailabilities of 30 to 38% (Table 1). The least efficient cyclodextrins with respect to nasal buserelin absorption were (in order of decreasing potency): carboxymethyl-β-cyclodextrin = γ-cyclodextrin > hydroxypropyl-β-cyclodextrin = maltosyl-β-cyclodextrin. No absorption enhancement of nasally administered buserelin was observed for carboxymethyl-α-cyclodextrin, hydroxypropyl-α-cyclodextrin and β-cyclodextrin sulfate [12].

The nasal absorption of leuprolide in rats increased largely, to a bioavailability of 36%, after co-administration with 5% α-cyclodextrin, but in humans a bioavailability of only 4% could be obtained using the same absorption enhancer [13].

2.2.2. Polypeptide and protein drugs

Cyclodextrins have been used as absorption enhancers for calcitonin, glucagon, insulin and recombinant human granulocyte colony stimulating factor.

Calcitonin, a polypeptide of 3.4 kDa, has been administered intranasally in rats and rabbits with methylated β-cyclodextrin derivatives as absorption enhancers (Table 2) [14]. In a concentration of 5%, they enhance the nasal absorption of salmon cal-

Table 2
Cyclodextrins in nasal delivery of polypeptides and proteins

Drug	M_w (kDa)	Cyclodextrin	CD amount/ concentration	Formulation	Species	Bioavailabiity (%)	Calcium reduction (%)	References
Calcitonin	3.4	–	–	Drops	Rat		3	[14]
		RAMEB	5%	Drops	Rat		24	
		DM-β-CD	10%	Drops	Rat		23	
		DM-β-CD	5%	Drops	Rat		22	
		DM-β-CD	3%	Drops	Rat		23	
		DM-β-CD	2%	Drops	Rat		19	
		DM-β-CD	1%	Drops	Rat		11	
		TM-β-CD	5%	Drops	Rat		15	
		DM-β-CD	5%	Drops	Rabbit		10	
Glucagon	3.5	–	0	Spray	Rabbit	4		[15]
		DM-β-CD	5%	Spray	Rabbit	83		
		DM-β-CD	2 mg/kg	Powder	Rabbit	82		
		DM-β-CD	2%	Spray	Rabbit	43		
		DM-β-CD	0.8 mg/kg	Powder	Rabbit	45		
rhG-CSF	19	–	–	Drops	Rabbit	5[a]		[30]
		DM-β-CD	10 mg/kg	Drops	Rabbit	16[a]		
		α-CD	10 mg/kg	Drops	Rabbit	11[a]		[31]
		β-CD	10 mg/kg	Drops	Rabbit	11		
		γ-CD	10 mg/kg	Drops	Rabbit	3[a]		

[a]Bioavailability estimated from given AUC data.
rhG-CSF, recombinant human granulocyte colony-stimulating factor; RAMEB, randomly methylated β-cyclodextrin; DM-β-CD, dimethyl-β-cyclodextrin; TM-β-CD, trimethyl-β-cyclodextrin; α-CD, α-cyclodextrin; β-CD, β-cyclodextrin; γ-CD, γ-cyclodextrin.

citonin, measured by the reduction of calcium blood levels. The effect of their nasal administration was comparable to the intravenous or subcutaneous administration of calcitonin. At concentrations of 2 and 3% dimethyl-β-cyclodextrin also resulted in substantial decrements in serum calcium levels, whereas 5% trimethyl-β-cyclodextrin was less potent. In rabbits the effect of coadministration of 5% dimethyl-β-cyclodextrin was also comparable to the effect of intravenously or subcutaneously administered calcitonin [14].

When liquid and powder formulations of glucagon (3.5 kDa) with dimethyl-β-cyclodextrin were sprayed into the nasal cavity, a nasal bioavailability of more than 80% (compared with subcutaneous administration) was found in rabbits (Table 2) [15]. Lower amounts of dimethyl-β-cyclodextrin in the formulations resulted in reduced glucagon absorption.

