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COMMENTARY

Intranasal Delivery—Modification of Drug Metabolism and Brain Disposition

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ABSTRACT Intranasal route continues to be one of the main focuses of drug delivery research. Although it is generally perceived that the nasal route could avoid the first-pass metabolism in liver and gastrointestinal tract, the role of metabolic conversions in systemic and brain-targeted deliveries of the parent compounds and their metabolites should not be underestimated. In this commentary, metabolite formations after intranasal and other routes of administration are compared. Also, the disposition of metabolites in plasma and brain after nasal administrations of parent drugs, prodrugs and preformed metabolites will be discussed. The importance and implications of metabolism for future nasal drug development are highlighted.

KEY WORDS active metabolite · brain distribution · central nervous system · drug delivery · intranasal · metabolism

INTRODUCTION

Intranasal route continues to be an attractive field of research on systemic and brain-targeted drug deliveries. Although nasal route is regarded as a path that could avoid the first-pass metabolism in liver and gastrointestinal (GI) tract, the role of metabolic conversions in systemic and brain-targeted deliveries of the parent compounds and their metabolites should not be underestimated. Metabolite formation after intranasal application has not received adequate attention in the past, probably due to following reasons: 1) It is a general belief that the nasal route could avoid the first-pass metabolism effects. Consequently, information about the formation and importance of metabolites after nasal delivery of the parent drugs is limited. 2) The amount of metabolite formed, particularly in brain, is usually low in both human and animals. The traditional analytical instruments might not be sensitive enough for quantification or even identification of metabolites in plasma and central nervous system (CNS) including cerebrospinal fluid (CSF), intracellular contents after homogenization of brain tissue, or extracellular microdialysis. In addition, the unstable metabolites might not withstand the storage and sample treatments. With the development of advanced analytical technologies, such as LC/MS/MS and LC/NMR (1), qualitative and quantitative monitoring of active metabolites is becoming more feasible in nasal drug development. In this commentary, we summarize the metabolite formations and the disposition of metabolites in plasma and brain after nasal administrations of parent drugs, prodrugs and preformed metabolites. The importance and implications of metabolism for future nasal drug development are highlighted.

METABOLITE FORMATION IN HUMAN AFTER NASAL APPLICATION

Nasal Metabolisms

Although the metabolic capacity in nasal cavity is lower than that in liver or GI tract, the nasally applied drugs might still be subjected to metabolisms in the nasal mucosa.

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Phase I enzymes, such as cytochrome P-450s (CYP) and epoxide hydroxylase, and conjugative Phase II enzymes, such as UDP-glucuronyltransferase and glutathione transferase, have been identified in human nasal tissue (2). Metabolisms in nasal cavity play important roles in bioactivation and deactivation of inhaled or systemically applied toxicants (3), which have long been the focus of nasal investigations. However, data regarding the metabolism of nasal drugs is limited. Besides biotransformations, nasal metabolism could enhance upper respiratory tract scrubbing capacity of vapors such as naphthalene (4).

Gervasi *et al.* found in human respiratory mucosa that the amount of CYPs was about 1/20 of those in liver (~0.025 nmol/mg protein in nasal mucosa and ~0.5 nmol/mg protein in liver), and the catalytic activities of CYPs in human nasal mucosa were in general lower than that in liver (5). However, CYP activity on diethylnitrosamine in nasal mucosa, expressed per nmol of CYPs, was found to be 10–25 times higher than that in human liver (6). In metabolism studies of the CYP substrates by human respiratory mucosa, it was found that ethoxyresorufin was not metabolized, and the metabolisms of both testosterone and loteprednol-etabonate were limited and markedly lower than that in the liver (7). On the contrary, metabolic clearance of testosterone was higher in olfactory mucosa than liver in rat (8). For enzymes such as glutathione transferase, epoxide hydroxylase and rhodanese, Gervasi's study suggested that their activities were comparable or lower than that of human liver, while the nasal UDP-glucuronyltransferase activity was undetectable (5).

Direct nose-to-brain delivery through the olfactory region has undergone intensive investigation since the 1990s. Along the olfactory pathway, the enzyme expressions and specific metabolic barriers involved in olfactory epithelium, olfactory bulbs and brain have been reviewed by Minn *et al.* (9). However, the significance of olfactory enzyme systems relative to the metabolism and disposition of the xenobiotics in brain after nasal application remains unknown in human. In laboratory animals, for most enzymes, olfactory mucosa has a greater level of activity than nasal respiratory mucosa (10), and the concentrations and activities of CYPs and other enzymes in both mucosae could be comparable or even higher than that of liver (2,11). The human olfactory epithelium, restricted to a small area in the roof of nasal cavity, only owns less than 10% of nasal cavity compared with 50% in rat (12) and 77% in dog (13). Thus, the animal nasal models might overestimate the metabolism of nasal drug by olfactory epithelium. Due to species differences in nasal enzyme expression and metabolic capacities between human and laboratory animals (10), nasal metabolic data obtained from animal models should be interpreted with caution.

The importance of nasal metabolism in clinical practices is exemplified by ciclesonide, a novel corticosteroid for the treatment of allergic rhinitis and asthma. After nasal administration of ciclesonide, it is activated by intracellular esterases (mainly carboxylesterases and cholinesterases) in human nasal and other airway cells to pharmacologically active metabolite desisobutyryl-ciclesonide (des-CIC). The highly lipophilic fatty acid conjugates (logD~13) of des-CIC are retained inside the nasal cells and serve as a reversible pool of des-CIC, which remains at a stable amount throughout a 24-hour post-treatment period, allowing once-daily dosing for allergic rhinitis and asthma (14,15).

