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Facilitated Uptake of Fentanyl, but Not Alfentanil, by Human Pulmonary Endothelial Cells

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Background: **Extensive pulmonary uptake of lipophilic basic amines, such as fentanyl, attenuates early blood drug concentrations after rapid intravenous administration. The basis of this phenomenon is poorly understood. The authors tested the hypothesis that fentanyl uptake into cultured human lung microvascular endothelial (HMVE-L) cells occurs by facilitated uptake in addition to passive diffusion. The authors compared fentanyl and alfentanil uptake with that of antipyrine, a diffusible marker of pulmonary tissue water. In addition, the authors determined the effect of verapamil, a nonspecific inhibitor of drug transport, and UIC2, a blocking antibody of the P-glycoprotein drug transporter, on the uptake of these drugs.**

Methods: **Human lung microvascular endothelial cells were incubated, with varying concentrations of antipyrine and fentanyl or alfentanil in the absence or presence of varying verapamil concentrations or of UIC2. Supernatants were collected and cells were rinsed and dissolved. Supernatant and cell-associated antipyrine, fentanyl, and alfentanil concentrations were measured. The data were fit to a model of cellular uptake that allowed for passive diffusion and facilitated uptake.**

Results: **Alfentanil uptake by HMVE-L cells was indistinguishable from that of antipyrine for the concentration ranges studied. In contrast, at low concentrations, fentanyl sequestration into HMVE-L cells was substantially greater than that of antipyrine. Facilitated fentanyl uptake was blocked by verapamil, but not by UIC2, in a concentration-dependent manner.**

Conclusions: **The differential HMVE-L uptake of fentanyl and alfentanil is consistent with the observed differences in the pulmonary uptake of these drugs. This suggests that specific fentanyl uptake and sequestration by HMVE-L cells may be the mechanisms of its extensive pulmonary uptake. (Key words: Antipyrine; facilitated drug uptake; P-glycoprotein blocking antibody.)**

THE lungs are anatomically unique in that they are located between the systemic venous and arterial circulations, and they are perfused by nearly the entire cardiac output. In addition, the pulmonary circulation contains almost half the endothelium in the entire body.[1] Thus, in addition to gas exchange, the lungs are well-designed for the regulation of blood concentrations of autacoids and xenobiotics.[2,3] Extensive pulmonary uptake of basic lipophilic amines, such as fentanyl,[4,5] lidocaine,[6,7] propranolol,[8] and sufentanil,[9] has been reported. Because of this capacitive effect, early arterial concentrations of these drugs are lower than those of other lipophilic drugs.

It has long been maintained that extensive pulmonary drug uptake is the result of "simple diffusion" of drugs from the intravascular space into lung tissues.[3] However, we recently demonstrated that the relative uptake of fentanyl by isolated bovine pulmonary artery endothelial cells was significantly greater at lower concentrations than would be expected by diffusion alone.[10] We explained these observations with use of a model of fentanyl uptake that includes passive diffusion and saturable, specific uptake mechanisms.

Although the pulmonary uptake of fentanyl is extensive,[4,5] that of the structurally related phenylpiperidine opioid analgesic alfentanil is minimal.[9] The purpose of the current study was to compare the uptake of fentanyl and alfentanil with that of antipyrine, a tissue water marker that has been used to estimate the volume of extravascular lung water,[11,12] in primary cultures of human lung microvascular endothelial (HMVE-L) cells. We tested the hypothesis that, although alfentanil and antipyrine are distributed into HMVE-L cells by diffusion alone, fentanyl uptake by HMVE-L cells occurs not

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only by passive diffusion, but also by a specific uptake mechanism that can be blocked by verapamil, an inhibitor of multidrug transporters.[13,14] To identify this transporter, we also tested the effect of UIC2, a blocking antibody of the P-glycoprotein drug transporter,[15] on the uptake of these drugs by HMVE-L cells.

Materials and Methods

Cell Culture

Human lung microvascular endothelial cells were obtained from Clonetics (San Diego, CA) and maintained in a supplemented endothelial growth medium (EGM-2-MV) supplied by Clonetics that contained human epidermal growth factor (hEGF), hydrocortisone, vascular endothelial growth factor (VEGF), human basic fibroblast growth factor (hFGF-B with heparin), long recombinant 3-insulin–like growth factor-1 (R3-IGF-1), ascorbic acid, heparin, gentamicin, amphotericin B, and 5% fetal bovine serum. Cells between passages 4 and 8 were seeded onto 12-well culture plates (3.8 cm^2/well) 2 days before experimentation. For experimentation, cells were more than 90% confluent, and the cell density was approximately 7×10^4 cells/well, as evaluated by use of a Coulter counter (Model ZM; Coulter Electronics, Hialeah, FL) for representative wells from each plate.