For insulin (5.8 kDa) the absolute bioavailability after nasal administration in rats was increased from no absorption without absorption enhancer, to about 100% with dimethyl-β-cyclodextrin (3 to 5%; Table 3) [16,17]. Studies in rats showed that dimethyl-β-cyclodextrin was a more efficient enhancer of nasal insulin absorption than α-cyclodextrin, dimethyl-α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin or hydroxypropyl-β-cyclodextrin [16,18]. In rabbits the absorption enhancing effects of dimethyl-β-cyclodextrin in an insulin liquid formulation were negligible [19]. However, powder formulations with dimethyl-β-cyclodextrin increased the insulin bioavailability to 13%. In contrast, the results of another nasal insulin absorption study in rabbits suggested that with a nasal liquid formulation a maximal bioavailability of 16% could be accomplished [20]. However, the total amount of cyclodextrin administered in this formulation was three times higher as in the formulation used in the first study [19]. When the concentration of dimethyl-β-cyclodextrin in the nasal liquid formulations was increased to 50%, the insulin bioavailability was again markedly reduced com-

Table 3
Cyclodextrins in nasal delivery of insulin (5.8 kDa)

Cyclodextrin	CD amount/concentration	Formulation	Species	Bioavailability (%)		Reference
–	0	Drops	Rat	0		[16]
DM-β-CD	5%	Drops	Rat	109		
α-CD	5%	Drops	Rat	28		
β-CD	1.8%	Drops	Rat	3		
γ-CD	5%	Drops	Rat	0		
–	–	Drops	Rat	0		[17]
DM-β-CD	1%	Drops	Rat	7		
DM-β-CD	2%	Drops	Rat	63		
DM-β-CD	3%	Drops	Rat	91		
DM-β-CD	4%	Drops	Rat	105		
DM-β-CD	5%	Drops	Rat	97		
–	–	Drops	Rabbit		35[a]	[20]
DM-β-CD	3.3 mg/kg	Drops	Rabbit		170[a]	
DM-β-CD	10 mg/kg	Drops	Rabbit		241[a]	
DM-β-CD	17 mg/kg	Drops	Rabbit		360[a]	
DM-β-CD	33 mg/kg	Drops	Rabbit		100[a]	
α-CD	10 mg/kg	Drops	Rabbit		120[a]	
γ-CD	10 mg/kg	Drops	Rabbit		76[a]	
HP-β-CD	10 mg/kg	Drops	Rabbit		69[a]	
β-CD	10 mg/kg	Drops	Rabbit		45[a]	
–	–	Drops	Rat	0		[18]
DM-β-CD	80 mM	Drops	Rat	24		
β-CD	1.85 g/dl	Drops	Rat	21		
DM-α-CD	80 mM	Drops	Rat	15		
α-CD	80 mM	Drops	Rat	12		
HP-α-CD	80 mM	Drops	Rat	5		
HP-β-CD	80 mM	Drops	Rat	4		
–	–	Drops	Rabbit	1		[19]
DM-β-CD	5%	Drops	Rabbit	1		
DM-β-CD	30%	Drops	Rabbit	3		
DM-β-CD	0.8 mg/kg	Powder	Rabbit	13		
DM-β-CD	0.25 mg/kg	Powder	Human	3.4[b]	5.1[c]	[21]

[a]AUC (h μU/ml).
[b]Healthy volunteers.
[c]Diabetes mellitus patients.
DM-β-CD, dimethyl-β-cyclodextrin; α-CD, α-cyclodextrin; β-CD, β-cyclodextrin; γ-CD, γ-cyclodextrin; HP-β-CD, hydroxypropyl-β-cyclodextrin; DM-α-CD, dimethyl-α-cyclodextrin; HP-α-CD, hydroxypropyl-α-cyclodextrin.

pared with lower concentrations of the cyclodextrin [20]. This decrease in absorption was possibly caused by mucus secretion from the nasal cavity, due to administration of extremely high concentrations of dimethyl-β-cyclodextrin, or an excess of cyclodextrin in solution interferes with the insulin transport across the nasal epithelium.