Compared to the nasal metabolism of small molecules, the impact of enzymatic degradation for nasal delivery of peptides is even more pronounced. *In vitro* human nasal metabolisms of peptides are summarized in Table II. In human nasal epithelium, peptide degradation by both exopeptidase (aminopeptidases, carboxypeptidases) and endopeptidases has been demonstrated. *In vitro* studies suggested that rate of enkephalin hydrolysis by nasal mucosa did not differ substantially from the ileal mucosa (16). Moreover, metabolism of opioid tetrapeptide was higher after intranasal delivery than intra-tracheal, pulmonary and intravenous applications (17), which suggested that enzymatic degradation could be one of the main barriers for nasal-delivered peptides and proteins. Leucine-enkephalin and methionine-enkephalin, the two model peptides for nasal metabolic studies, were mainly degraded via cleavage of tyrosine at the N-terminus by aminopeptidases (18).

As demonstrated in Table II, discrepancies in results from metabolic studies have been observed, e.g. in leucine-enkephalin (19), which could be attributed to four possible factors. First, species differences of nasal enzymes such as aminopeptidase and esterase (20) had been identified. As an example, the degradation product of desamino[1], D-arginine[8]-vasopressin rapidly formed in rabbit nasal mucosa (21) and rat intestinal mucosa (22) *in vitro* was not identified in human plasma after nasal administration of the drug (21). Since the predictability of animal metabolic studies remains controversial, we have only summarized data from human trials (Table I) and *in vitro* human nasal metabolism (Table II). Second, different experimental models have been applied in nasal metabolic studies. Among the four human nasal primary culture systems, peptidase activity was in the order of sequential monolayer-suspension > air-liquid interface > immersion > floating collagen (19). While nasal lavage contains mainly the extracellular enzymes, homogenization of the nasal tissue might liberate and/or degrade certain nasal enzymes (23). Thus, the rate and/or extent of peptide metabolism could vary between different *in vitro* metabolic incubation systems. Third, saturation of

nasal peptidases may lead to a certain portion of the nasal drug escaping from nasal metabolisms when the peptide dose chosen exceeds the metabolic capacity of the model (24,25). Fourth, the nasal cavity might not be the only region for drug absorption and metabolism *in vivo*, and part of the peptides could deposit in the oropharynx and be swallowed into GI tract, which will be discussed in the coming section.

Contribution of GI Absorption and Metabolism After Intranasal Administration

GI absorption and metabolism after nasal administration is unavoidable due to the following two situations. First, the mucus layer is propelled by the cilia towards the nasopharynx and then to the GI tract with a mucociliary transit time about 12 to 15 mins in humans (26). Therefore, a portion of the drug might be cleared into the GI tract before it is completely absorbed in the nasal cavity. Second, when the volume of solutions applied to the nasal cavity exceeds 100 μl in human and 20 μl in rat (12), significant amount of the drug might be swallowed and subjected to metabolisms in GI tract and liver.

In human trial by Fattinger *et al.* (27), after intranasal administration of cocaine solution, the fraction absorbed through the nasal mucosa was estimated to be 19%, which contributed 31% of total systemic cocaine exposure. By giving oral-activated charcoal, which can block drug absorption in GI, the direct absorption of zolmitriptan through nasal mucosa after administration via nasal spray was found to contribute only 29% of total zolmitriptan exposure. After intranasal administration of zolmitriptan, it was found that there was 30% of the total dose converted to 183C91, its active metabolite, via GI metabolism, whereas no zolmitriptan or 183C91 was detected in patients with charcoal treatment after ingestion of zolmitriptan tablet (28). Through similar strategy, the actual nasal absorption of beclomethasone dipropionate was suggested to be less than 1% after intranasal dosing. With oral charcoal treatment, concentration of the active metabolite, beclomethasone 17-monopropionate dropped significantly to a very low level in plasma (29) (Table I).

Further mechanistic studies suggested that highly permeable drugs, such as methotrexate and sulfanilic acid, were primarily absorbed through the nasal mucosa before it is cleared to the GI tract, and poorly permeable drug, such as inulin, was absorbed neither from the nasal nor the GI tract (30). It is suggested that the primary absorption site of drug after nasal application is determined by rate of mucociliary clearance and the extent of absorption through the nasal mucosa.

In summary, the significance of GI absorption and metabolism cannot be overlooked. Several strategies to minimize the GI disposition after nasal administration have been investiagated, including utilization of bioadhesive materials and microspheres to increase nasal residence time so as to enhance the absorption in nasal cavity and bioavailability (26,31). Clinical trials on desmopressin suggested that using spray device and applying the drug in divided doses to each nostril (2×50 μl rather than 1×100 μl) would enhance nasal absorption, bioavailability and clinical efficacy (32,33).

Example: Intranasal Opioids

The human plasma dispositions of parent drugs and metabolites after nasal administrations are summarized in Table I. Based on the well documented activities of both parent drug and resultant metabolites (34), opioids are used as examples to demonstrate the role of metabolism in intranasal delivery. For review, refer to ref. (35,36).

Pharmacokinetic study of heroin (diacetylmorphine or diamorphine) indicated that heroin is rapidly hydrolyzed in plasma by esterases to 6-monoacetylmorphine and then to morphine, which is further glucuronidated to morphine-3-glucuronide (M3G, with no analgesic activity) or morphine-6-glucuronide (M6G, active metabolite) primarily by UGT2B7 (37). After intranasal administration of heroin, a second peak in plasma concentrations of both morphine and M3G was observed in several subjects. The investigators suggested that it could be due to transient storage of parent drug or metabolite in nasal cavity, or swallowing of the intranasal dose with resultant delay in absorption and metabolism (38–40). After intranasal administration of morphine prepared with chitosan, the levels of its metabolites were comparable to that after intravenous, which were much lower than after oral route (41). Such results could be served as the evidence for direct uptake of morphine from nasal cavity to systemic circulation, escaping from first-pass metabolism in GI tract and liver, although chitosan, an absorption enhancer, could be a contributing factor.

In summary, Table I provides some insights on the metabolite formation after nasal drug application. Most of the drugs listed in Table I are subjected to significant first pass metabolisms in GI tract and liver after oral intake. If plasma $AUC_{metabolite}/AUC_{parent\ drug}$ after intranasal administration is higher than that after parenteral route, or similar to that after ingestion, a considerable portion of the nasal drug might actually be swallowed and then metabolized, as in the case of zolmitriptan. For most of the drugs contribution of metabolism in nasal cavity is largely unknown, except for the intranasal corticosteroids (e.g. ciclesonide) which are extensively metabolized by both nasal and GI enzymes, leading to negligible systemic bioavailabilities of the parent drugs.

