

Transporter-Mediated Disposition of Opioids: Implications for Clinical Drug Interactions

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ABSTRACT Opioid-related deaths, abuse, and drug interactions are growing epidemic problems that have medical, social, and economic implications. Drug transporters play a major role in the disposition of many drugs, including opioids; hence they can modulate their pharmacokinetics, pharmacodynamics and their associated drug-drug interactions (DDIs). Our understanding of the interaction of transporters with many therapeutic agents is improving; however, investigating such interactions with opioids is progressing relatively slowly despite the alarming number of opioids-mediated DDIs that may be related to transporters. This review presents a comprehensive report of the current literature relating to opioids and their drug transporter interactions. Additionally, it highlights the emergence of transporters that are yet to be fully identified but may play prominent roles in the disposition of opioids, the growing interest in transporter genomics for opioids, and the potential implications of opioid-drug transporter interactions for cancer treatments. A better understanding of drug transporters interactions with opioids will provide greater insight into potential clinical DDIs and could help improve opioids safety and efficacy.

KEY WORDS opioid abuse · opioid DDI · opioid drug transporters · opioids and P-gp

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ABBREVIATIONS

6-MAM	6-monoacetylmorphine
AAPCC	American Association for Poison Control Centers
AUC	Area under the curve
BBB	Blood–brain barrier
BCRP	Breast cancer resistance protein
CL _{in}	Permeability clearance into the brain
CNS	Central nervous system
DDI	Drug-drug interaction
ED	Emergency department
FDA	Food and Drug Administration
GLUT	Glucose transporters
HEK293	Human embryonic kidney 293
IC ₅₀	Half maximal inhibitory concentration
K _i	Inhibition constant
K _{p,uu}	Ratio of unbound drug in the brain to unbound drug in the blood
M3G	Morphine-3-glucuronide
M6G	Morphine-6-glucuronide
MDMA	3,4-methylenedioxy-methamphetamine
MMT	Methadone maintenance treatment
MOR	μ-opioid receptor
MRP	Multidrug resistance-associated proteins
NSDUH	National Survey on Drug Use and Health
OAT	Organic anion transporters
OATP	Organic anion-transporting polypeptides
OCT	Organic cation transporters
PD	Pharmacodynamics
P-gp	P-glycoprotein
PK	Pharmacokinetics
V _{u,brain}	Volume of distribution within the brain

INTRODUCTION

Opioids are the standard analgesics of choice for the treatment of chronic and severe pain, as well as cancer-pain

management (1, 2). In addition to their clinical applications in pain management, opioids have a long history of illicit abuse. Opioids comprise a large family of both naturally occurring and synthetic molecules that are classified by their interactions with the three opioid receptors: μ , δ , and κ (3). μ -opioid receptor (MOR) agonists are known to produce the euphoric and analgesic effects most commonly associated with physical dependence and abuse (4).

The United States (US) is presently facing a severe epidemic of drug overdose deaths which appears to be driven primarily by opioid abuse (5–8). The number of deaths related to opioid overdose has more than quadrupled since 1999, directly correlating with the dramatic increase in the sale of opioid pharmaceuticals (7, 9, 10). In 2010 an estimated 46 Americans were dying each day from opioid overdoses while 210 million opioid prescriptions were dispensed by U.S. retail pharmacies (10, 11). Further, in 2011 alone over 190,000 emergency department visits were believed to be due to adverse events resulting from narcotic pain relieving medications, and 400,000 emergency department visits were related to opioid analgesic abuse (12). Hence, greater measures are urgently needed to increase the drug safety of opioids, including greater understanding of their pharmacokinetic mechanisms and potential drug-drug interactions (DDIs).