With an intranasal insulin/dimethyl-β-cyclodextrin powder formulation a mean absolute bioavailability of 3.4% in healthy volunteers and of 5.1% in diabetes mellitus patients was obtained (Table 3) [21]. From these studies it was concluded that the nasal absorption of insulin in humans is not sufficient and reproducible enough for therapeutic purposes [21]. The nasal administration of insulin using various absorption enhancers in humans has been

extensively reviewed [21–23]. So far, the nasal insulin bioavailability is insufficient even after co-administration with effective absorption enhancers. Also the long-term safety of the enhancer is uncertain. With 1% sodium deoxycholate, a nasal insulin bioavailability of 10% was obtained [24]. Sodium glycocholate at 1% resulted in a nasal insulin bioavailability of 12.5% [25]. For 1% sodium taurodihydrofusidate, insulin bioavailabilities between 7.1 and 9.5% were observed in healthy volunteers after nasal administration [26]. For the phospholipid didecanoyl-L-α-phosphatidylcholine (2%) encouraging results were found for the nasal administration of insulin in volunteers [27,28]. However, in a long-term study with insulin-dependent diabetes mellitus patients the results were negative. The insulin nasal bioavailability was low and the rate of therapeutic failure was high [29]. For the moment, intranasal insulin treatment cannot be considered a realistic alternative to subcutaneous insulin injections.

Recombinant human granulocyte colony-stimulating factor, a protein with a molecular weight of 19 kDa, was administered intranasally to rabbits with cyclodextrins [30,31]. Without enhancer the absolute bioavailability was about 5%. When α-cyclodextrin, β-cyclodextrin and dimethyl-β-cyclodextrin were added as absorption enhancers the bioavailability increased two- to threefold, to 11% for α-cyclodextrin and β-cyclodextrin, and to 16% for dimethyl-β-cyclodextrin (Table 2). No effect was observed with γ-cyclodextrin.

3. Species differences in nasal drug absorption

Species differences between rat, rabbit and man have been observed for the nasal administration of peptide and protein drugs with cyclodextrins as absorption enhancers. In rabbits a twofold increase in bioavailability of Org2766 was obtained with 5% dimethyl-β-cyclodextrin, whereas in rats the increase in bioavailability was about fivefold (Table 1) [11]. The intranasal administration of calcitonin with 5% dimethyl-β-cyclodextrin caused a 22% reduction in calcium levels in rats, but in rabbits the hypocalcemic effect of intranasal calcitonin was lower (10%; Table 2) [14].

For leuprolide administered as drops, using α-cyclodextrin as absorption enhancer, a bioavailability of 36% was achieved in rats versus 4% in humans (Table 1) [13]. Nasal insulin absorption with dimethyl-β-cyclodextrin has been extensively investigated. The intranasal bioavailability of a liquid insulin formulation was 100% in rats [16,17], but 0% in rabbits and man [32]. However, an insulin powder formulation provided a bioavailability of about 13% in rabbits and only about 5% in humans (Fig. 3) [19,21].

The observed species differences in nasal absorption are caused by several factors. For the differences in absorption between liquid drop and powder formulations of insulin, the deposition of the formulation and the different local concentrations can result in different bioavailabilities. A similar difference in nasal absorption between drops and powders for insulin administration was observed with the absorption enhancer sodium taurodihydrofusidate in sheep [33]. However, when liquid and powder formulations of glucagon with dimethyl-β-cyclodextrin were administered as sprays in rabbits, the nasal absorption was comparable for both formulations [15].

Differences in nasal anatomy and surface area can also result in different nasal absorption between species. Furthermore, anaesthesia of the animal can decrease the nasal mucociliary clearance [34,35]. Rats are usually under complete anaesthesia during an absorption experiment, whereas rabbits are generally only sedated and humans are not sedated. In

Species / Formulation Effects

insulin bioavailability

species	insulin + DM-β-CD solution	insulin + DM-β-CD powder
rat	100 %	100 % (expected)
rabbit	0 %	13 %
man	0 %	5 %

Fig. 3. Interspecies differences and formulation effects in the absorption of nasal drug formulations as demonstrated for insulin [16,19,21,32].

completely anaesthetized rats the nasal insulin absorption was increased, compared with rats that were only sedated [36]. Due to the large species differences between rats, rabbits and man in nasal drug absorption, the animal model for nasal absorption studies should be chosen carefully and the results obtained considered in the proper perspective.

Based upon animal studies, it can be concluded that the most effective absorption enhancing cyclodextrins for peptides and proteins are the methylated β-cyclodextrin derivatives, dimethyl-β-cyclodextrin and randomly methylated β-cyclodextrin. They are active at relatively low concentrations ranging between 2 and 5% (w/v). Also α-cyclodextrin can substantially increase the bioavailability of a variety of peptides and proteins, but it is less potent.