Table I Comparison of Parent Drugs, Plasma Levels and Formation of Metabolites Between Nasal and Others Routes in Human

Nasal drug (bold) and active/inactive metabolites formed (italic)	Study subjects	Nasal formulations, dose and volume [a]	Plasma AUC / total amount [a]	Plasma C_{max} [a]	Comment	Ref.
Opioids and derivatives						
Heroin (Diamorphine)	Regular heroin users after 3 days abstinence	▪ 6 mg as powder mix with lactose	IM >> IN IM >> IN	IM >> IN IM >> IN	▪ The minor metabolite M6G was only detected after 12 mg IN but not 6 mg IN or IM	(39)
➔ *6-acetylmorphine (AM, active)*						
➔ *Morphine (active)*			IM >> IN	IM > IN		
➔ *Morphine-3-glucuronide (M3G, inactive)*			IN > IM	IM >> IN		
Morphine	Healthy adults	▪ 10 mg with chitosan powder or chitosan solution	IV > IN PO >> IN > IV	IV >> IN IV > IN	▪ Similar ratios (AUC_{M3G} / AUC_{M6G}) for IV (3.8) and IN (3.4)	(41)
➔ *Morphine-6-glucuronide (M6G, active)*						
➔ *Morphine-3-glucuronide (M3G, inactive)*		▪ 125 μl to each nostril	PO >> IN ≈ IV	IV >> IN	▪ No significant difference between two nasal formulations	
Butorphanol	Healthy adults	▪ 1 mg in solution given as Stadol nasal spray	IN ≈ IV	IN ≈ IV	▪ Based on 2 studies: IN 1 mg (97) and IV 1 mg (98)	(97,98)
➔ *Hydroxybutorphanol (HO-B, inactive)*		▪ 100 μl to one nostril	NA	IV > IN	▪ HO-B exhibited elimination-rate-limited kinetics in IN study	
Methadone (MET)	Healthy adults	▪ 10 mg in solution given with Pfeiffer BiDose sprayer	IV > PO ≈ IN	IV > IN >> PO	▪ Similar metabolic ratios (AUC_{EDDP}/AUC_{MET}) for different routes (~0.14)	(99)
➔ *2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium (EDDP, inactive)*		▪ 100 μl to each nostril	PO ≈ IV > IN	PO > IV > IN	▪ Not significant presystemic metabolism	
Oxycodone (OXY)	Healthy adults	▪ IN (0.1 mg/kg solution, ~350 μl to each nostril) *vs* IV (0.05 mg/kg)	IV > IN IN > IV	IV > IN IN > IV	▪ Metabolic ratio (AUC_{NOR-O}/AUC_{OXY}) higher in IN (0.34) than IV (0.2)	(100)
➔ *Noroxycodone (NOR-O, inactive)*						
Other drugs						
Beclomethasone dipropionate (BDP)	Healthy adults	▪ IN 1344 μg (16 sprays to each nostril) *vs.* IV 1000 μg *vs.* IH 1000 μg *vs.* PO 4000 μg (without PO charcoal treatment)	IV >> IH	IV >> IH	▪ Direct nasal mucosa absorption <1% ▪ BDP not detected after IN or PO ▪ Similar IN and PO bioavailabilities of BMP (~40%)	(29)
➔ *Beclomethasone 17-monopropionate (BMP, active)*		▪ Without PO charcoal	IV > IH > IN > PO	IV >> IH > PO > IN		
➔ *Beclomethasone 17-monopropionate*		▪ With PO charcoal	IH >> IN > PO	IH >> PO > IN		
➔ *Beclomethasone (BEC, inactive)*		▪ Without PO charcoal	IV >> IH > IN > PO	NA		
➔ *Beclomethasone*		▪ With PO charcoal	IH >> IN > PO	NA		
Ciclesonide (CIC)	Healthy adults	▪ IN aqueous spray (AQ, 300 μg, 3 sprays to each nostril) *vs* IN aerosol (AE, 300 μg) Vs orally IH (320 μg)	IH >> IN (AE) >> IN(AQ)	IH >> IN (AE) >> IN(AQ)	▪ Compared with IH, systemic exposure of DES-C was 10× lower after AE and at least 40× lower after AQ	(101)
➔ *Desisobutyryl ciclesonide*			IH >> IN	IH >> IN	▪ CIC was detectable up to 2 hrs after AE and up to	

