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NASAL ADMINISTRATION:

A TOOL FOR TOMORROW'S SYSTEMIC ADMINISTRATION OF DRUGS

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ABSTRACT

By its anatomy and physiology, due to the great amount of air treated there, the nasal route represents a very interesting possibility for the administration of products degraded in the gastro-intestinal tract or inhibited by the first hepatic pass. The nasal dosage forms most studied are bioadhesive hydrogels and microspheres, especially for the systemic administration of peptides.

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INTRODUCTION

The nose is the visible organ of respiration, but it is also that of olfaction. These two functions require the permanent passage of a large amount of air and its treatment, according to each of the above functions. In any case, the respiratory function is the most important one, not only because from the fundamental standpoint it ensures life, but because it requires such a large amount of inhaled air, that it leads to the existence, at the nasal level, of a mucosa far greater than the olfactory mucosa: almost 120 cm^2 compared with 2 cm^2 for the latter. Inhaled air is intended for ensuring the ventilation of almost 100 m^2 of pulmonary surface, taking into account the alveola. Consequently, the inhaled air must be warmed up, humidified and above all purified because it should normally be sterile when it arrives in the lungs.

Because of its valuable surface and of its accessibility, the nasal mucosa represents an interesting administration route, not only for products with local activity, but also for products with systemic activity. Unfortunately, the clearance function might be a hindrance to this administration route.

ANATOMY AND PHYSIOLOGY OF THE NASAL PASSAGE

Description

Nasal holes extend from the nostrils to the rhinopharynx (figure 1). They are separated by a partition: the septum. The vestibule in fact represents the nostrils. It is cone-shaped with the narrow part towards the nasal valva (internal ostium), situated at almost 15 mm from the opening of the nostrils and constituting the boundary between the skin and the mucosa. The atrium (preturbinate), which follows this constriction, is a transition zone to the turbinates.

The structure of nasal holes is such that the air, which penetrates vertically with a velocity of 2 to 3 m/s, is accelerated at the ostium level and, because

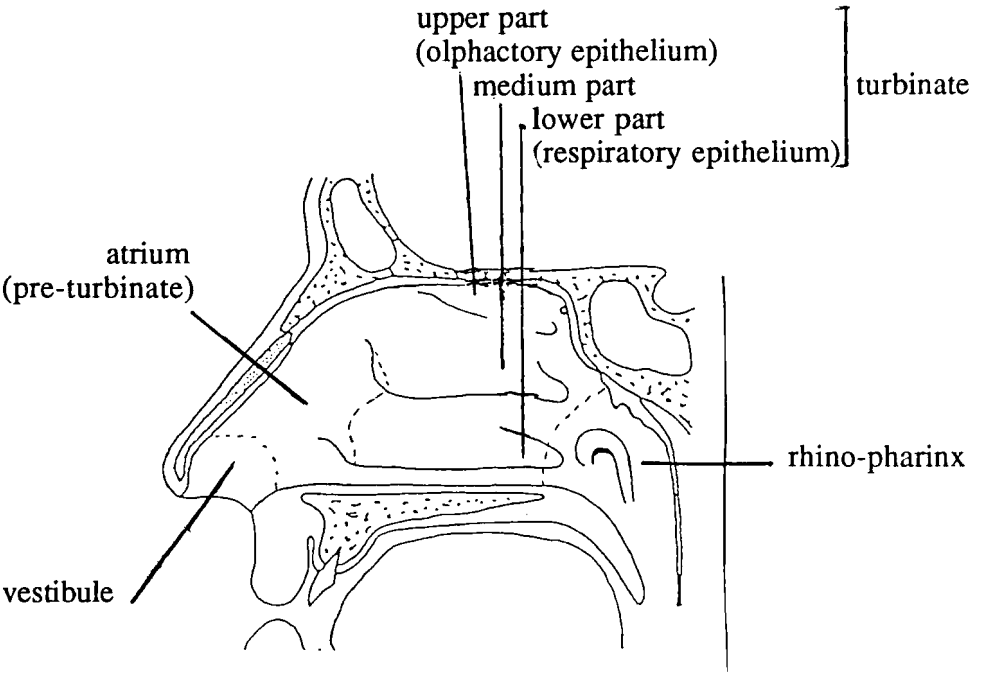

Figure 1. Scheme of the nasal passage

of the convergence it undergoes, its velocity is at that moment from 12 to 18 m/s. On arriving in the nasal cavity, the air is directed horizontally and its velocity is reduced to 2 to 3 m/s, because of the enlargement of this cavity. The impaction ratio is very important at this level because it concerns almost all the particles with a diameter greater than 10 µm (those that escape will be stopped on the posterior surface of the pharynx or in the trachea).