4. Toxicity of cyclodextrins in nasal drug delivery

4.1. Local effects

The safety of cyclodextrins as nasal absorption enhancers is determined by two factors: first by their direct effects on the nasal epithelium, i.e. local toxicity, and second by their systemic effects after absorption of the cyclodextrins through the nasal epithelium. Several models have been used to investigate the potential local toxic effects of cyclodextrins, such as changes in nasal morphology in vivo, effects on ciliary beat frequency in vitro, release of marker compounds in vivo or in situ, erythrocyte hemolysis test in vitro, and cytotoxicity in vitro. The validity of these models is discussed taking into account the experimental conditions of the various models and the real conditions in human nasal drug delivery.

4.1.1. Nasal morphology in vivo

The acute histological effects of cyclodextrins on the epithelium of the nasal cavity have been investigated in rats with light microscopy [37,38]. For instance, after a single nasal administration of 2% randomly methylated β-cyclodextrin or 2% dimethyl-β-cyclodextrin, only minor changes were observed in the appearance of the cilia and the apical cell membranes, and small amounts of mucus were excreted into the nasal cavity [37]. These effects were quite similar to those of the control (physiological saline; 0.9% NaCl), in which the absorption enhancers were dissolved. The morphological changes of the nasal mucosa caused by administration of methylated β-cyclodextrins were smaller than those caused by 0.01% benzalkonium chloride, which is a world-widely accepted preservative in nasal drug formulations. Furthermore, the effects of dimethyl-β-cyclodextrin, randomly methylated β-cyclodextrin, and benzalkonium chloride were small compared with those of two other absorption enhancers studied: the bile salt sodium glycocholate 1%, and the phospholipid L-α-lysophosphatidylcholine 1%. These enhancers showed severe nasal membrane damage and a single nasal dose of 1% L-α-lysophosphatidylcholine even caused complete epithelial removal [37].

With a novel visualization technique, confocal laser scanning microscopy, also no changes in cell morphology were observed after a single intranasal administration of 2% randomly methylated β-cyclodextrin, whereas 1% sodium taurodihydrofusidate resulted in swelling of the cells and substantial mucus extrusion [39].

Histological evaluation of γ-cyclodextrin and hydroxypropyl-β-cyclodextrin in rats showed that the effects of 8% hydroxypropyl-β-cyclodextrin were slightly more toxic than those of the control (a phosphate buffer), and that 5% γ-cyclodextrin was comparable to the control. Both cyclodextrins were much less toxic than the surfactant laureth-9 at 1% [38].

4.1.2. Ciliary beat frequency in vitro

The effects of cyclodextrins on the nasal mucociliary clearance have been studied, because it is essential that they do not have adverse effects on this primary defense system of the respiratory tract [40]. By measuring the influence of drugs and excipients on the ciliary beat frequency (CBF) in vitro, the effects of these substances on the ciliary function and mucociliary clearance can be estimated [41,42].

Nasal drug formulations should not interfere with the self-cleaning capacity of the nose, effectuated by the ciliary epithelium. The coordinated beating of the cilia results in the movement of the upper mucus layer towards the nasopharynx. The combined action

of cilia and mucus layer is called mucociliary clearance, in which the ciliary movement plays an important role. It is, therefore, relevant to study the effect of drugs and pharmaceutical excipients for nasal drug delivery on CBF, because it helps to design formulations with a minimal or acceptable toxicity profile. The measurement of the CBF in vitro is very accurate, reproducible and sensitive. It should be emphasized that the ciliostatic effect of a compound measured under these in vitro conditions is much more pronounced than that in vivo. In vitro, the ciliated tissue is directly exposed to the compounds investigated, whereas in vivo the cilia are protected by the mucus layer. Moreover, under in vivo conditions the nasally administered drug formulation will be diluted by the mucus and subsequently removed by the mucociliary clearance within a short period of time.