Experimental Protocol

Each experiment was performed in triplicate with use of cells from at least two different donors. At the beginning of the experiment, the cells were rinsed twice with 1 ml of Hank's balanced salt solution that contained 0.5% bovine serum albumin (HBSS; GIBCO, Grand Island, NY) and then incubated for 10 min at 37°C, with HBSS containing varying concentrations of verapamil, ^{14}C-labeled (Sigma Chemical, St. Louis, MO) and unlabeled (Sigma Chemical) antipyrine, and ^{3}H-labeled (Research Diagnostics, Flanders, NJ) and unlabeled (Sigma Chemical) fentanyl or ^{3}H-labeled (Research Diagnostics) and unlabeled (Janssen Pharmaceutica, Piscataway, NJ) alfentanil. To study the effects of verapamil on the uptake of antipyrine and the opioids, we measured uptake during control conditions (*i.e.*, the HBSS contained no verapamil) and in the presence of 1.0, 10, and 100 μM verapamil. ^{14}C-Antipyrine (0.0015 mM) was used alone and with unlabeled antipyrine at concentrations ranging from 0.00106 to 10.6 mM. ^{3}H-fentanyl (0.0071 μM) was used alone and with unlabeled fentanyl at concentrations ranging from 0.0946 to 946 μM. ^{3}H-Alfentanil (0.005 μM) was used alone and with unlabeled alfentanil at concentrations ranging from 0.024 to 94.5 μM. Preliminary experiments showed that equilibrium between the cells and the supernatant was achieved by 10 min.

After 10 min of incubation, the supernatant was removed from the wells and the cells were rinsed twice with HBSS that contained excess unlabeled antipyrine and fentanyl or alfentanil to prevent back-diffusion of cell-associated, labeled drug. The cells were then dissolved using 1 N NaOH. The cell-associated and free ^{14}C-antipyrine and ^{3}H-fentanyl or ^{3}H-alfentanil were measured using a dual-label, liquid-scintillation technique with external standard quench correction, as previously described.[16] Counts that were less than twice the background count were considered to be below the lower limit of detection; the coefficient of variation of replicate sample analysis by the assay was less than 3%. Cell-associated antipyrine and fentanyl or alfentanil data were normalized for antipyrine and fentanyl or alfentanil per cell by dividing the antipyrine and fentanyl or alfentanil concentrations by the mean number of cells/well for that day's experiments.

Data Analysis

To enable description of drug uptake as passive, facilitated, or both, we evaluated HMVE-L cellular drug uptake with use of a model that includes both a diffusional pathway and a saturable facilitated uptake pathway, as previously described[10] and as illustrated in figure 1. Cellular uptake by passive diffusion is characterized by rapid partitioning between the supernatant fluid (C_S) and a cellular diffusion compartment (C_D) with use of a partition coefficient H:

$$H = C_D/C_S \quad (1)$$

At equilibrium, specific, facilitated drug uptake into the cellular transporter compartment (C_T) is characterized by a bimolecular reaction rate constant (k_t) for binding to the number of available transporter sites (R, which is the difference between the total transport capacity, R_{max}, and the drug in the cell arriving by the transport mechanism, C_T), assuming dissociation of drug from the transporter, characterized by k_o, results in immediate equilibration with the drug partitioned into the cell by diffusion alone, C_D:

$$C_T = (k_t/k_o) \cdot C_S \cdot R \quad (2)$$

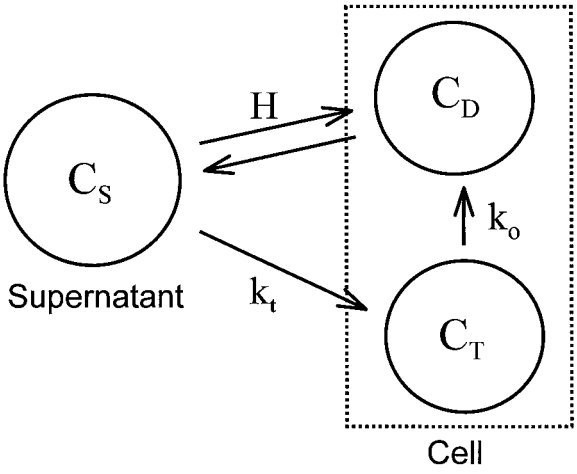

Fig. 1. The model of drug uptake from supernatant (C_S) into human lung microvascular endothelial cells by diffusion (C_D) and by facilitated transport (C_T). The drug can enter the cells by passive diffusion, characterized by a partition coefficient (H), or by facilitated transport, characterized by a rate constant for association with a specific cellular or subcellular binding site (k_t). Intracellular transfer between C_T and C_D is assumed to move only in the direction characterized by the transfer rate constant (k_o).