(DES-C, active)			(AE) > IN (AQ)	(AE) > IN (AQ)	4 hrs after IH, but not detectable after AQ	
Cocaine (COC) ➔ *Benzoylecgonine (BZE, active)*	Cocaine users	▪ IN (32 mg COC HCl powder) *vs* IV (25 mg COC HCl) *vs* SM (42 mg COC free base)	IN ≈ SM ≈ IV IN > IV ≈ SM	IV ≈ SM > > IN IN > IV ≈ SM	▪ Similar metabolic ratios (AUC_{BZE}/AUC_{COC}) between IN and IV (~4.1) but lower for SM (2.8)	(102)
Ketamine (KET) ➔ *Norketamine (NOR-K, active)*	Halothane anesthetized children	▪ IN solution 3 mg kg^{-1} (~0.6 ml each nostril) *vs* IV 3 mg kg^{-1} ; IN 9 mg kg^{-1} *vs* PR 9 mg kg^{-1} (~1.8 ml each nostril)	IV > IN > PR PR > IN	IV > IN > PR IV, PR > IN	▪ NOR-K appeared earlier after PR than IN ▪ Some solution might be swallowed due to the large IN volume ▪ Anesthesia reduced sneezing, coughing or swallowing of the nasal dose	(103)
Ketamine (KET) **- (R)-enantiomer** **- (S)-enantiomer** ➔ *Norketamine (actve)* *- (R)-enantiomer* *- (S)-enantiomer*	Healthy adults	▪ Racemic KET IN solution 25 mg *vs* IV 20 mg *vs* PO 50 mg *vs* SL 50 mg *vs* PR 50 mg	IV > SL ≈ PR > IN ≈ PO IV > SL > PR > IN > PO PO > SL > PR > IV > IN PO > SL > PR > IV > IN	IV > SL > PR ≈ PO > IN IV > SL > PO ≈ PR > IN PO > SL > PR > IV > IN PO > SL > PR > IV > IN	▪ IN achieved the highest bioavailability (~45%, adjusted by dose) and low metabolites level ▪ Metabolic ratios (AUC_{NOR-K}/AUC_{KET}): PO > SL > PR > IN > IV ▪ No significant difference in pharmacokinetics of KET and NOR-K between each route, and between the (R)- and (S)-enantiomer	(104)
Midazolam (MID) ➔ *1-hydroxymidazolam (OH-M, active)*	Healthy adult (105); adult surgical patients (106)	▪ Aqueous spray solution: 5 mg, 90 μl to each nostril (105) ▪ 0.15 mg kg^{-1}, ~1 ml to each nostril (106)	IV > IN IV > IN	IV >> IN IV > IN	▪ Similar metabolic ratios (AUC_{OH-M}/AUC_{MID}) between IN and IV (~0.13) (105)	(105,106)
Zolmitriptan (ZOL) ➔ *N-desmethyl-Zolmitriptan (183C91, active)*	Healthy adults	▪ 100 μl IN spray 2.5 mg (pH7.4) *vs* IN spray 2.5 mg (pH5) *vs* PO tablet 2.5 mg (68) ▪ IN spray 2.5/5 mg *vs* PO tablet 2.5/5 mg (67)	PO ≥ IN PO ≥ IN	PO ≥ IN PO ≥ IN	▪ No significant difference between two pH ▪ Compared with ZOL, appearance of 183C91 was delayed in IN ▪ Similar metabolic ratios (AUC_{183C91}/AUC_{ZOL}) between IN and PO (~0.5)	(67,68)

AUC area under concentration-time curve, C_{max} maximum concentration, *IH* inhalation, *IM* intramuscular, *IN* intranasal, *IV* intravenous, *NA* not available, *PO* oral, *PR* rectal, *SL* sublingual, *SM* smoked

[a] Unless specified, doses of other routes are the same as that of IN route, and AUC and C_{max} are compared at the same dose.

Table II *In Vitro* Human Nasal Metabolisms of Various Peptides

Models	Enzymes, substrates (bold) and metabolites (italic) monitored	Inhibitors or absorption enhancers studied	Results	Comparison between different species and models	Reference
Metabolism in cell culture with transport studies					
Human nasal epithelium primary culture monolayer	**ME** ➔ *Des-Tyr-ME*	▪ Bestatin ▪ Puromycin	▪ Tiny amount of Phe-Met also detected as degradation product ▪ Co-incubation with enkephalin metabolite analogs reduced enzymatic hydrolysis of ME	NA	(107)
		▪ Glycocholate (GC) ▪ Dimethyl-β-cyclodextrin (DMβCD) ▪ Metabolite analogs	▪ Permeation of ME in the presence of puromycin alone or in combination with DMβCD, GC was higher than in the presence of bestatin, respectively		
	LE ➔ *Des-Tyr-LE*	▪ Bestatin ▪ Puromycin	▪ Both inhibitors induced a significant rebound AP activity, which can be associated with protein leakage, although only puromycin permeated the human nasal epithelium	▪ Rebound AP activity comparable to that in rat *in situ* perfusion by Hussain (108)	(109)
		▪ Glycocholate (GC) ▪ Dimethyl-β-cyclodextrin (DMβCD)	▪ Combination of puromycin with GC or DMβCD resulted in higher transport enhancement of LE and Des-Tyr-LE than when absorption enhancers were combined with bestatin or when inhibitors were used alone		
	Thyrotropin-releasing hormone (TRH) ➔ *TRH free acid* **ME** ➔ *Des-Tyr-ME*	NA	▪ Transport and metabolism of TRH (by cytosolic endopeptidase, with enzyme saturation) occurred in parallel ▪ Ca. 20% of Des-Tyr-ME (formed by APs on apical side) transported to serosal side ▪ TRH, ME and Des-Tyr-ME transported mainly through paracellular route	▪ TRH deavage rates in homogenates of rabbit nasal mucosa similar to those in human nasal epithelium (110)	(111)
Metabolism with nasal provocation studies					
Excised human nasal mucosa; nasal lavage fluid	i. Alanine-AP: **Ala-*p*-nitroanilide** ➔ *nitroanilide*	▪ Bestatin & Puromycin (APs inhibitors)	▪ Enzyme activities were higher in membrane-rich fraction than cytosolic fraction except CPs ▪ LE-degrading-AP and NEP originate from glands, while ACE and CPs originate from plasma ▪ Enzymatic activities in lavage might be provoked by nasal challenges of methacholine, histamine or allergen	NA	(18,112–114)
	ii. LE-degrading-AP: **LE** iii. Angiotensin-converting enzyme (ACE): **Benzoyl-Gly-Gly-Gly** ➔ *Benzoyl-Gly*; also **LE** iv. Carboxypeptidases (CPs) **Benzoyl-Gly-Lys** ➔ *Benzoyl-Gly*	▪ Captopril (ACE inhibitor) ▪ Mergetpa (CPs inhibitor) ▪ Phosphoramidon (NEP inhibitor)	▪ Bestatin showed stronger inhibition on APs than puromycin		