Although not as well recognized as metabolizing enzymes, membrane drug transporters are being increasingly acknowledged as important determinants of drug pharmacokinetics (PK), pharmacodynamics (PD) and DDIs (13). Regulatory agencies from the US, European Union and Japan have all released guidances in recent years addressing the emerging clinical significance of drug transported-mediated DDIs (14–17). The 2012 US Food and Drug Administration (FDA) Guidance for Industry on drug interaction studies outlined 7 transporters with which a developing drug should be evaluated for interactions with. These transporters of interest are P-glycoprotein (P-gp/MDR1, ABCB1), Breast Cancer Resistance Protein (BCRP, ABCG2), the hepatic uptake Organic Anion Transporting Polypeptides (OATP) 1B1 and 1B3, and the renal Organic Anion Transporter (OAT) 1 and 3, and Organic Cation Transporter 2 (OCT2) (16). In addition, there are multiple emerging transporters of interest, such as members of the multidrug and toxic compound extrusion (MATE) family, as well as a number of yet to be fully identified transporters (18–20)(Fig. 1).

The anti-diarrheal loperamide is a prime example of the impact drug transporters may have on the PK/PD of opioids. Loperamide exhibits minimal central nervous system (CNS) activity due to being a substrate of P-gp, an efflux transporter with broad substrate specificity, at the blood–brain barrier (BBB) (21). Clinically significant transporter-mediated DDIs have already been demonstrated for a number of commonly used medications including multiple statins, cimetidine, digoxin, metformin, anti-epileptics and anti-cancer agents, to name

a few (16). Thus, the role of drug transporters in drug PK/PD, toxicity, human disease, and inter-individual variability in drug response is an emerging field of interest, however their contributions to the disposition of opioids in humans remains largely unexplored (17).

In order to most accurately predict their potential clinical implications, it is essential to have mechanisms in place that can quantitatively evaluate transporter-mediated DDIs. For example, transporter-mediated DDIs that occur in preclinical studies may not be applicable to the clinic due to the unsafe or non-clinically relevant doses at which they occur. Since opioids are primarily used for their centrally acting analgesic effects, their drug transporter interactions at the BBB are of particular interest. Previous studies have proposed that the rate and extent of drug delivery to the brain *in vivo* can be comprehensively described by three parameters: the ratio of concentration of unbound drug in the brain to the unbound concentration in the blood ($K_{p,uu}$), the permeability clearance into the brain (CL_{in}), and the volume of distribution within the brain ($V_{u,brain}$) (22).

The purpose of this review article is to highlight the influence of drug transporters on the disposition of opioids to predict clinical implications and to improve both their safety and efficacy. When applicable, we provide for each opioid a systematic review of *in vitro*, *in vivo* (animal) and clinical data regarding drug-transporter interactions, and highlight the potential clinical implications of these interactions that warrant further investigation.

OPIOIDS OF ABUSE

Morphine

Morphine is considered the prototypical opioid and gold standard to which other strong analgesics are compared (23). The major metabolite of morphine is morphine-3-glucuronide (M3G), which has no analgesic properties but elicits neuro-excitatory effects. Approximately 10% of morphine is metabolized to morphine-6-glucuronide (M6G) which has much greater analgesic effects than its parent compound. Both M3G and M6G are products of morphine metabolism by UGT2B7 in the liver (24). Although markedly more potent than morphine at the μ -opioid receptor, M6G exhibits poor blood–brain BBB permeability (25). Morphine itself exhibits a lower CL_{in} than most opioids, though it is at least 10-fold higher than M6G. Similarly, morphine's $K_{p,uu}$ and $V_{u,brain}$ appears to be many folds higher than M6G's (26–28). An estimated 4 million morphine containing prescriptions were dispensed in 2009 (29). In 2011 nearly 35,000 emergency department (ED) visits were reportedly due to abuse of morphine products, representing an over 100% increase from 2004 (12).