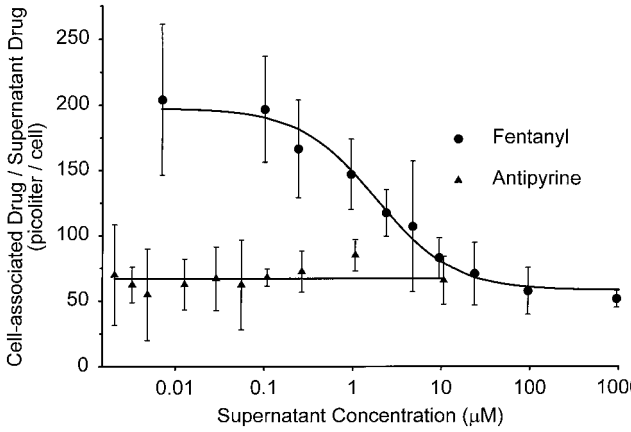

Fig. 2. Fentanyl and antipyrine uptake by human lung microvascular endothelial cells. The symbols represent the ratio (K_{EQ}) of total cell-associated drug concentrations (nM/cell) to supernatant drug concentrations plotted as a function of supernatant drug concentration (C_S, μM). The symbols (circles = fentanyl; triangles = antipyrine) represent the mean value from three experiments; the error bars represent the SD; the solid line represents the best fit of the model to the data for fentanyl. For antipyrine, the solid line represents the mean value for K_{EQ} for the concentration range studied.

The equilibration between cell-associated drug and extracellular drug can be given by

$$K_{EQ} = (C_D + C_T)/C_S = H + R_{max}/[(k_o/k_t) + C_S] \quad (3)$$

where the ratio k_o/k_t is the free drug concentration that leads to 50% occupancy of the transporters.[10]

The ratio (K_{EQ}) of total (labeled plus unlabeled) cell-associated drug ($C_D + C_T$), normalized to the number of cells/well, to supernatant drug concentration (C_S) is plotted as a function of supernatant drug concentration (C_S). Data from each experiment were fit to equation 3 using a constant weight and TableCurve2D (SPSS, Chicago, IL). From the nonlinear least-squares fit, values for H, R_{max}, and the ratio k_o/k_t were obtained. If the nonlinear least-squares fit (equation 3) did not reach the criterion for rejection of the null hypothesis, $P < 0.05$, the data were pooled for the concentration range studied and represented by the average value for K_{EQ}.

Results

Specific Uptake of Fentanyl by Human Lung Microvascular Endothelial Cells

If a drug is equilibrated with cells by simple diffusion, the relative amount of uptake (*i.e.*, the partition coefficient) should be constant with increasing concentrations. As shown in figure 2, the uptake of antipyrine was constant for the concentration range, suggesting uptake by simple diffusion alone. However, the relative uptake of fentanyl is much higher at the lower concentrations. At the higher concentrations, the relative fentanyl uptake decreases and approaches that of antipyrine, suggesting saturation of facilitated uptake. Similar to antipyrine, the relative uptake of alfentanil was constant for the entire concentration range (fig. 3).

As indicated in equation 3, at the highest concentrations the value of the equilibration constant K_{EQ} approaches the value of the diffusional partition coefficient H. The diffusional partition coefficient in this relation can be interpreted similarly to a volume of distribution for the cells. The diffusional partition coefficient H for fentanyl based on the best fit of equation 3 to the control data was 57.2 picoliters/cell, whereas the H derived from the average K_{EQ} for antipyrine was 66.8 picoliters/cell. The H derived from the average K_{EQ} for alfentanil was 64.6 picoliters/cell. The similarity of the H for fentanyl and the average K_{EQ} for antipyrine and alfentanil would suggest that the drugs exhibit similar nonspecific partitioning, but that there is an additional mechanism that facilitates fentanyl uptake and becomes saturated at high concentrations.