	v. Neutral endopeptidase (NEP): **LE**				
Metabolisms in excised tissues and primary cell cultures					
Human nasal epithelium primary culture monolayer; excised human nasal epithelium	Specific substrates for i. APN: **L-Ala-4-methyl-coumaryl-7-amide** ii. APB: **L-Arg-4-methyl-coumaryl-7-amide** iii. DD dipeptidyl dipeptidase: **Gly-L-Pro-4-methyl-coumaryl-7-amide** ➔ *7-amino-methyl coumarin*	▪ Bestatin ▪ Puromycin	▪ Except for APN, the tissue culture conditions did not significantly alter the functional (K_m, V_{max}, response to inhibitors) and mRNA expression of the APs ▪ Saturation of metabolite formation in DD observed (within 15 min) ▪ Bestatin showed stronger inhibition on APs than puromycin ▪ DD showed higher resistance than other enzymes to inhibitory effects by inhibitors	NA	(115)
Different human nasal primary culture systems	**LE** ➔ *Des-Tyr-LE*	▪ Bestatin ▪ Puromycin ▪ Metabolite analogs (Tyr-Gly, Phe-Leu, Tyr-Gly-Gly, Gly-Phe-Leu)	▪ Degradation products other than Des-Tyr-LE not observed (except a tiny amount of Phe-Leu) ▪ Degradation followed first-order kinetic ▪ Bestatin showed stronger enzymatic inhibition than puromycin ▪ Co-incubation with enkephalin metabolite analogs, particularly Gly-Phe-Leu, reduced hydrolysis of LE	▪ Differences in LE kinetics between species and experimental models	(19)
Human RPMI 2650 cell culture sheet; excised human nasal tissue	**4-Methoxy-2-naphthylamides (MNA) of Leu, Ala, Arg and Glu** ➔ *MNA* **Hoe 140**	NA	▪ AP pattern in culture sheets similar to excised tissue, probably included Leu-AP, APN, APA, APB, and lysosomal AP ▪ Higher enzyme activities in cell cultures for Leu-, Ala-, and Arg-MNA ▪ Glu-MNA and Hoe 140 stable in both models	NA	(116)
Human nasal epithelium primary culture monolayer	**LE** ➔ *Des-Tyr-LE* **ME** ➔ *Des-Tyr-ME*	NA	▪ Formation rates of the products Des-Tyr-LE and Des-Tyr-ME were similar ▪ Some of the Des-Tyr-LE were probably further degraded	▪ Formation rate of Des-Tyr-LE in agreement with the in-situ rat nasal perfusion study by Hussain (117)	(118)
Excised human nasal tissue	**1-Leu-4-methoxy-2-naphthylamide** ➔ *MNA*	NA	▪ Saturable nonlinear kinetics observed for metabolite formation	▪ Excised bovine and human nasal tissue showed similar metabolic rates at different substrate conc. and overall trends ▪ APs activities of bovine and human tissues dropped after 4 hours post excision	(119)
Enzymatic degradations in nasal wash					
Incubation with freshly collected human nasal wash	**Thyrotropin-releasing hormone**	Glycocholate (GC)	▪ No enzymatic degradation observed in human nasal wash	▪ Degradation in rabbit nasal mucosa could be substantially inhibited by small amount of GC	(110)
	Angiopeptin	NA	▪ No enzymatic degradation observed in human nasal wash ▪ Degradation probably caused by hydrolysis only ▪ Enzymatic stability might be due to alkylated N-terminus, amide-	▪ No enzymatic degradation observed in rabbit nasal mucosa tissue	(120)

Table II (continued)

Models	Enzymes, substrates (bold) and metabolites (italic) monitored	Inhibitors or absorption enhancers studied	Results	Comparison between different species and models	Reference
			altered C-terminus and having D- instead of L-configuration in some of the amino acids		
	Human insulin	NA	▪ Degradation rate in human nasal wash ≤ 0.02 *μg*/min, i.e. ≤ 0.5% of an intranasally applied insulin dose might be destroyed during the time of absorption (≈30 min)	▪ No significant degradation by pure leucine AP, porcine APN, and isolated enzymes from rabbit or pig nasal mucosae	(121)
	LE → *Des-Tyr-LE* **Des-Tyr-LE**	NA	▪ LE and Des-Tyr-LE followed first-order degradation, and all original peptides were hydrolyzed to other fragments ▪ Amount of Des-Tyr-LE formed from LE was unquantifiable	▪ LE was quantitatively converted to Des-Tyr-LE in rat *in situ* model (117)	(122)

AP aminopeptidase, *APA* aminopeptidase A, *APB* aminopeptidase B, *APN* aminopeptidase N, *LE* leucine-enkephalin (Tyr-Gly-Gly-Phe-Leu), *ME* methionine-enkephalin (Tyr-Gly-Gly-Phe-Met), *NA* not applicable

BRAIN DEPOSITION OF DRUGS AND THEIR METABOLITES

Nasal Pathways to CNS

Mechanistic studies on nose-to-brain transport proposed that drug may reach the CNS mainly through three pathways after nasal instillation: olfactory, trigeminal and systemic pathways (42,43). The olfactory epithelium is located at the very top of the nasal cavity, and the drug may cross the olfactory region by either neuronal or extraneuronal routes and reaches the brain parenchyma and CSF. The trigeminal neural pathway provides an additional route for transporting the drug to brain tissues. Both the olfactory and trigeminal pathways provide direct nose-to-brain delivery of the drug, with the extraneuronal pathway delivering the drug much faster (reaches brain within minutes) than the transneural pathways (44). The systemic pathway by which the drug is absorbed into the systemic circulation followed by brain entry via the blood-brain barrier (BBB) is an indirect pathway for delivering nasal-administered drug to brain and confers no selective advantage on CNS-targeted delivery. The metabolites formed during circulation might also enter the brain through BBB. The nasally applied drugs, thus, could reach the brain/CSF by means of one or multiple transport mechanisms (45).

Brain Distribution After Nasal Uptake

Quantitative studies about the brain dispositions of metabolites after nasal applications of parent compounds are rather limited and are presented in Table III.