A number of *in vitro* studies have demonstrated that morphine (30–34) and M6G (35) are likely P-gp substrates. Conversely, Tournier *et al.*, suggested morphine is neither a P-gp nor BCRP substrate (36), and Wandel *et al.*, suggested that neither M3G nor M6G are P-gp substrates (33). Both Morphine and codeine were shown to inhibit OCT1 mediated uptake at 100 μM in HEK293 cells overexpressing OCT1 (37), while morphine, but not codeine, was shown to be an OCT1 substrate in this same model cell line as well as in primary hepatocytes. Further, a number of medications that are cationic at physiological pH were shown to inhibit morphine's OCT1 mediated uptake in a concentration-dependent manner at clinically relevant doses, such as irinotecan ($\text{IC}_{50}=1.5 \mu\text{M}$), ondansetron ($\text{IC}_{50}=1.17 \mu\text{M}$), and verapamil ($\text{IC}_{50}=1.6 \mu\text{M}$) (38). *In vitro* studies have also suggested that M6G and M3G are both multidrug resistance-associated protein 2 (MRP2) and MRP3 substrates in hepatocytes (39, 40).

Although there is evidence of an active influx at the BBB (41, 42), it appears that morphine largely undergoes a net efflux at the BBB *in vivo* (41, 43–45). A number of animal studies have demonstrated morphine to be a likely P-gp substrate. P-gp inhibitors were shown to increase CNS concentrations and antinociceptive effects of morphine (46–49). Similarly, P-gp knock-out mice administered morphine had significantly increased analgesia (50, 51) and brain distribution (30, 41, 52). A negative correlation was also found between P-gp expression levels in the brains of rats and morphine-induced analgesia (53). Correspondingly, P-gp was found to be up-regulated in the brains of morphine tolerant rats (47) and the down-regulation of rat Pgp1 with antisense prevented morphine tolerance development and reduced morphine's brain-to-blood efflux (54). Morphine was also shown to significantly inhibit the proton-coupled antiporter transport of clonidine at the BBB of mice (20 reduction in clonidine brain transport at a concentration of 10 mM). However, this does not represent a clinically relevant concentration of morphine and the authors have suggested that morphine is unlikely to be an efficient substrate of this emerging transporter (19).

Rats administered M6G intravenously along with a known P-gp inhibitor were found to have significantly increased M6G antinociception and a 3-fold increase in CNS M6G relative to those not administered the inhibitor (55). However, Bourasset *et al.*, demonstrated M6G to be neither a P-gp nor Mrp1 substrate, but rather a GLUT1 and/or possibly a weak Oatp2-like substrate at the BBB in mice (56). Other knockout mice studies have suggested that Mrp2 and Mrp3 play important roles in the hepatic excretion of M3G and M6G and modulate the antinociception of M6G (39, 40). Another study indicated that probenecid significantly decreased the systemic elimination by 22% but had no effect on the BBB transport of M6G in rats, suggesting M6G may be

a substrate of a probenecid-sensitive transporter (OAT) outside the CNS (57).

A number of clinical studies have supported the hypothesis that morphine and its glucuronide metabolites are P-gp substrates. One case report noted that concomitant administration of known P-gp inducer rifampin with morphine resulted in noticeably decreased morphine blood levels (58). Patients deemed to have P-gp positive tumors from immunohistochemistry (IHC) results were found to require higher doses of morphine for pain control, relative to those with P-gp negative tumors (59). However, another study investigating the CNS effects of morphine coadministered with a known P-gp inhibitor found no clinically significant differences relative to controls. The plasma pharmacokinetics of morphine and M6G were unaffected by P-gp inhibition, while the area under the curve (AUC) and half-life ($t_{1/2}$) of M3G were significantly increased (60). Concomitant use of known P-gp inhibitor cyclosporine with morphine in healthy volunteers resulted in significantly increased morphine_{AUC} relative to controls, though the authors proposed this may not have any major clinical effects (61).