Dimethyl-β-cyclodextrin and randomly methylated β-cyclodextrin at concentrations of 2% were found to have minor effects on CBF, similar to those of physiological saline (Fig. 4) [43]. The effects of both cyclodextrins were smaller than those observed for the preservative benzalkonium chloride, in a concentration of 0.01%. Severe ciliostatic effects were found for the enhancers sodium taurodihydrofusidate at 1% and L-α-lysophosphatidylcholine at 1% [42,44]. For dimethyl-β-cyclodextrin the effects on the CBF are concentration dependent [44]. After rinsing with Locke–Ringer solution the effect is reversible. At a concentration of 5% the cilio-inhibition of dimethyl-β-cyclodextrin and α-cyclodextrin was comparable, while the cilio-inhibition of 5% hydroxypropyl-β-cyclodextrin and 5% γ-cyclodextrin was smaller [44]. At the following concentrations comparable cilio-inhibition was observed: 1.8% β-cyclodextrin = 5% hydroxypropyl-β-cyclodextrin = 1–2% dimethyl-β-cyclodextrin [44]. It is important to realise that only methylated-β-cyclodextrins are effective in increasing the nasal absorption of peptides and proteins, whereas 1.8% β-cyclodextrin and 5% hydroxypropyl-β-cyclodextrin are not [11,12,16,18,20].

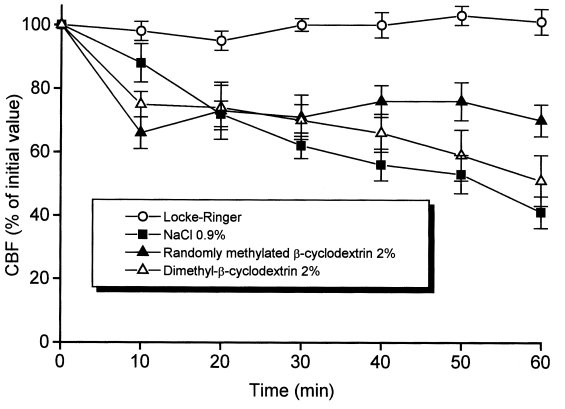

Fig. 4. The effects on the ciliary beat frequency (CBF) of chicken embryo trachea in vitro. Control solutions: Locke–Ringer (○) and physiological saline (0.9% NaCl; ■). Cyclodextrins: randomly methylated β-cyclodextrin 2% (▲) and dimethyl-β-cyclodextrin 2% (△). Data are expressed as the mean±S.E.M. of 6–11 experiments. Adapted from [43].

4.1.3. Release of marker compounds: in vivo versus in situ

By measuring the release of marker compounds from the nasal cavity after nasal application of substances, information on the effect of these substances on the nasal mucosa can be obtained [45]. In an in vivo rat model, a small volume of the absorption enhancer solution (20 μl) is administered intranasally, and 15 min after nasal instillation the released substances are determined by washing the nasal cavity with physiological saline via an esophageal cannula. With this method, 2% randomly methylated β-cyclodextrin and 2% dimethyl-β-cyclodextrin were found to release larger amounts of protein and cholesterol than the control (physiological saline). However, the amounts released by these cyclodextrins were significantly lower than observed for the absorption enhancers 1% sodium taurodihydrofusidate, 1% laureth-9 and 1% L-α-lysophosphatidylcholine [45]. For these latter three enhancers the release of an intracellular enzyme, acid phosphatase, was also shown, which suggests severe nasal membrane damage. In contrast, no intracellular enzyme release could be detected after intranasal administration of 2 and 5% methylated β-cyclodextrin. The results of the in vivo release of marker compounds, after a single nasal dose, correspond with those of morphological and ciliary beat frequency studies [37,42–45].

Another method to investigate the release of marker compounds from the nasal cavity is the so-called in situ perfusion model. The release of the

compounds is measured by continuously perfusing the nasal cavity of a rat with 5 ml absorption enhancer solution during 2 h [46,47]. Samples of the perfusate are taken and analyzed for protein, cholesterol and intracellular enzyme contents. Obviously, the experimental conditions of this method are not physiological and unrealistic for nasal drug administration in humans [48]. Firstly, because the perfusion time (90–120 min) is excessively long, considering that the nasal mucociliary half-time is about 15 min [49]. Secondly, the perfusion of large volumes of fluid through the nasal cavity is completely different from the installation of μl amounts of a nasal formulation in the nasal cavity. During the perfusion the protective mucus layer is washed away, thus exposing the nasal epithelium directly to the perfusion fluid. Furthermore, if inflammatory mediators are released from the mucosa [50–52] because of the presence of absorption enhancers, they will be taken up in the perfusate and recirculated through the nasal cavity, possibly resulting in secondary toxic effects. Finally, the amount of cyclodextrin present in 5 ml perfusate is about 250-times larger than the amount administered to rats in a volume of 20 μl. When the conditions of in situ perfusion in rats are extrapolated to humans, the deviation of this model from reality is evident. In a rat, having a nasal volume of 0.4 ml [49], a volume of 5 ml is perfused during 2 h. In humans the comparable volume would be 250 ml, because the human nasal volume is about 20 ml [49]. The clinical reality is that a volume of 100 μl is administered nasally, which will be removed quickly by the nasal mucociliary clearance [49]. Therefore, the in situ model has an aberration of about 20 000 times from the clinical reality (Fig. 5). This implies that the results from in situ perfusion studies with cyclodextrins cannot be extrapolated to the clinical application of nasal drug formulations, and that conclusions about their safety based on these studies are questionable.