The nasal absorption and the subsequent brain/CSF uptake are related to the lipophilicity and molecular size of the drug (46). The distribution of drug in brain after nasal uptake also partly depends on these physiochemical properties. Graff and colleagues (47) demonstrated in P-glycoprotein (P-gp)-deficient mice that [^{3}H]-sucrose showed very limited brain uptake and did not distribute beyond the olfactory region. In addition, no [^{3}H]-sucrose was detected in the brain after intravenous administration, as this bulky, hydrophilic compound was unable to cross the BBB. [^{14}C]-diazepam, the most lipophilic compound tested, exhibited the highest total brain exposure, which was probably due to efficient diffusion through the olfactory epithelium into the brain. The amount delivered to brain correlated significantly with the log P values of the four model compounds tested. Nasal delivery resulted in preferential brain exposure in rostral portion, and the exposure decreased consistently from rostral to caudal portions. These results indicate that, in the absence of transporter-mediated flux such as P-gp, physicochemical

properties of the compound (i.e., lipophilicity) serve as the primary determinant of brain uptake and distribution after nasal application.

As the metabolites, particularly the phase-II conjugates, are more hydrophilic and bulky, it is expected that they might have a more limited distribution within the brain than their parent compounds if active transporters are not involved. Intranasal administrations of both cocaine (48) and benzoylecgonine (its *O*-demethylated active metabolite) (49) have been conducted and compared with that from intravenous administrations. It was found that the expected enhancement in brain-to-plasma ratio via nasal administrations is more significant for hydrophilic benzoylecgonine (logP 0.15) than hydrophobic cocaine (logP 7.6), which could be due to their lipophilicity differences and relative blood-brain barrier permeabilities.

Considering the CNS-targeting potentials of intranasal route after nasal application of the parent drugs, the active metabolites of these CNS drugs could contribute to the therapeutic effects in CNS even though they might have lower abilities to cross the BBB than the parent compounds. M6G is a good example to demonstrate this concept. Although M6G has direct analgesic effects, and the potency of M6G ranged from 0.3 to 808 times of that of morphine in rat or mice pain models (50), morphine was found to be present in human CSF at several folds higher concentration than its glucuronide metabolites after different routes of administrations (51). Klipatrick and Smith suggest that, although M6G has a lower efficiency in crossing the BBB than morphine, probably as a result of lower lipophilicity, other features of M6G, including metabolic stability, high unbound levels in fluid compartments on both sides of the BBB, low distribution from brain extracellular to intracellular fluid, and differences in affinities for transporter proteins may compensate and contribute to the observed *in vivo* efficacy of M6G (50).

Graff and colleagues also demonstrated that P-gp, which is present in both nasal cavity, olfactory epithelium and olfactory bulb, attenuated brain uptake and facilitated brain removal of intranasally administered P-gp substrates (52), which might be reversed by intranasal P-gp inhibitor (52,53). The magnitude of the influence of P-gp on substrate residence in brain depended on the region of brain (47). Therefore, both lipid solubility and efflux transporters are important factors in determining nasal absorption and subsequent distribution in brain.

In addition to Pgp, lipophilicity and molecular size of the parent compounds as well as their metabolites, metabolic characteristics of different animal models could also affect the brain dispositions of parent drugs and their metabolites after nasal delivery. Studies in both mice and rats demonstrated that diazepam is metabolized by CYPs to *N*-desmethyldiazepam, which is further hydroxylated to oxazepam. Compared to diazepam, its metabolites enter the brain more slowly after systemic administration but exert comparable anticonvulsant activity. These studies also showed that the level of diazepam is similar in both species, while there is an accumulation of *N*-desmethyl metabolites in brain of mice rather than rats, providing longer lasting anticonvulsant effect (54–56). After intravenous injection of diazepam to mice, diazepam is no longer detectable 60 min post-injection. Although *N*-desmethyl metabolites are still detectable in brain and plasma 24 hours after injection of diazepam to mice (57,58), they are only transiently present in trace amount in rat brain after diazepam injection (54). Therefore, a significant portion of the radioactivity in mice brain at 6 hours post-dose (when peak radioactivity occurred) in Graff's study on nasally delivered [^{14}C]-diazepam (47) could be constituted by these metabolites. On the contrary, Kaur and Kim concluded that there was no significant direct nose-to-brain transport of diazepam via olfactory epithelium since they observed homogenous distribution patterns of the unchanged diazepam in various brain regions within 60 min after intranasal and intravenous administrations in both S/D rats and rabbits (59). Therefore, species difference should be considered in nasal delivery studies. Even within the same species, inter-strain differences in diazepam metabolism by liver (60) and kidney (61) had been identified in rats.

IMPLICATIONS OF METABOLISM FOR NASAL DRUG DEVELOPMENT

Pharmacokinetic and Pharmacodynamic Monitoring of Nasal-Delivered Drugs

Active metabolites are common for various CNS drugs, and their roles in opioids (34), antidepressants (62) and antipsychotic drugs (63) have been well documented. From a drug-development perspective, active metabolites appear to be a mixed blessing. Although they can be developed as "new" drugs in their own right (64), for the safety evaluation of new drug products, certain metabolites have to be monitored in systemic circulation such as plasma and excreta such as urine and feces (65,66). Which metabolites and how they should be assessed remain a matter of study and debate.

The pharmacokinetics of metabolites is more complex than that of parent drug. More effort should be put into monitoring the active metabolite profiles and investigating the correlation of active metabolites levels with pharmacodynamic effects after nasal application, which could provide further clinical utilities. For instance, as morphine has lower clearance rate than heroin and 6-acetylmorphine, morphine is present in the body for a longer period of time. So

Table III Brain Dispositions of Parent Compounds and Their Metabolites After Nasal Applications in Rats and Mouse