ABCB1, the gene that encodes P-gp, has been reported to have certain polymorphisms which may predispose patients to morphine-induced adverse events (62) and variable pain relief (63). However, Coulbault *et al.*, demonstrated that ABCB1 polymorphisms appeared to have no association with morphine doses post-operatively, and only borderline association ($p=0.07$) with morphine-induced side effects (64). In line with the group's *in vitro* findings, Tzvetkov *et al.*, demonstrated that following oral administration of codeine, the AUC of morphine, but not codeine, was greatly affected by OCT1 polymorphisms. Patients homozygous for OCT1 loss-of-function polymorphisms were found to have 1.5 fold higher morphine_{AUC} relative to those heterozygous and homozygous for functional OCT1 alleles (38). Similarly, a clinical study of children undergoing adenotonsillectomy found that subjects homozygous for OCT1 loss-of-function variants had significantly lower (20%) morphine clearances than wild-types or those heterozygous for such variants (65).

Despite numerous studies there currently appears to be no definitive consensus regarding the role of P-gp in the disposition of morphine or its glucuronidated metabolites. Preclinical evidence mostly suggests morphine is a P-gp substrate, while clinical data appears inconsistent. One transporter of increasing interest in morphine's disposition, particularly its hepatic efflux, is OCT1. *In vitro* and clinical data are in agreement that OCT1 plays an important role in the pharmacokinetics of morphine. These findings may imply a host of potential transporter-based clinical DDIs with other OCT1 substrates or inhibitors (Table I), some of which have already been demonstrated *in vitro* at clinically relevant concentrations (38). OCT1-mediated DDIs between morphine and other substrates/inhibitors could lead to impaired hepatic clearance

Table 1 Summary of Opioid-Drug Transporter Interactions and Their Potential Implications

Drug Name	Transporter	Evidence	Clinical implications
Morphine	P-gp	<p>In vitro: Morphine is a substrate (30–34) Morphine is not a substrate (36) M6G is a substrate (35) M3G and M6G are not a substrates (33)</p> <p>In vivo: Morphine is a substrate (30, 41, 46–54) M6G is a substrate (55) M6G is not a substrate (56)</p> <p>Clinical: Morphine is a substrate (58, 59, 61) Morphine and M6G are not substrates, while M3G is a possible substrate (60) ABCB1 polymorphisms impact morphine PK/PD (62, 63) ABCB1 polymorphisms have no impact on morphine PK/PD (64)</p>	The role of P-gp in the disposition of morphine and its glucuronidated metabolites is unclear.
	BCRP	<p>In vitro: Morphine is not a substrate (36)</p>	Limited evidence suggests morphine may not be a BCRP substrate
	OCT1	<p>In vitro: Morphine is an inhibitor (37) Morphine is a substrate (38)</p> <p>Clinical: OCT1 polymorphisms impact PK of morphine (38, 65)</p>	<p>Morphine appears to be a likely OCT1 substrate/inhibitor; inferring potential clinical DDIs with other OCT1 substrates which may include acyclovir (66), metformin (67–72), imatinib (73), oxaliplatin (74), ranitidine, (75) and inhibitors such as quinidine (75), atropine, diphenhydramine (76), amitriptyline (68), memantine, cocaine (77), prazosin (78), rosiglitazone (79), pioglitazone (68), verapamil (78), tramadol (80), and at clinically relevant doses of irinotecan ($IC_{50} = 1.5 \mu M$) and ondansetron ($IC_{50} = 1.17 \mu M$) (38). Impaired hepatic clearance of morphine could lead to longer morphine exposures in patients and increase their risks of adverse events such as respiratory depression, severe hypotension, myoclonic spasms, constipation, and others (29).</p>
	OCT3	<p>In vitro: Morphine is not a substrate (38)</p>	Limited evidence suggests morphine is not an OCT3 substrate.
	GLUT-1	<p>In vivo: M6G is a substrate at the BBB (56, 81)</p>	Heroin and morphine may affect glucose transport at the BBB
	MRP1	<p>In vivo: M6G is not a substrate (56)</p>	MRP2 and MRP3 appear to be involved in hepatic excretion of morphine glucuronidated metabolites.
	MRP2	<p>In vitro & in vivo: M3G is a substrate (39, 40)</p>	This could infer transporter-based DDIs with other MRP2 and MRP3 substrates and inhibitors.
	MRP3	<p>In vitro:</p>	Possible MRP2 substrates include olmesartan (82), valsartan (83), etoposide (84), irinotecan (85).