The best-fit curve for fentanyl also gave the number of transporters/cell ($R_{max} = 1.55 \times 10^8$/cell) and the con-

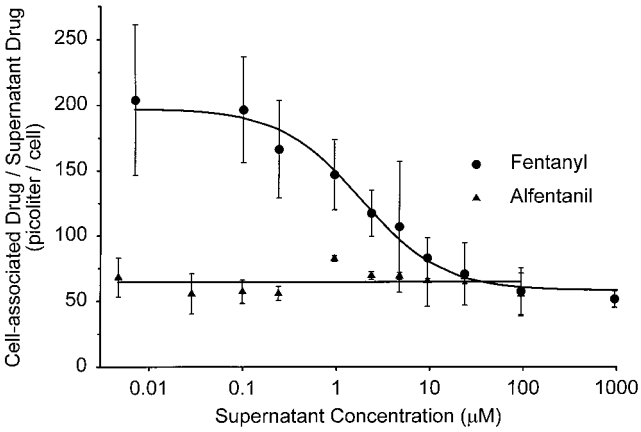

Fig. 3. Fentanyl and alfentanil uptake by human lung microvascular endothelial cells. The symbols represent the ratio (K_{EQ}) of total cell-associated drug concentrations (nM/cell) to supernatant drug concentrations plotted as a function of supernatant drug concentration (C_S, μM). The symbols (circles = fentanyl; triangles = alfentanil) represent the mean value from three experiments; the error bars represent the SD; the solid line represents the best fit of the model to the data for fentanyl. For alfentanil, the solid line represents the mean value for K_{EQ} for the concentration range studied.

centration for 50% occupancy of transporters (k_o/k_t = 1.84 μM).

Verapamil Blocks Uptake of Fentanyl by Human Lung Microvascular Endothelial Cells

If a transporter facilitates fentanyl uptake by HMVE-L cells, it should be possible to block that uptake with verapamil, a nonspecific competitive inhibitor of drug transport. Although verapamil partially inhibited facilitated fentanyl uptake at the lowest concentration tested (1 μM), higher concentrations (10 and 100 μM) substantially shifted the uptake curves downward (fig. 4). The downward shift indicates a loss of available transporter sites because the data for the highest concentration of verapamil could not be fit with the dual pathway model. Treatment of cells with verapamil had no effect on the uptake of either antipyrine or alfentanil (data not shown).

Because verapamil significantly blocked facilitated fentanyl uptake, we investigated whether P-glycoprotein was responsible for the enhanced uptake in HMVE-L cells with use of a blocking antibody. The UIC2 antibody (Immunotech, Marseille, France) was used as a blocking antibody against P-glycoprotein to determine whether enhanced fentanyl uptake could be blocked. UIC2 is highly specific and a potent inhibitor of P-glycoprotein. In an early study,[15] UIC2 reacted with all tested cell lines that expressed the human *MDR1* gene product, but not

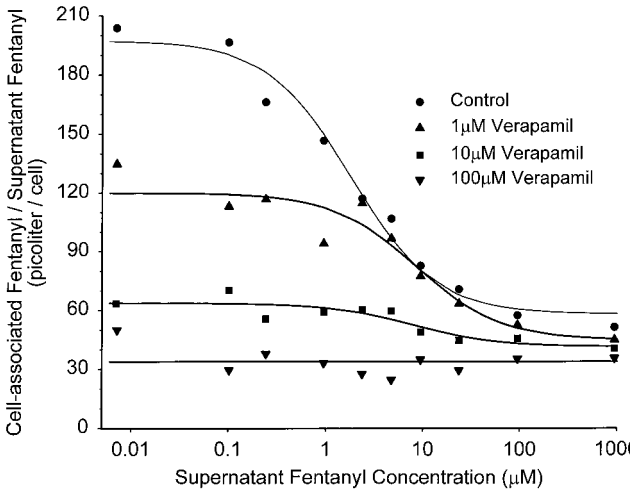

Fig. 4. Verapamil inhibition of facilitated fentanyl uptake by human lung microvascular endothelial cells. The symbols represent the ratio (K_{EQ}) of total cell-associated drug concentrations (nM/cell) to supernatant drug concentrations plotted as a function of supernatant concentration (C_S, μM). The symbols (circles = control; triangles = 1 μM verapamil; squares = 10 μM verapamil; inverted triangles = 100 μM verapamil) represent the mean value from three separate experiments; the solid lines represent the best fit of the model to the data, except for 100 μM verapamil, for which the line represents the mean value for K_{EQ} for the concentration range studied.