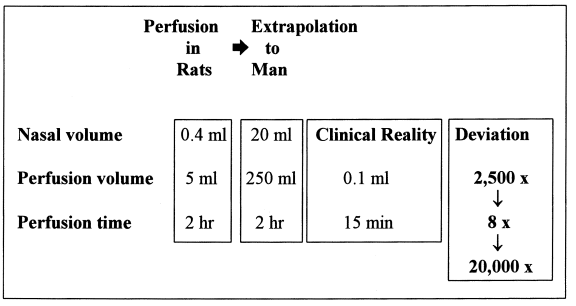

Fig. 5. The rat in situ perfusion technique as a model for nasal drug absorption or nasal toxicity studies may lead to questionable results, because of the large difference in experimental conditions of this model and nasal drug administration in clinical reality [48].

4.1.4. Erythrocyte hemolysis test in vitro

Erythrocyte hemolysis is commonly used as a model to investigate membrane interactions, because erythrocytes are readily available and their lysis is easily measured. The interaction of different absorption enhancers with erythrocyte membranes can be compared with this method. Moreover, erythrocyte lysis has been used to obtain preliminary indications of the potentially toxic interactions of substances with membranes. For example, the hemolysis of human erythrocytes in vitro, caused by cyclodextrins, has been proposed as a measure of their membrane damaging effects [53–56].

The order of the hemolytic effect of cyclodextrins and their derivatives has been reported to be (in increasing order): γ-cyclodextrin < hydroxypropyl-β-cyclodextrin = α-cyclodextrin < randomly methylated β-cyclodextrin = trimethyl-β-cyclodextrin < dimethyl-β-cyclodextrin [56–60]. For dimethyl-β-cyclodextrin hemolysis is initiated at a concentration of 0.07%, and the EC_{50} is 0.14% [54]. These concentrations are one or two orders of magnitude lower than the concentration at which dimethyl-β-cyclodextrin is used in nasal drug formulations, e.g. 2 to 5%. The hemolytic activity of cyclodextrins is probably the result of membrane disruption, caused by the removal of membrane components such as phospholipids, proteins and cholesterol [54].

The value of the erythrocyte lysis model in a plasma free medium to predict cytotoxicity in vivo is questionable, because it is a very sensitive method. The concentrations at which hemolysis occurs in vitro are orders of magnitude lower than those actually used in vivo [61]. This is evident from the hemolytic values obtained for the drug chlorpromazine, which is administered intravenously. The hemolytic concentration of chlorpromazine in vitro is 30-times lower than the concentration administered to patients intravenously [61]. More important, hemolysis by cyclodextrins, easily detectable in

plasma free medium in vitro, cannot be observed in vivo [62].

Two factors can explain the discrepancy between the toxic concentrations for erythrocytes in vitro and the concentrations used in vivo without adverse effects. Firstly, hemolytic activity is usually determined with erythrocytes suspended in a buffer solution or in physiological saline. However, it has been reported that the hemolytic activity of cyclodextrins is 4 to 10 times smaller in serum than in buffer or physiological saline [61,63,64]. The competition for cyclodextrin inclusion between serum lipids and erythrocyte cell membrane components reduces the cytotoxicity of cyclodextrins in serum. Secondly, it was demonstrated that the rate of cholesterol extraction from cells in suspension (such as erythrocytes) is higher than the cholesterol extraction from cell culture monolayers [63].