Table 1 (continued)

Drug Name	Transporter	Evidence	Clinical implications
	Digoxin-sensitive transporter	<p><i>In vitro</i>: a substrate (39, 40)</p> <p><i>In vivo</i>: a weak substrate at the BBB (56)</p> <p>M3G is a substrate (39, 40)</p>	<p>methotrexate (86), vinblastine (87), and inhibitors include cyclosporine A, daunorubicin, etoposide, vincristine (87), efavirenz, emtricitabine, delavirdine (88), indomethacin, ketoprofen (86).</p> <p>Possible MRP3 substrates include fexofenadine (89), methotrexate, folic acid, leucovorin (90), and inhibitors efavirenz, emtricitabine, and delavirdine (88)</p> <p>The identity of this transporter has not yet been established, but may be OATP1B1. This could imply potential DDIs with OATP1B1 substrates such as pitavastatin, rosuvastatin (91), simvastatin (92), docetaxel (93), paclitaxel (94), fexofenadine (95), glyburide (96), and digoxin (97), as well as likely OATP1B1 inhibitors such as ritonavir (97, 98), atazanavir, darunavir, indinavir, lopinavir (98), and verapamil (92)</p> <p>The identity of this transporter has not yet been established.</p> <p>If M6G is an OAT substrate, there may be potential DDIs between morphine and other OATP substrates such as</p> <p>acyclovir (99), cidofovir (100), a number of cephalosporins such as cefaclor (101), cefonicid (102), ceftriaxone (103), and cephradine (101), ciprofloxacin (104), dicloxacillin (105), famotidine (106), and furosemide (107), as well as with OAT inhibitors such as probenecid (105) and sulfamethoxazole-trimethoprim (108).</p> <p>Morphine was found to significantly reduce the brain transport of clonidine by 20% at a concentration of 20 mM. This does not represent a clinically relevant concentration and so clinical DDIs may be unlikely. Further studies are warranted.</p> <p>Role of OCT1 is not clear</p>
	Probenecid-sensitive transporter (OAT)	<p><i>In vivo</i>:</p> <p>M6G is a possible substrate outside the BBB (57)</p>	
	Proton-coupled antiporter	<p><i>In vivo</i>:</p> <p>Possible weak inhibitor/substrate of the proton-coupled antiporter of clonidine at the BBB (19)</p>	
Codeine	OCT1	<p><i>In vitro</i>:</p> <p>Inhibitor (37)</p> <p>Not a substrate (38)</p> <p><i>In vivo</i>:</p> <p>Possible substrate at intestinal epithelium and BBB (109)</p>	
	Proton-coupled antiporter		<p>Possible substrate of this yet to be fully identified transporter.</p> <p>The cellular uptake of codeine (1.2 nM) was potentially inhibited in the presence of pyrilamine, clonidine, diphenhydramine, propranolol, verapamil, as well as a numbers of drugs of abuse, including D- and</p>

Table 1 (continued)