with P-glycoprotein–negative cells or with cells expressing the closely related human *MDR2* gene product. In addition, UIC2 strongly inhibited P-glycoprotein–mediated efflux of several cytotoxic drugs, including vinblastine, vincristine, colchicine, taxol, doxorubicin, etoposide, actinomycin D, puromycin, and gramicidin D. UIC2 had maximal inhibitory effect at 10 μg/ml for cells expressing high levels of P-glycoprotein.[15] When HMVE-L cells were incubated with 0, 0.4, 4.0, and 40.0 μg UIC2 antibody/ml, there was no effect on fentanyl uptake (data not shown). These results suggest that a mechanism other than the P-glycoprotein transport is responsible for facilitating the uptake of fentanyl.

Discussion

Various mechanisms have developed in animals to protect them from xenobiotics to which they are exposed.[17] Some of the most widely known of these protective mechanisms are the xenobiotic-metabolizing enzymes, including the cytochrome P-450 3A enzymes, that have wide substrate specificity and are responsible for molecular oxidation in preparation for conjugation with hydrophilic moieties and subsequent excretion. Another

protective mechanism is the mammalian xenobiotic transporting P-glycoprotein, located on the plasma membrane of the cell, that can transport xenobiotics against a concentration gradient, thereby reducing intestinal toxin absorption, limiting drug distribution into the brain, and facilitating drug elimination into the bile and urine.[17,18] The cytochrome P-450 3A enzymes and the P-glycoproteins have considerable overlap in substrate specificity.[19] Because the extensive pulmonary uptake of autacoids and xenobiotics is potentially protective,[2,20] the possibility exists that P-glycoprotein is involved in facilitating their uptake. Similarly, because fentanyl and alfentanil are metabolized by cytochrome P-450 3A4,[21,22] the possibility exists that they are both substrates for P-glycoprotein transport. Verapamil, a P-450 3A4 and 3A5 substrate,[23] is not only a substrate for P-glycoprotein, but is also the prototypical P-glycoprotein competitive inhibitor.[24]

To test the hypothesis that fentanyl and alfentanil are transported by P-glycoprotein into pulmonary endothelial cells, we began by measuring the uptake of each drug in isolated HMVE-L cells. The equilibrium binding of antipyrine and alfentanil was consistent with a purely diffusion-driven uptake mechanism: as more drug was added to the extracellular medium, the fractional uptake (i.e., partition coefficient) remained constant (figs. 2 and 3). In the case of fentanyl, however, there was significantly higher fractional uptake at the lower supernatant concentrations than at the higher concentrations (figs. 2 and 3). As the fentanyl concentration was increased in the medium, there was a decrease in the fractional uptake that approached the uptake levels of antipyrine and alfentanil. This decrease can be interpreted as a saturation of the specific uptake-facilitating mechanism and can be explained by our model. In equation 3, as the C_S of fentanyl becomes much greater than k_o/k_t, the second term on the right becomes smaller and contributes less to the overall equilibrium. The equilibrium partitioning is then largely controlled by the diffusional partition coefficient H. Conversely, when C_S is small or comparable to k_o/k_t, facilitated uptake contributes significantly to the overall cellular uptake. Thus, despite the expectation that fentanyl and alfentanil might be substrates for facilitated cellular uptake because they are substrates for CYP 3A4, they are not; the overlap of CYP 3A and P-glycoprotein substrate specificities has been shown recently to be more fortuitous than indicative of a fundamental relation.[25] As discussed in the next paragraph, the relative uptake of fentanyl, alfentanil, and antipyrine by isolated HMVE-L cells is consistent with the differences in pulmonary uptake observed in vivo.

Recirculatory compartmental pharmacokinetic modeling[26] of the disposition of fentanyl, alfentanil, and antipyrine in human volunteers quantitated the difference in the pulmonary uptake of these drugs in terms of pulmonary distribution volume (unpublished observations). Although the pulmonary distribution volume of alfentanil (0.5 l) was similar to the extravascular lung water volume defined by antipyrine (0.3 l), that of fentanyl (8.4 l) was 28 times as big. Differences in pulmonary uptake may be a result of the fact that fentanyl is a basic amine, whereas alfentanil is not. Although fentanyl and alfentanil are structurally similar lipophilic amines (their octanol–water partition coefficients are 816 and 128, respectively[27]), with similar plasma protein binding (84 and 92%, respectively[27]), at physiologic pH the degree of ionization of fentanyl ($pK_a = 8.4$) is 91.5%, whereas that of alfentanil ($pK_a = 6.5$) is only 11%.[27] The requirement that a molecule be a lipophilic basic amine for efficient pulmonary uptake to occur is well-known[2] and is shown by contrasting the extensive uptake of the basic lipophilic amine methadone with the minimal uptake of the nonbasic lipophilic amine diazepam by the isolated perfused rat lung.[28]