Nature of the epithelium

The vestibule, in other words the nostrils, in contact with external air, is lined with a cutaneous layer: stratified, keratinized and squamous epithelium, with sweat and sebaceous glands, and hairs. Behind the ostium, the epithelium loses its keratine and becomes a mucosa, first

without microvilli then progressively covered with microvilli in the turbinate.

The olfactory epithelium (at the upper part) is cylindrical, pseudostratified, ciliated, and characterized by the existence of Schultze sensory cells. Rigid and short cilia have an essentially sensory rôle. The epithelium thickness is between 60 and 70 μm.

The respiratory epithelium (in the medium and lower parts) is of the cylindrical, pseudostratified and ciliated type. This epithelium is appreciably less thick than the previous one: 20 to 30 μm. The respiratory epithelium is composed of basal cells which are precursors of cylindrical cells (ciliated and non-ciliated) and of goblet cells. Goblet cells are especially numerous in parts subjected to the air flow. Their surface includes some microvilli but they are more especially characterized by the existence of mucus granules. Non-ciliated cylindrical cells have microvilli of about 3 μm in length and 0.1 μm in diameter. Ciliated cells are the most numerous: almost 70 to 80% of superficial cells. They are about 20 μm high. They are characterized by the presence, in the middle of the microvilli, of numerous vibratile cilia constituting a quasi-continuous layer.

Not all the junctions between the epithelial cells are of the same structure. Hence, connections between goblet cells, or between goblet cells and ciliated cells are weaker than those between ciliated cells. Consequently, the protection of the epithelial barrier varies according to the epidermal region.

Irrigation of nasal mucosa

The nasal mucosa is highly vascularized [1]. The veinous flow escapes to the portal system during its first pass, allowing the blood to reach the various tissues and organs before the liver, thus preventing eventual degradation.

The nasopharyngeal region possesses a very rich lymphatic plexus. The lymphatics play an important rôle in the nasal absorption of substances such as pituitary hormone [2].

Mucociliary clearance

The mucociliary apparatus [3], especially responsible for respiratory route clearance, is basically constituted of two indispensable and physiologically indissociable elements: mucus and vibratile cilia.

In the present state of knowledge, the nasal mucus has the general composition of every mucus [4], in other words it contains essentially a glycoprotein (mucin), electrolytes, lipids, enzymes (especially proteolytic enzymes), antibodies, sloughed epithelial cells, bacterial products and obviously water. It represents a protective barrier against the penetration of various molecules, because of the polymeric network constituted by mucin, and also because of the enzymatic degradations which can occur in the penetrating molecules. The mucus is organized in two layers: the lower one, more fluid with a low viscosity, and the upper one more visco-elastic and susceptible of moving on the previous one because of the cilia movements. The mucus layer forms a continuous film.

Vibratile cilia [3] are almost 200 for one ciliated cell. Their dimensions are 6 to 8 µm in height , and 0.25 µm in diameter. They move with rhythmic flutterings. Each cycle consists of two active phases separated by one of rest. The cycle begins by a preparatory phase occurring in the lower layer of mucus, following a pseudo-circular clockwise movement . The curve of the cilium starts at its base and reaches its extremity, while pulling it back after describing a 180 degree angle. In the propulsion phase, the stretched cilium sweeps a given amount of liquid in a plane almost perpendicular to the cell surface. The motion is fast, the curve of the cilium deals only with its base and brings it abruptly to its rest position, in which its stays streched, its extremity pointing in the direction of mucus flow, thus decreasing the resistance to this mucus flow.

During the preparatory phase, each cilium stimulates its neighbours by hustling them on the side where it beats, thus setting off their own movement. Due to the differences in cilia height compared with the cell surface, according to the beating zone, cilia in the propulsion phase seem higher than those in the preparatory phase. The movement propagation wave of the whole cilia extends in a metachronic way [1]: the cilia combined movement, which resembles that of grass in the wind, results from the phase shift between adjoining cilia.