Drug Name	Transporter	Evidence	Clinical implications
Diacetylmorphine	P-gp	<p>In vivo: Not a substrate (49)</p> <p>In vivo: Possible inhibitor of the proton-coupled antiporter of dionidine at the BBB (19)</p>	<p>L-amphetamine, cocaine, and methamphetamine (all at 2 mM) (109). More studies are necessary to determine if these DDIs occur at clinically relevant concentrations. Diacetylmorphine and 6-MAM do not appear to be P-gp substrates Possible inhibitor of this yet to be fully identified transporter at the BBB. Could be a source of a number of clinical DDIs between diacetylmorphine and other potential substrates/inhibitors including clonidine, diphenhydramine, tramadol and nicotine, codeine, morphine, cocaine, and MDMA (19, 20)</p>
Oxycodone	P-gp	<p>In vitro: Substrate (110)</p> <p>In vivo: Substrate (110, 111) Not a substrate (112)</p> <p>Clinical: C3435T and G2677T/A ABCBI polymorphisms linked to less adverse effects of oxycodone (113) Mothers with at least one 2677T copy had increased risk of oxycodone-induced sedation (114) C3435T polymorphism had no effects on oxycodone PK (115)</p> <p>In vivo: Inducer (117)</p>	<p>Role of P-gp is highly controversial (116)</p>
	BCRP		<p>Concomitant use of oxycodone with other BCRP substrates could result in transporter-mediated DDIs, which may include daunorubicin, doxorubicin, methotrexate, imatinib, lapatanib, irinotecan, and topotecan (16), as well as non-chemotherapy agents such as nitrofurantoin, prazosin, glyburide (118), rosuvastatin, sulfasalazine (16), and fellow drug of abuse, marijuana (36, 119, 120)</p>
	Proton-coupled antiporter	<p>In vitro & in vivo: Substrate of the proton-coupled antiporter of pyrilamine at the BBB (121, 122)</p>	<p>Possible substrate of this yet to be fully identified transporter at the BBB. <i>In vitro</i> results suggest significantly inhibited BBB uptake of oxycodone (30 μM) in the presence of pyrilamine (1 mM), quinidine (1 mM), verapamil (1 mM), amantadine (1 mM) (121), amitriptyline ($K_i = 13 \mu\text{M}$), fluvoxamine ($K_i = 65 \mu\text{M}$), and mexiletine ($K_i = 44 \mu\text{M}$) (122) However, these DDIs occurred at very high concentrations and may not be clinically relevant. Further investigations are warranted.</p>

Table 1 (continued)

Drug Name	Transporter	Evidence	Clinical implications
Oxycodone	P-gp	In vitro: Substrate of the proton-coupled antiporter of diphenhydramine at the BBB (123, 124)	<i>In vitro</i> results suggest oxycodone and diphenhydramine share this yet to be fully identified active transporter at the BBB. However, <i>in vivo</i> results suggest oxycodone and diphenhydramine do not have a significant DDI at the BBB at non-toxic concentrations, making a clinical DDI unlikely at therapeutic doses (123)
	Unidentified BBB transporter	In vitro: Not a substrate (111)	Limited evidence suggests oxycodone is not a likely P-gp substrate
		In vivo: Oxycodone undergoes active transport at BBB by a yet identified transporter (125)	Further research is needed
Fentanyl	P-gp	In vitro: Substrate (126, 127) Not a substrate (33)	Current role of P-gp is not clear
		In vivo: Substrate (51, 127–130)	
		Clinical: Intestinal but not BBB substrate (131) 1236TT, 2677TT, and 3435TT genotypes linked with increased fentanyl-induced respiratory depression (132) ABCB1 polymorphisms have no effect on fentanyl-induced sedation or respiratory depression (133), or coma/delirium (134)	
	OATP	In vitro: Not OATP1B1 substrate (135)	Fentanyl does not appear to be an OATP1B1 substrate
		In vivo: Substrate (136)	
		Clinical: Not a substrate (135)	
	Yet identified transporter at BBB	In vitro: Substrate at the BBB (126)	More research is needed into this transporter which <i>in vitro</i> evidence suggests plays a more prominent role than P-gp in oxycodone disposition at the BBB
Methadone	P-gp	In vitro: Substrate (32, 36, 111, 137–145)	<i>In vitro</i> and <i>in vivo</i> evidence clearly support methadone as a P-gp substrate, but clinical evidence is conflicting. The current role of P-gp is unclear
		In vivo: Substrate (51, 111, 130, 146–148)	
		Clinical: Substrate (149–151) Possible intestinal, but not BBB substrate (131) Unlikely substrate (95, 152–154) ABCB1 polymorphisms influence methadone dosing (155–157) and PK (158, 159) ABCB1 polymorphisms have little/no influence methadone dosing (160–162) or PK (163–165)	