With the recent discoveries of drug transporters that function to establish concentration gradients of drugs across cellular interfaces,[17] including the blood–brain barrier,[29] we hypothesized that such a transport mechanism may exist in the pulmonary endothelium. Because many lipophilic compounds are substrates for P-glycoprotein,[30] including several opioids,[31] we hypothesized that P-glycoprotein may transport fentanyl. Although its functional role has not been elucidated, there have been two previous reports of P-glycoprotein expression in the human lung,[32,33] including a report of expression in bronchial capillary endothelial cells.[33] Verapamil, a calcium-channel blocker, is a prototypical competitive blocker of P-glycoprotein and other drug transporters.[13,14] When we incubated isolated cells with use of verapamil, we found a verapamil concentration–dependent decrease in fentanyl uptake (fig. 4). At the highest verapamil concentration (100 μM) the fentanyl HMVE-L cellular uptake curve was flat and resembled the uptake of alfentanil and antipyrine.

These results support facilitated fentanyl uptake by a cellular transporter such as P-glycoprotein. However, when we incubated cells with a blocking antibody specific for P-glycoprotein, UIC2,[15] it did not affect the facilitated uptake of fentanyl (data not shown). There-

fore, we hypothesize that an unidentified, facilitated uptake mechanism exists in human pulmonary endothelium that is capable of mediating significant uptake of fentanyl and other drugs. Henthorn *et al.*[13] reached a similar conclusion in a recent study of fentanyl uptake by isolated bovine brain microvascular endothelial cells. They found that, although there was outwardly directed P-glycoprotein–mediated extrusion of fentanyl, it was small compared with the facilitated uptake by an unidentified transporter, and the transport of fentanyl by both could be blocked by verapamil. This is not unexpected because P-glycoprotein is primarily responsible for active removal of drugs from cells rather than for transport into them.[34] Numerous transporters responsible for substrate import and export have been identified, and many more remain to be identified,[35] one of which may be responsible for transport of fentanyl into pulmonary vascular endothelial cells. However, other mechanisms exist whereby cellular drug uptake is facilitated and may be responsible for the facilitated uptake of fentanyl.

Data from the current study were interpreted in terms of linear, passive diffusion and saturable, facilitated uptake of drug into pulmonary vascular endothelial cells. Although the model fit our data well, the data could just as easily be interpreted as representing a combination of weak, linear, cellular binding and saturable, high-affinity binding of amphiphilic, high pK_a bases to subcellular components such as lysosomes[36] or mitochondria.[37] In the absence of studies of initial uptake rate, it is difficult to distinguish between active transport and subcellular trapping.[36] However, data from our bovine pulmonary artery endothelial cell (BPAEC) column study[10] showed rapid fentanyl efflux from the cells, findings that are consistent with *in vivo* human data obtained with use of frequent arterial blood sampling. In that study, monolayers of the bovine pulmonary artery endothelial cells were grown on microcarrier beads and placed in a chromatography column with a nonrecirculating perfusate. The perfusate was sampled at frequent intervals after bolus doses of fentanyl and antipyrine, and a nondiffusible marker was injected at the inlet of the columns. These results suggest that the drug transport model[10] is more appropriate because drug trapping creates a slowly effluxable drug pool.[36,37]

The k_o/k_t value, which is the concentration of fentanyl leading to 50% occupancy of transporters, for isolated cells was 1.84 μM or 619 ng/ml and is well above the clinical range of arterial fentanyl concentrations.[38] This suggests that fentanyl, at normally observed clinical concentrations in humans, should be subject to facilitated pulmonary uptake.