The mucus lower layer comes and goes around the cilia, forward in the propulsion phase, backward in the preparatory phase [3]. During the propulsion phase, the cilia extremity scrapes the upper layer of mucus, penetrating it almost 0.5 μm. Zones of ciliary activity then occur from place to place in the mucus film at intervals of some 10 μm. In the case of interference with the propulsion phase, at any level of the muco-ciliary carpet, the cilia situated backward, due to their pressure, help to overcome the obstacle. The cilia movement results in the transport of the mucus on the top of the cilia towards the nasopharynx, thus preventing nasal absorption of the drug carried.

Necessity of maintaining the muco-ciliary function

The defence mechanism of the air ways against external aggression is mostly assured through the muco-ciliary function. Thus it is obvious that, even if this protection sytem is unfavourable for the administration of some drugs, it must be maintained. From this point of view, it is necessary to avoid possible toxicity of the active ingredients or of the accompanying additives.

It should be noted that sympathomimetics leads always to an acceleration of the muco-ciliary function, which could be the consequence either of a direct action on the cilium, or of an increase in secretions [5]. Parasympathomimetics directly stimulates ciliary action, when parasympatholytics slows down muco-ciliary transport [5].

It was shown with a number of local congestion-relieving products that active ingredients can lead to less inhibitory effects than the corresponding specialties, this difference being the result of the stability additives [5].

FACTORS INFLUENCING NASAL ABSORPTION

Administration by the nasal route is actually the subject of growing interest. It presents a great asset for active ingredients undergoing either degradation at the hepatic first pass, or metabolization at the level of the intestinal wall, or destruction by digestive liquids. It was shown for some products, such as propranolol, that nasal administration leads to a bioavailability quite comparable to intravenous administration [6].

Despite this, it must be remembered that the muco-ciliary clearance phenomenon is very often an obstacle to be taken into account, and that the presence of various enzymes, in the nasal mucus, especially peptidases (varying according to animal species), can result in a check for the nasal absorption of some drugs.

Main physicochemical factors

Although the influence of rate of perfusion and volume of perfusate on nasal absorption has been the subject of studies [7], these parameters are not the aim of the present paper, because the dosage forms which are the subject of actual research tend to simplify the mode of administration , and thus they do not appeal to perfusion.

The pH effect on nasal absorption has been studied especially on benzoic acid [7]. This effect is not negligible since at pH 2.5 the quantity absorbed after one hour of perfusion is 44% for only 13% at pH 7.19. However it is not possible to conclude that benzoic acid is absorbed only on the un-ionized form, since at pH 7.19 where 99.9% is ionized, there is still 13% of absorption. On the other hand, if the two forms, ionized and un-ionized, are effectively absorbed, it is the un-ionized one which absorbed the more rapidly.

Table 1

Comparison of the nasal absorption of barbiturates and chloroform/water partition coefficient of the undissociated drug (According to [7])

Barbiturate	Partition coefficient	Percent absorbed	
		Nasal	Gastro-intestinal
Barbital	0.7	5.0 ± 3.00	12
Phenobarbital	4.8	10.6 ± 3.88	20
Pentobarbital	28.0	20.3 ± 4.65	30
Secobarbital	50.7	23.9 ± 3.38	40

Normally, the absorption of a product through biological membranes depends on its lipophilicity. The influence of this factor has been studied on a series of four barbiturates at pH 6 corresponding to the existence of the un-ionized form (pKa: 7.6) [7]. As seen in Table 1, the amount absorbed is related to the chloroform/water partition coefficient However, fifty-fold variations in the partition coefficient result in relatively low variations in absorption rates, and furthermore these absorption values always remain lower than that of gastro-intestinal absorption.

A study of the same type has been carried out on a series of progestational steroids [8]. The partition coefficient octanol/water does not represent the lipophilicity of progesterone and its hydroxylated derivatives and does not correlate with their behaviour towards the nasal mucosa. On the other hand, the systemic bioavailability of progesterone can be correlated with the nasal mucosa/buffer partition coefficient.