Table 1 (continued)

Drug Name	Transporter	Evidence	Clinical implications
Buprenorphine	P-gp	<p>In vitro: Inhibitor: buprenorphine and norbuprenorphine (36) Substrate: norbuprenorphine (36, 166) Not a substrate: buprenorphine (111, 166, 167)</p> <p>In vivo: Substrate: buprenorphine (168) and norbuprenorphine (166, 169) Not a substrate: buprenorphine (111)</p> <p>Clinical: Not a substrate (59)</p> <p>In vitro: Inhibitor: buprenorphine and norbuprenorphine (36)</p>	<p><i>In vitro</i> and <i>in vivo</i> evidence appear to suggest buprenorphine is not, while norbuprenorphine is a P-gp substrate. One clinical study available suggests buprenorphine is not a P-gp substrate (59), but this finding has been contested by others (170)</p> <p>Limited evidence suggests buprenorphine and norbuprenorphine are BCRP inhibitors. This could lead to clinical DDIs with likely BCRP substrates such as the chemotherapy agents mitoxantrone, methotrexate, imatinib, lapatanib, irinotecan, and topotecan (16), as well as non-chemotherapy agents such as nitrofurantoin, prazosin, glyburide (118), rosuvastatin, sulfasalazine (16), and fellow drug of abuse, marijuana (36, 119, 120)</p> <p>Tramadol and O-desmethyiltramadol not appear to be P-gp substrates</p>
Tramadol	P-gp	<p>In vitro: (+)-tramadol, (–)-tramadol and (+)-O-desmethyiltramadol are not substrates (171)</p> <p>In vivo: Tramadol is not a substrate (172)</p> <p>Clinical: MDR1 C3435T polymorphisms influence tramadol C_{max} in CYP2D6 poor metabolizers (173) MDR1 C3435T polymorphisms have no influence on tramadol-induced post-operative analgesia (174) P-gp inhibitor verapamil had no influence on the PK of tramadol or its DDI with tidopadine (175)</p> <p>In vitro: (+)-tramadol, (–)-tramadol and (+)-O-desmethyiltramadol are substrates of a yet identified pH dependent efflux transporter during GI absorption and renal excretion (171)</p> <p>In vitro: Substrate at the BBB (176)</p> <p>In vivo: Substrate at the BBB (20)</p>	<p>Tramadol and O-desmethyiltramadol not appear to be P-gp substrates</p> <p><i>In vitro</i> evidence suggests DDIs at the BBB between tramadol and morphine, codeine, oxycodone, apomorphine, clonidine, pyrilamine, diphenhydramine, amantadine, memantine, verapamil, and quinidine (176). <i>In vivo</i> evidence suggests DDI at the BBB between tramadol and nicotine or diphenhydramine (20)</p> <p>Tramadol appears to be a likely OCT1 substrate, inferring potential clinical DDIs with other OCT1 substrates such as acyclovir (66), metformin (67–72), imatinib</p>
	Proton-based efflux transporter		
	Proton-coupled antiporter		
	OCT1		

Table 1 (continued)

Drug Name	Transporter	Evidence	Clinical implications
Hydrocodone Hydromorphone		No transporter information currently available	(73), oxaliplatin (74), ranitidine, (75) and inhibitors such as quinidine (75), atropine, diphenhydramine (76), amitriptyline (68), memantine, cocaine (77), prazosin (78), rosiglitazone (79), pioglitazone (68), verapamil (78), and morphine (38), as well as with potential OCT1 inhibitors such as MDMA, amantadine, famotidine (75), memantine (77), amitriptyline (68), cimetidine, ranitidine (78), clonidine (76, 177), cocaine, D-amphetamine, ketamine, phenocyclidine (77), diphenhydramine (76), glyburide, metformin (75), pioglitazone (68), rosiglitazone (79), inclinavir, nelfinavir, ritonavir, saquinavir (177), prazosin, procainamide, quinidine (78), simvastatin (68), and verapamil (78)