Because verapamil is used regularly as a calcium-channel blocker, the question arises as to whether patients with therapeutic verapamil concentrations are at risk for complications when fentanyl is administered because of increased peak fentanyl concentrations resulting from decreased pulmonary fentanyl uptake. Our results suggest that verapamil would have little effect on the pulmonary uptake of fentanyl at blood concentrations less than 1 μM (454.6 ng/ml), which is more than 3 times typical therapeutic verapamil concentrations (120 $\pm$ 20 ng/ml).[39]

In a previous report,[10] we demonstrated facilitated transport of fentanyl by bovine pulmonary artery endothelial cells. In the current study, we compared the uptake of fentanyl and alfentanil to that of antipyrine, a marker of extravascular lung water,[11,12] by HMVE-L cells. Alfentanil uptake by HMVE-L cells was indistinguishable from that of antipyrine for the concentration ranges studied. In contrast, at low concentrations, fentanyl sequestration into HMVE-L cells was substantially greater than that of antipyrine, suggesting fentanyl uptake by HMVE-L cells is a result of diffusion and facilitated transport. A drug transporter located in the pulmonary endothelium that functions to temporarily remove xenobiotics from the circulation would be uniquely situated to serve as a control mechanism for drug disposition. Our results show for the first time that facilitated uptake of fentanyl occurs in human lung endothelial cells. Identification and characterization of the transporter may lead to better understanding of factors that influence pulmonary drug uptake and interindividual differences in early arterial blood drug concentrations.

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References

1. Simionescu M: Lung endothelium: Structure-function correlates. Lung: Scientific Foundations. Edited by Crystal RG, West JB. New York, Raven Press, 1991, pp 301–21

2. Bend JR, Serabjit-Singh CJ, Philpot RM: The pulmonary uptake, accumulation, and metabolism of xenobiotics. Ann Rev Pharmacol Toxicol 1985; 25:97–125

3. Roerig DL, Ahlf SB, Dawson CA, Linehan JH, Kampine JP: First pass uptake in the human lung of drugs used during anesthesia. Adv Pharmacol 1994; 31:531–49

4. Roerig DL, Kotrly KJ, Vucins EJ, Ahlf SB, Dawson CA, Kampine JP: First pass uptake of fentanyl, meperidine, and morphine in the human lung. ANESTHESIOLOGY 1987; 67:466–72

5. Taeger K, Weninger E, Schmelzer F, Adt M, Franke N, Peter K: Pulmonary kinetics of fentanyl and alfentanil in surgical patients. Br J Anaesth 1988; 61:425–34

6. Post C: Studies on the pharmacokinetic function of the lung with special reference to lidocaine. Acta Pharmacol Toxicol 1979; 44(suppl 1):1–53

7. Krejcie TC, Avram MJ, Gentry WB, Niemann CU, Janowski MP, Henthorn TK: A recirculatory model of the pulmonary uptake and pharmacokinetics of lidocaine based on analysis of arterial and mixed venous data from dogs. J Pharmacokinet Biopharm 1997; 25:169–90

8. Howell RE, Lanken PN: Pulmonary accumulation of propranolol in vivo: Sites and physiochemical mechanism. J Pharmacol Exp Ther 1992; 263:130–5

9. Boer F, Hoeft A, Scholz M, Bovill JG, Burm AG, Hak A: Pulmonary distribution of alfentanil and sufentanil studied with system dynamics analysis. J Pharmacokinet Biopharm 1996; 24:197–218

10. Waters CM, Avram MJ, Krejcie TC, Henthorn TK: Uptake of fentanyl in pulmonary endothelium. J Pharmacol Exp Ther 1999; 288: 157–63

11. Brigham KL, Ramsey LH, Snell JD, Merritt CR: On defining the pulmonary extravascular water volume. Circ Res 1971; 29:385–97

12. Effros RM, Mason GR, Reid E, Graham L, Silverman P: Diffusion of labeled water and lipophilic solutes in the lung. Microvasc Res 1985; 29:45–55

13. Henthorn TK, Liu Y, Mahapatro M, Ng K-Y: Active transport of fentanyl by the blood-brain barrier. J Pharmacol Exp Ther 1999; 289: 1084–9

14. Aszalos A, Ross DD: Biochemical and clinical aspects of efflux pump related resistance to anti-cancer drugs. Anticancer Res 1998; 18:2937–44

15. Mechetner EB, Roninson IB: Efficient inhibition of P-glycoprotein-mediated multidrug resistance with a monoclonal antibody. Proc Natl Acad Sci U S A 1992; 89:5824–8

16. Bowsher DJ, Krejcie TC, Avram MJ, Chow MJ, DelGreco F, Atkinson AJ Jr: Reduction in slow intercompartmental clearance of urea during dialysis. J Lab Clin Med 1985; 105:489–97

17. Schinkel, AH: The physiological function of drug transporting P-glycoproteins. Sem Cancer Biol 1997; 8:161–170

18. Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM: Biochemical, cellular, and pharmacological aspects of the multidrug transporter. Annu Rev Pharmacol Toxicol 1999; 39: 361–98