Table 2

Relationship between nasal bioavailability of peptides and their molecular weight (number of amino acids) (According to [14])

Peptides	Absorption (%)	Number of amino acids
Thyrotropin-releasing hormone (TRH)	20	3
Vasopressin analogues	6 - 12	9
LHRH, agonists and antagonists	1 - 10	9 - 10
ACTH analogues	12	17 - 18
Growth hormone-releasing factor (GRF)	1 - 2	40 - 44

Absorption of peptides

The mechanism of peptide absorption by the nasal route is not yet elucidated. In fact, absorption depends partly, as for many kinds of molecule [9-13], on the molecular weight (Table 2) [14], but this is not confirmed in every cases. Thus, the tripeptide: L-pyro-2-aminodipyl-L-histidyl-L-thiazoline-4-carboxamide has an absorption of only 7% compared with intravenous administration [7].

The relatively high polarity of peptides was thought to be responsible for this low absorption. In order to verify this hypothesis, hydroxyl groups of L-tyrosine were esterified by acyl groups. However the rate of nasal absorption of this compound was not significantly modified (Table 3) [7]. This result was attributed to the fact that L-tyrosine, as well as its O-acetyl or O-valeryl derivative is in zwitterionic form in the pH range studied.

Table 3

Apparent partition coefficients (octanol/pH 7.4) and observed nasal absorption rate constants (k_{obs}, min^{-1}) of L-tyrosine and its O-acyl derivatives (According to [7])

Compound	Apparent partition coefficient	k_{obs}, min^{-1}
L-tyrosine	0.0256	0.0023
O-acetyl-L-tyrosine	0.0468	0.0026
O-valeryl-L-tyrosine	1.17	0.0029

Table 4

Apparent partition coefficients (octanol/pH 7.4) and calculated nasal absorption rate constants (k_a, min^{-1}) of L-tyrosine carboxylic acid esters and N-acetyl-L-tyrosine (According to [7])

Compound	Apparent partition coefficient	k_a, min^{-1}
L-tyrosine methyl ester	1.97	0.0116
L-tyrosine ethyl ester	5.20	0.0254
L-tyrosine n-propyl ester	20.79	0.0230
L-tyrosine t-butyl ester	62.50	0.0105
N-acetyl-L-tyrosine	0.0256	0.0024

Consequently, an attempt was made to improve absorption by masking the ionic character of L-tyrosine on the carboxylic and/or amino groups [7]. Acetylation of the amino functional group does not modify its partition coefficient (octanol/pH 7.4 buffer), or its nasal absorption. On the other hand, the esterification of acid carboxylic functional groups leads to an increase in the partition coefficient and in the absorption constants, without any correlation between these two factors (Table 4).

In order to verify the influence of masking of the negative charge of peptides, a similar modification was carried out on L-tyrosyl-L-tyronine. However, for L-tyrosyl-L-tyronine as well as for its methylated derivative, hydrolysis occurs on the nasal mucosa [7]. Hydrolysis of the same type was observed also for L-glycyl-L-tyrosine and L-glycyl-L-tyrosine amide [7], for enkephalin-leucine and its metabolite des-tyrosine-leucine-enkephalin [15]. Methionine enkephalin [15, 16], leucine enkephalin [16], des-tyrosine leucine enkephalin [15], substance P [17], insulin and proinsulin [17] are among the peptide or protein drugs susceptible to hydrolysis by the nasal route. However, despite the fact that the nasal proteolytic barrier can significantly reduce the bioavailability of peptide and protein drugs, the level of aminopeptidase present seems to be much lower than in the case of the gastro-intestinal tract [18, 19].

Nasal absorption of L-tyrosine in its zwitterionic form seems to be a carrier-mediated process [7]. Such a mechanism might be characteristic of peptides.

It can be concluded that nasal absorption of peptides depends on a whole set of factors, among which one must not forget the possibility of complexation by immunoglobulin (Igs) leading to an increase in the molecular weight and a greater difficulty to pass the biological membrane. Furthermore, the enzymatic barrier, even if it is weaker than that of the gastro-intestinal tract, must be considered among the reasons for some very low absorption rates.