Nasal compound (bold) and metabolites formed (italic)	Nasal formulations	Disposition in brain	Comment	Ref
Small molecules				
Morphine (MOR)	IN solution (50 μl) to the right nostril, compared with equivalent IV dose	▪ IN: Right OB > Left OB > Right Hem ≈ Left Hem ▪ IV: Left OB ≈ Right OB ≈ Right Hem ≈ Left Hem ▪ M3G was detected in the right OB 15 min and 60 min (0.8 and 1.0 nmol/g tissue, respectively) after IN but not detectable in other brain tissue after IN or IV	▪ AUC of MOR at right OB 5× higher after IN than IV ▪ Metabolic ratio in plasma (AUC_{M3G}/AUC_{MOR}) higher after IN (5.3) than IV (1.2)	(123)
➔ *Morphine-3-glucuronide (M3G)*				
[a]**Toluene**	Inhalation of volatile vapor	NA	▪ Metabolic pathway: toluene ➔ BA ➔ HA	(124)
➔ *Benzoic acid (BA)*		Nasal mucosa > OB > Cerebrum hem		
➔ *Hippuric acid (HA, glycine-conjugated)*		Nasal mucosa > OB > Cerebrum hem	▪ HA levels much lower than BA	
[a]**para-Xylene**	Inhalation of volatile vapor	NA	▪ Metabolic pathway: para-xylene ➔ TA ➔ MHA	(124)
➔ *para-meta-Toluic acid (TA)*		Nasal mucosa > OB >> Cerebrum hem (not detectable)	▪ Selective accumulation of MHA but not TA in OB despite similar conc. in nasal mucosa	
➔ *Methylhippuric acid (MHA, glycine-conjugated)*		Nasal mucosa > OB >> Cerebrum hem (not detectable)		
Peptides				
Semax (Met-Glu-His-Phe-Pro-Gly-Pro)	IN aqueous solution (20 μl) to both nostrils	▪ From 2 min to 60 min after IN, percentage of semax in brain decreased from 77% to 23% (of total semax and metabolites radioactivity)	▪ Rapid enzymatic hydrolysis in plasma and to a lesser extent in brain by proteases (carboxypeptidases) to shorter peptides, mainly to tripeptide Pro-Gly-Pro	(125)
➔ *Sem-3 fraction (mainly Pro-Gly-Pro)*		▪ From 2 min to 60 min after IN, percentage of Sem-3 in brain increased from 2% to 52%	▪ Semax in brain was 10–15 times higher after IN than IV	
Prodrugs				
D4T-acetate (D4T-Ac, prodrug) ➔ *D4T*	IN solution (100 μl) to one nostril, compared with a halved IV dose	▪ Much higher conc. of D4T (2′,3′-didehydro-3′-deoxythymidine) in CSF 15 min after IN than IV ▪ Most of the prodrug reached CSF as regenerated D4T	▪ D4T and D4T-Ac well absorbed from nasal mucosa ▪ A large part of D4T-Ac might be hydrolyzed to D4T before or during transportation to CSF	(84)
D4T-hemi-succinate (D4T-Su, prodrug) ➔ *D4T*	IN solution (100 μl) to one nostrils, compared with equivalent IV dose	▪ IN D4T-Su reached CSF in intact form, which was not detected in CSF after IV D4T-Su	▪ D4T-Su has higher hydrophilicity and stability in rat nasal tissue homogenate than D4T-Ac ▪ Slower absorption of D4T-Su from nasal cavity than D4T-Ac	(84)
3-DMABE$_2$HCl (3DE, ester prodrug) ➔ *17β-Estradiol*	IN solution (100 μl) to one nostril, compared with equivalent IV dose	CSF ratio of 17β-estradiol (IN/IV) 8.8	▪ 3DE and 17DE are rapidly hydrolyzed in rat brain homogenate and plasma ($t_{1/2}$ ≈1–2 min) ▪ 3DE and 17DE have 100× higher aqueous solubility than estradiol	(81)
17-DMABE$_2$HCl (17DE, ester prodrug) ➔ *17β-Estradiol*	(same)	CSF ratio 17β-estradiol (IN/IV) 4.7		(81)

L-dopa Butyl Ester (prodrug) → *L-dopa (L-DA)* →*Dopamine (DA)*	IN solution (100 μl) to one nostril, compared with equivalent IV dose	▪ Conc. of L-DA and DA in CSF and OB were several times higher after IN ▪ L-DA is further decarboxylated to DA in brain and peripheral circulation	▪ Prodrug has 400× higher solubility than L-DA ▪ Prodrug is rapidly hydrolyzed in rat brain and plasma ($t_{1/2}$ ≈0.7 min) but is stable in CSF and nasal perfusate ($t_{1/2}$ 33 and 144 min, resp.)	(80)
Nipecotic Acid n-Butyl Ester (prodrug) → *Nipecotic Acid*	IN solution (25 μl) to each nostril, compared with equivalent IV dose	Total brain exposure to nipecotic acid was not significantly different after IN and IV	▪ Ester hydrolysis in rat brain is rate limiting for nipecotic acid brain delivery ▪ Tissue trapping of the nipecotic acid formed in brain	(83)

[a] Study using mouse. *CSF* cerebrospinal fluid, *Hem* hemisphere, *IN* intranasal, *IV* intravenous, *NA* not available, *OB* olfactory bulb

after intranasal heroin administration in human, morphine's time course coincided most closely with that observed for drug-induced effects (38). After intranasal administration, zolmitriptan appeared in human plasma more rapidly (2 min) compared with that after oral tablet (10 min), reflecting rapid absorption across nasal mucosa and that first-pass metabolism may be initially bypassed. However, the appearance of its active metabolite 183C91 was delayed after intranasal administration (67,68). In such case, nasal application of zolmitriptan might have a dual advantage of faster onset of action against the acute migraine attack through the parent compound, with sustained relief and protection against recurrence of migraine attack symptoms via its active metabolite 183C91 (67).

In addition to therapeutic benefits, potential interactions among parent drugs and the active metabolites could exist. M3G does not bind to opioid receptors and is devoid of analgesic activity. However, M3G might antagonize M6G-analgesia and morphine-analgesia in rat (69,70). Intranasal midazolam has been extensively investigated for its therapeutic effects in both children and adults. The pharmacodynamic competitive interaction between midazolam and its active metabolite 1-hydroxy-midazolam has also been characterized *in vivo* (71).