19. Wacher VJ, Wu CY, Benet LZ: Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: Implications for drug delivery and activity in cancer chemotherapy. Mol Carcinog 1995; 13:129–34

20. Upton RN, Doolette DJ: Kinetic aspects of drug disposition in the lungs. Clin Exp Pharmacol Physiol 1999; 25:381–391

21. Labroo RB, Paine MF, Thummel KE, Kharasch ED: Fentanyl metabolism by human hepatic and intestinal cytochrome p450 3A4: Implications for interindividual variability in disposition, efficacy, and drug interactions. Drug Metab Dispos 1997; 25:1072–80

22. Kharasch ED, Russell M, Mautz D, Thummel KE, Kunze KL, Bowdle TA, Cox K: The role of cytochrome P450 3A4 in alfentanil clearance: Implications for interindividuality variability in disposition and perioperative drug interactions. ANESTHESIOLOGY 1997; 87:36–50

23. Tracy TS, Korzekwa KR, Gonzalez FJ, Wainer IW: Cytochrome P450 isoforms involved in metabolism of the enantiomers of verapamil and norverapamil. Br J Clin Pharmacol 1999; 47; 545–52

24. Yusa K, Tsuruo T: Reversal mechanism of multidrug resistance by verapamil: Direct binding of verapamil to P-glycoprotein on specific sites and transport of verapamil outward across the plasma membrane of K562/ADM Cells. Cancer Res 1989; 49:5002–6

25. Kim RB, Wandel C, Leake B, Cvetkovic M, Fromm MF, Dempsey PJ, Roden MM, Belas F, Chaudhary AK, Roden DM, Wood AJJ, Wilkinson GR: Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. Pharmaceut Res 1999; 16:408–14

26. Krejcie TC, Henthorn TK, Niemann CU, Klein C, Gupta DK, Gentry WB, Shanks CA, Avram MJ: Recirculatory pharmacokinetic models of markers of blood, extracellular fluid and total body water administered concomitantly. J Pharmacol Exp Ther 1996; 278: 1050–7

27. Bovill JG: Pharmacokinetics of opioids, The Pharmacologic Basis of Anesthesiology. Edited by Bowdle TA, Horita A, Kharasch ED. New York, Churchill Livingston, 1994, pp 37–81

28. Roerig DL, Dahl RR, Dawson CA, Wang RIH: Effect of plasma protein binding on the uptake of methadone and diazepam in the isolated perfused rat lung. Drug Metab Disp 1984; 12:536–42

29. van Asperen J, Mayer U, van Tellingen O, Beijnen JH: The functional role of P-glycoprotein in the blood-brain barrier. J Pharm Sci 1997; 86:881–4

30. Schinkel AH, Wagenaar E, Mol CA, van Deemter: P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J Clin Invest 1996; 97:2517–24

31. Callaghan R, Riordan JR: Synthetic and natural opiates interact with P-glycoprotein in multidrug-resistant cells. J Biol Chem 1993; 268:16059–64

32. Bagrij T, Hladky SB, Stewart S, Scheper RJ, Barrand MA: Studies of multidrug transport proteins in cells derived from human lung samples. Int J Clin Pharmacol Ther 1998; 36:80–1

33. Lechapt-Zalcman E, Hurbain I, Lacave R, Commo F, Urban T, Antoine M, Milleron B, Bernaudin JF: MDR-1Pgp 170 expression in human bronchus. Eur Resp J 1997; 10:1837–43

34. Leveille-Webster CR, Arias IM: The biology of the P-glycoproteins. J Membrane Biol 1995; 143:89–102

35. Higgins CF: ABC transporters: From microorganisms to man. Annu Rev Cell Biol 1992; 8:67–113

36. MacIntyre AC, Cutler DJ: The potential role of lysosomes in tissue distribution of weak bases. Biopharm Drug Dispos 1988; 9:513–26

37. Miniati M, Paci A, Cocci F, Ciarimboli G, Monti S, Pistolesi M: Mitochondria act as a reservoir for the basic amine HIPDM in the lung. Eur Respir J 1996; 9:2306–12

38. Bailey PL: Clinical pharmacology and applications of opioid agonists, The Pharmacologic Basis of Anesthesiology. Edited by Bowdle Ta, Horita A, Kharasch ED. New York, Churchill Livingstone, 1994, pp 83–119

39. McTavish D, Sorkin EM: Verapamil: An updated review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in hypertension. Drugs 1989; 38:19–76