DEVELOPMENT OF PEPTIDE DOSAGE FORMS FOR NASAL ADMINISTRATION

Due to the degradation of peptides in the gastro-intestinal tract, the possibility of their nasal administration is of major value. However, because of the muco-ciliary clearance and of possible enzymatic hydrolysis, which reduce the contact time between the intact active ingredient and the mucosa, the development of a nasal form includes two essential points: the choice of an absorption enhancer and the design of a dosage form with a sensitivity to clearance phenomena as low as possible.

Absorption enhancers

Numerous absorption enhancers have been described for the nasal route [20] and their efficacy is very often obvious, although variable, according to the peptide considered. Different mechanisms of action have been proposed such as: alteration in the mucus rheology [21, 22], reduction in the nasal ciliary beat frequency [23, 24, 25], enhancement of transcellular transport by affecting membrane lipids and proteins [21], enhancement of paracellular transport [26], suppression of proteolytic activity [23], or enhancement of thermodynamic activity of peptides and proteins [26].

During the absorption study of human growth hormone (Table 5) [22], palmitoyl-DL-carnitine chloride proved to be a mean absorption enhancer. This should be explained by the fact that this enhancer, which acts almost as a fatty acid carrier through the mitochondrial membranes, is not well adapted for improving growth hormone absorption.

Dealing with aminopeptidase inhibitor, amastatin hydrochloride is a rather good enhancer compared with bestatin hydrochloride which has no effect. N-acetyl-L-cystein, which is a powerful mucolytic, has a notable effect on maximum plasma concentration, but a poor effect on the area under the curve. Furthermore it must be used at high concentrations (20%). The most efficient product is L-a-lysophosphatidyl-choline (lysolecithin), which is an amphiphile surfactant that lowers the mucus viscosity [27].

Table 5

Effect of some absorption enhancers on the bioavailability of human growth hormone (According to [22])

Enhancer	C max (ng/ml)	Increase in C max (%)	Increase in AUC (%)	Bioavailability compared with SC (%)
none	11.2			7.4
lysophospha-tidylcholine	61.6	450	250	25.8
palmitoyl-DL-carnitine	35.4	216	199	22.1
amastatin hydrochloride	42.4	278	291	28.9
bestatin hydrochloride	5.4	-	-	1.9
N-acetyl-L-cystein	40.4	260	64	12.2

The rôle of lysophosphatidylcholine has also been demonstrated on insulin absorption [27]. Numerous other enhancers have been investigated with regard to insulin, especially polyoxyethylene-9-lauryl ether and sodium taurodihydrofusidate [28]. This last product, which could act by increasing membrane permeability [29, 30], and probably also by inhibiting the enzymatic degradation [30], is particularly effective [28].

Some other absorption enhancers have been investigated by the nasal route, among which are biliary salts such as sodium glycocholate and dehydrocholate. Their action probably results from a lowering of the

membrane potential when this is penetrated [31]. Another mechanism of action could be related to their ability to release proteins from isolated epithelium [32].

Finally, dimethyl ß-cyclodextrin has been proposed as an absorption enhancer for 17-ß-estradiol [25, 33] and progesterone [33].

The toxicity of absorption enhancers has been the subject of various investigations, dealing especially with the possibility of membrane lesion and effect on ciliary movement. The disruptive effect of polyoxyethylene-9-lauryl ether (laureth-9) and taurodihydrofusidate [34, 35, 36] is discussed [28]. Sodium glycocholate, as well as didecanoyl-L-a-phosphatidylcholine, are without any effect on mucociliary transport [27]. On the other hand, lysophosphatidylcholine, polyoxyethylene-9-lauryl ether and sodium dihydrofusidate [27, 33], as well as sodium desoxycholate [33], should have a ciliostatic effect, which varies according to the concentration employed.

Dosage forms

The classic and ancient dosage form for drugs administered by the nasal route is obviously the liquid form. If it can eventually be effective for local disinfection, it is,on the other hand, very often inadequate to ensure the correct absorption of products intended for systemic activity. In fact it is easily subjected to mucociliary clearance and generally requires more or less repeated infusions and not just simple sprays.