The erythrocyte lysis model is not only too sensitive to extrapolate obtained results to in vivo situations, but the model also does not have any physiological relevance to predict effects on the nasal epithelium protected by the mucus layer. Therefore, the use of the erythrocyte model to evaluate the toxicity of excipients for nasal drug delivery without performing other, more relevant in vivo experiments is not recommended.

4.1.5. Cytotoxicity studies

In vitro cytotoxicity tests have shown that hydroxypropyl-β-cyclodextrin and methylated β-cyclodextrin can extract cholesterol from different types of cell cultures and thus perturb the cell membrane [65]. However, other membrane components, such as adenine, were not released when cholesterol was extracted from the cell cultures [65]. The release of adenine was only observed after exposure times of more than 8 h. In cultures of fibroblasts and intestinal cells no effects on intracellular calcium levels and pH were observed after application of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, dimethyl-β-cyclodextrin or randomly methylated β-cyclodextrin, even at high concentrations (20–40 mM) [61].

The cytotoxicity of dimethyl-β-cyclodextrin has also been investigated in intestinal Caco-2 cell monolayers, by measuring the effects on mitochondrial dehydrogenase activity and on cytoplasmic or nuclear staining by membrane impermeable probes [66,67]. The results of the mitochondrial enzyme activity test of these two studies were different. In the first study application of 1% and 2% dimethyl-β-cyclodextrin resulted in no or small decreases in enzyme activity [66]. However, in the second study, 1.5% dimethyl-β-cyclodextrin resulted in a large decrease of the enzyme activity [67]. This difference in effects is probably due to the different experimental conditions employed in these studies. For cytoplasmic or nuclear staining by membrane impermeable probes, similar results were found in both studies. After 1 h about 1% of the cells were stained intracellularly when dimethyl-β-cyclodextrin (2.5–3%) was applied, and 4% of the cells were stained after 5% dimethyl-β-cyclodextrin [66,67]. Only after incubation with 5% dimethyl-β-cyclodextrin for 3 h, a substantial effect on the intracellular staining was observed [66]. The results of these studies seem not relevant for cyclodextrins used in nasal drug delivery, because only small amounts are used and the residence time in the nose is very limited.

4.2. Systemic effects

Several reviews extensively discuss the systemic toxicity of cyclodextrins [54,68–73]. Two major toxic side-effects have been reported after systemic administration of cyclodextrins: renal toxicity and hemolysis. However, the risk of systemic side-effects of cyclodextrins after nasal administration depends on how much cyclodextrin will be absorbed. Therefore, establishing how much of the cyclodextrin is absorbed is essential, before an appraisal of a potential systemic effect of nasally administered cyclodextrins can be made.

After nasal administration of a drug–cyclodextrin formulation, only the drug is absorbed by the nasal epithelium, but not the highly water-soluble cyclodextrin and its complex. In humans dimethyl-β-cyclodextrin was hardly absorbed after nasal administration of a spray containing 2 and 5% dimethyl-β-cyclodextrin. Only 2.5 to 4% of the nasally administered concentration was recovered in the urine [74].

The fraction of the cyclodextrin dose that is not absorbed from the nasal cavity is removed by the nasal mucociliary clearance system. The mucociliary

clearance system transports the cyclodextrin toward the oesophagus where it is swallowed. Methylated β-cyclodextrins are probably absorbed in minute amounts from the gastrointestinal tract because of their highly water-soluble properties. In rabbits only 2.7% of an orally administered dimethyl-β-cyclodextrin dose was excreted in the urine within 24 h [75]. In rats less than 10% of the orally administered dose of dimethyl-β-cyclodextrin was absorbed, and the absorption seemed to be independent of the cyclodextrin dose [76]. When cyclodextrins are administered orally, they do not cause acute toxicity [77]. Oral administration of dimethyl-β-cyclodextrin in doses up to 3 g/kg in mice did not result in toxic effects [70].

Based on the low nasal and oral absorption of cyclodextrins, only a small fraction of a nasally administered dose of methylated β-cyclodextrin would be absorbed into the systemic circulation. Considering the usual dose of methylated β-cyclodextrin in nasal drug formulations, the total amount available for absorption is quite low. For instance, a nasal formulation containing 2 to 5% methylated β-cyclodextrin is administered in a volume of 100 to 150 μl in humans, resulting in a dose of 2 to 7.5 mg methylated β-cyclodextrin per single administration. Even in the very unlikely situation that complete nasal absorption would take place, a maximal dose of about 0.1 mg/kg methylated β-cyclodextrin might be absorbed systemically.