Considering the above-mentioned complexities resulted from intranasal administration, it is believed that the clinical effects cannot be predicted merely from plasma level of the parent drug, and the use of biomarkers should be considered. Biomarkers are objective physical signs or laboratory measurements occurring in association with a pathological process and have putative diagnostic and/or prognostic utility (72). For instance, intranasal corticosteroids are intended for the local treatment of allergic rhinitis. However, the systemic availabilities of different intranasal corticosteroids could range from less than 0.1% to 100%; thus, systemic toxicity is a concern (73,74). Different biomarkers have been used as surrogate measurements for the systemic effects, including hypothalamic-pituitary-adrenal axis activity, bone metabolism and growth after intranasal corticosteroids treatment (75,76). Therefore, the relationships between plasma levels and brain levels of drug, metabolites, biomarkers and clinical outcomes should be further assessed for nasal-delivered drugs.

Nasal Drug Design

Prodrug and Structural Modification Approaches

As metabolizing enzymes are present in the nasal mucosa, use of prodrug has been adopted as a strategy to enhance nasal drug delivery of small molecules and peptides (77). Esterification is one of the most common approaches, as esterases are present and exhibit high activity in the nasal

mucosa (20,78). Among the intranasal corticosteroids, ciclesonide and beclomethasone dipropionate (Table I) are inactive ester prodrugs, which would be activated by esterases in nasal cells for local anti-inflammatory actions.

Prodrugs confer particular advantage to CNS-target delivery, and nose-to-brain delivery of several prodrugs have been reported (79) and summarized in Table III. Hydrophilicities and enzymatic stabilities of the prodrugs could be manipulated by modifying the chemical structure. The ester prodrugs of L-dopa (80) and 17β-estradiol (81) have 100 to 400 times higher aqueous solubilities than the original drugs, and all these ester prodrugs are rapidly hydrolyzed in brain and plasma. Compared with intravenous dosing, nasal deliveries of all these prodrugs achieved higher brain exposure of original drugs. On the contrary, direct nasal administration of L-dopa did not enhance brain disposition (AUC_{brain}/AUC_{plasma}) compared with intravenous and oral routes (82). Nipecotic acid, a CNS active zwitterions, had only 14% systemic availability after nasal dosing, which is possibly due to its highly polar nature (logP 0.006). However, with a better hydrophilic-hydrophobic balance, the *n*-butyl ester of nipecotic acid (logP 0.93) could achieve a systemic availability of 92% after nasal delivery (83). Deliveries of 2′,3′-didehydro-3′-deoxythymidine (D4T) and its acetate and hemi-succinate prodrugs to CSF via nasal route indicated that a large part of the acetate prodrug might be hydrolyzed in the nasal cavity to D4T prior or during transport to CSF, whereas the more hydrophilic hemi-succinate prodrug reached CSF slowly and mainly as intact form due to its higher enzymatic stability in nasal tissue (84).

Considering protease inhibitors' ciliotoxicity (85) and their interferences on the physiological functioning of endogenous proteases, structural modification of peptides could be an alternative approach to enhance enzymatic stability and nasal absorption of peptides. For instance, L-tyrosine was absorbed from the nasal cavity in its zwitterionic form with limited absorption. Although the esters of L-tyrosine studied had higher partition coefficients than tyrosine, only the carboxylic acid esters, but not the *O*-actyl esters, exhibited higher absorption rates with only a small portion being hydrolyzed to tyrosine. The enhancement of nasal absorption by esterification is therefore attributed to the masking of negative charge on carboxylate moiety of the amino acid rather than the increase in lipophilicity (86). The more lipophilic methyl ester of L-tyrosyl-L-tyrosine was also found to be stable in nasal cavity with similar absorption rate to that of the original peptide (87). Study on a series of hexapeptides also illustrated that nasal absorption of peptides might not correlate closely with their lipophilicities (88).

Besides esterification, the effects of other structural modification on nasal metabolism and absorption of peptides had also been reported. Changing the N-terminal amino acid of leucine-enkephalin from tyrosine to aspartic acid provided excellent stability against aminopeptidases while maintaining similar nasal absorption rate (89). Substitution of natural L-amino acid with unnatural D-amino acid could also enhance the stability of the peptide against nasal peptidase (16,88). Polyethyleneglycol conjugation on salmon calcitonin could not only protect the peptide against nasal peptidases (90), but also lead to delayed time to maximal concentration and prolonged elimination half life after nasal administration (91), probably because the pegylated peptide is retained in the nasal cavity and serves as a reservoir of sustained release (92).

Direct Application of the Active Metabolites

When given to humans or animals, a synthetic, preformed metabolite's kinetic behavior could differ from that of the corresponding metabolite generated endogenously from its parent compound (93,94). Nasal application of preformed metabolites, which are usually more hydrophilic and more bulky (if such metabolites are phase-II conjugation products) than the parent compounds, might result in lower contribution of nasal cavity absorption (relative to GI absorption) to total exposure (30). Therefore, the pharmacokinetic profile should be carefully studied if the preformed metabolite is used for nasal delivery.

Direct applications of preformed active metabolites have been reported. The pharmacokinetic and pharmacodynamic effects of intranasal cocaethylene, an active metabolite of cocaine, had been studied in human. Using the same dose of intranasal cocaine to compare, intranasal cocaethylene resulted in similar euphoria with shorter absorption half life but longer elimination half life (95). Nasal application of M6G in sheep resulted in a bioavailability of 31% with no morphine or M3G detected in plasma (96). Nose-to-brain delivery of benzoylecgonine, the active metabolite of cocaine, had also been studied in rat (49) as discussed in the previous section.

SUMMARY

There is a need for further investigation in metabolite formation and disposition after nasal application. It is difficult to predict the overall efficacy of the nasal drug as both the formations and/or ratios of parent drugs and active metabolites in plasma and brain could be modified by the nasal route. The clinical effects cannot be estimated merely from the pharmacokinetic parameters from human or animal models. Therefore, concurrent pharmacodynamic investigations are necessary to establish the potential utilities of the nasal drug. Biomarkers from plasma, CSF or

other tissues could be used and should be investigated simultaneously if possible. The relationships between levels of parent drug, metabolites, biomarkers and clinical responses should be verified. More effort should be put on the pharmacokinetic-pharmacodynamic correlations of active metabolites, which would facilitate the development of nasal medicines in forms of parent drugs, prodrugs or preformed metabolites.

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