Studies have been carried out with the main objective of evaluating the favourable effect resulting from an increase in viscosity [37, 38]. An increase in viscosity not only increases the particle size but also leads to a more localized *in vivo* deposition in the vestibule anterior part [37]. On the other hand, with regard to mucociliary clearance, results are difficult to interpret. According to some authors, an increase in viscosity results in a decrease in clearance rate [38]. For others, a progressive increase in viscosity leads first to a slowdown of clearance followed by an increase

Table 6
Influence of viscosity (% of methylcellulose)
on the bioavailability of desmopressin administered nasally
(According to [39])

Methylcellulose (%)	AUC pg/ml.h	C_{max} pg/ml	t_{max} min	$t_{1/2}$ h
0	2143±780	507±198	35±16	3.0±0.5
0.25	2364±896	490±292	58±21	2.8±0.7

[37]. Another factor must also be taken into account: the diffusion rate of the active ingredient. This one is progressively lowered [39], resulting in delayed release without any improvement in bioavailability (Table 6).

Directly deriving from viscous liquids, hydrogels have been studied. For example, in the case of a bioadhesive hydrogel containing polyacrylic acid for insulin nasal administration [40], if the bioadhesive character conferred by polyacrylic acid is favourable, on the other hand, too high a concentration to this polymer slows down insulin release and a delayed effect is observed.

In attempts at nasal vaccination by *Tetanus toxoid* [41], classic bioadhesive gels have proved their inefficacy, whereas an aqueous gel of silicic acid and anionic detergent led to an immunization comparable to that obtained by parenteral administration.

Solid forms have also been the subject of studies especially for insulin administration. A bioadhesive powder obtained by mixing the freeze-dried product of a viscous solution of insulin, polyacrylic acid and hydroxypropyl cellulose, with crystalline cellulose [42, 43], led to a

hypoglycaemia equivalent to one/third of that obtained by intravenous injection of the same insulin dose.

In fact, the greater part of studies deal with bioadhesive microspheres, especially for the administration of insulin [44, 45, 46], human growth hormone [47], oxytocin [48], desmopressin [49], or propranolol [50].

Three kinds of microsphere are more especially studied [51]: microspheres of DEAE-dextran, serum albumin and degradable starch. DEAE-dextran microspheres are prepared by the cross-linking of DEAE-dextran by epichlorohydrin, and they have a diameter of almost 40 to 150 μm. Serum albumin microspheres are obtained by an emulsification of rabbit serum albumin solution in petroleum ether and olive oil. Microspheres are stabilized by the addition of glutaraldehyde. After separation and washing they are freeze-dried. Their diameter is 40 to 60 μm [51]. Degradable starch microspheres are obtained by the cross-linking of starch by epichlorohydrin [52], and their mean diameter is 48 μm.

Work on Lomudal® (sodium chromoglycate) has revealed that DEAE-dextran microspheres lead to the most significant decrease in nasal clearance, followed by degradable starch and albumin microspheres [51]. In fact, with technetium-labelled microspheres, three hours after administration, there is still 60% of initial activity for DEAE-dextran microspheres, for 50% for starch or albumin microspheres, and only 20% for a solution or a powder [51].

A well-known bioadhesive polymer is polyacrylic acid (Carbopol®). Microspheres have been prepared by cross-linking Carbopol 907 with maltose in a w/o emulsification process [53]. It appeared that an increase in curing time of microspheres gave rise to an increased *in vitro* release rate of oxytocin. This result might be due to the fact that an increased curing time leads to a heavily cross-linked polymeric network inside the microspheres, and that swelling on hydration becomes severely limited. The oxytocin will therefore be rather surface-associated instead of being uniformly distributed throughout the microsphere.

CONCLUSION

Due to many advantages such as a high vasularization allowing good bioavailability, absence of first pass effect, low aminopeptidase activity, the nasal route reveals itself to be especially interesting for the systemic administation of products such as peptides. However, due to mucociliary clearance and still some possibilitiy of hydrolysis, the design of a nasal dosage form needs to slow down the clearance effect and to increase the absorption rate of the active ingredient. Bioadhesive forms, especially microspheres, containing absorption enhancers, seem to be a good answer to the pharmacolotechnical problem raised.

At present, a number of very promising works have already been carried out on peptides and proteins: insulin, human growth hormone, oxytocin, desmopressin, *Tetanus toxoid.* Their results allow the hope that the nasal route will be able to replace the parenteral route, leading to better patient comfort.

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