Renal toxicity of cyclodextrins has been observed after parenteral administration of cyclodextrins. After daily subcutaneous, intraperitoneal or intravenous injections of β-cyclodextrin in doses higher than 3 g/kg renal necrosis developed [78,79]. The nephrotoxicity is probably due to accumulation and recrystallization of poorly aqueous soluble β-cyclodextrin–cholesterol complexes in the kidneys [80]. In rats, higher nephrotoxicity was observed for intravenously administered β-cyclodextrin, than for the much more water-soluble hydroxypropyl-β-cyclodextrin [80]. After intravenous dosing of mice with 50 mg/kg β-cyclodextrin irreversible histopathological changes were observed, while for the same dose of dimethyl-β-cyclodextrin no histopathological changes were found [70]. The LD_{50} values of 2,6-dimethyl-β-cyclodextrins in rats are 220 mg/kg after intravenous administration and 330 mg/kg after subcutaneous administration [70]. In toxicity experiments it is relevant whether highly purified specific 2,6-dimethyl-β-cyclodextrin or a randomly methylated dimethyl-β-cyclodextrin has been used, because the latter does not crystallize in vivo. Randomly methylated β-cyclodextrins (degree of substitution 1.8), even at daily oral doses of 1000 mg/kg body weight, did not affect the glomeruli [81]. The systemic dosages at which nephrotoxicity was observed are several thousand times higher than the amount that can be absorbed maximally after nasal administration (estimated to be maximally 0.1 mg/kg in humans, as calculated above). In rat studies, no toxic effect level of 100 mg/kg/day for randomly methylated β-cyclodextrin has been described [81]. These data demonstrate the safety of the nasal administration of methylated β-cyclodextrins in low concentrations.

4.3. Safety of cyclodextrins as nasal excipients

Toxicity models to detect the local and systemic safety of cyclodextrins as nasal formulation excipients should be performed in conditions comparable to nasal drug delivery. They should have physiological relevance, and should use appropriate concentrations. When the results of such models described in this review are compared, the effects of randomly methylated β-cyclodextrin and dimethyl-β-cyclodextrin are mild and not indicative of local nasal toxicity. The observed effects of the methylated β-cyclodextrins were always smaller than those of the preservative benzalkonium chloride.

These toxicity studies discuss primarily the acute local effects of cyclodextrins. No comprehensive studies have yet been published in which the chronic effects of cyclodextrins as nasal excipients have been performed. Because most peptide and protein drugs are to be administered more than once, or even chronically, such studies are needed to establish the safety of long-term administration of cyclodextrins. After a single administration of a nasal insulin/dimethyl-β-cyclodextrin powder in healthy volunteers and diabetes patients, no serious side effects but only slight nasal itching has been reported [21]. Moreover, after chronic nasal administration of sprays containing a methylated β-cyclodextrin with

estradiol during 6 months in women, no adverse effects were observed [3,4].

Based on toxicity tests and these results of nasal administration in humans, methylated β-cyclodextrins can be considered safe and efficient nasal absorption enhancers in humans. However, the safety of chronic nasal administration of cyclodextrins has to be confirmed in comprehensive clinical studies.

5. Conclusions

Some cyclodextrins, in particular the methylated β-cyclodextrins, have shown to be useful excipients in nasal drug delivery. The results in humans published so far demonstrate that they can largely improve the nasal absorption of some lipophilic drugs and of oligopeptides. Their efficacy in human subjects is much less in the case of polypeptides and proteins like insulin.

Based on morphological studies, the effects on the ciliary beat frequency and the release of marker compounds, both dimethyl-β-cyclodextrin and randomly methylated β-cyclodextrin appeared to be safe excipients. Their effects are quite similar to that of physiological saline and smaller than those of benzalkonium chloride, a world-widely used preservative for nasal drug formulations.

The most important issue in nasal drug delivery studies is the fact that large interspecies differences have been found between animals and men in the nasal absorption of drug formulations with cyclodextrins as excipients to improve the absorption. It is therefore advised to do human absorption studies in an early stage, because for instance results in rat studies may look promising, but their clinical relevance is often doubtful.

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