of morphine, resulting in prolonged systemic exposure and increased risk of morphine-induced serious adverse events such as respiratory depression, severe hypotension, myoclonic spasms, constipation and others (29). MRP2 and MRP3 may be important transporters in the hepatic efflux of M3G and M6G *in vivo* and if clinically accurate could also be sources of transporter-mediated DDIs. The clinical utility of the highly potent analgesic M6G is limited by its poor BBB permeability, and preclinical evidence appears conflicting on which transporter(s) may be involved in its efflux from the BBB. Given the frequent clinical use of morphine, a greater understanding of its transport mechanisms and that of its metabolites may be essential for improving its safety and efficacy.

Codeine

Like morphine, codeine is a naturally occurring opiate found in *Papaver somniferum*. Codeine is the pro-drug of morphine and considered the gold standard of cough suppressants (178). Codeine is primarily metabolized to codeine 6-glucuronide with less than 10% of codeine being metabolized to morphine (179). Rat studies have demonstrated that codeine undergoes rapid and largely passive transport across BBB, and that the ratio of unbound codeine in the brain to unbound codeine in the blood is essentially 1 (180). Codeine has also demonstrated a much higher $K_{p,uu}$ and $V_{u,brain}$ compared to morphine (22). Although not among the most frequently abused opioids, codeine overdose remains a serious public health concern. In 2011 an estimated 1.7 million codeine containing prescriptions were dispensed (181), and the abuse of codeine products accounted for nearly 10,000 ED visits. In 2009 adverse reactions to codeine containing pharmaceuticals accounted for over 18,000 ED visits (12).

Codeine was shown to inhibit OCT-1 mediated uptake in HEK293 cells over-expressing OCT1 at 100 μ M (37). However, another *in vitro* study found that codeine uptake was not affected by OCT1 overexpression, but rather that codeine exhibits a high level of transporter-independent cell permeability relative to morphine (38). In addition, codeine uptake in intestinal and brain endothelial cells was found to be pH dependent and possibly mediated through a yet to be identified proton-coupled antiporter that may incur a number of DDIs (109). *In vivo* evidence from an *in situ* mouse brain perfusion study supported this finding, as codeine was found to significantly inhibit the BBB transport of nicotine (29% reduction in transport rate at a concentration of 10 mM), which may also be a substrate of this same proton-coupled antiporter (20). The clinical relevance of these reported interactions is unclear however, as they appear to occur at supra-therapeutic concentrations (182).

From the limited information available codeine appears to undergo a large degree of passive diffusion and is not a substrate of the same transporters as morphine. There appears to

be contrasting evidence on the role of OCT1 in codeine's disposition. *In vitro* and *in vivo* evidence suggest that codeine may be a substrate of a yet to be identified pH dependent proton-coupled antiporter at the BBB. There is presently not enough transporter information available for codeine to warrant predictions of potential DDIs without further studies.

Diacetylmorphine

Diacetylmorphine (heroin) was originally synthesized from morphine in the early 20th century as a classical analgesic. Today it is a Schedule I controlled substance and considered one of the most addictive and problematic drugs of abuse (183, 184). Despite its highly addictive properties, diacetylmorphine exhibits a low-binding affinity and efficacy at μ -opioid receptors (185). Thus, it has been proposed that diacetylmorphine behaves primarily as highly lipophilic pro-drug, able to cross the BBB (186) and then be subsequently converted to active metabolites with high μ -opioid receptor affinities (187). Diacetylmorphine is rapidly deacetylated to its more active metabolites 6-monoacetylmorphine (6-MAM) and then morphine within 10 min of administration (188, 189). There were an estimated 12–14 million heroin users world-wide in 2009 (184). Addiction to heroin has shown an alarming increase in the US with an estimated 500,000 addicts in 2009 (190). Between 2002 and 2010, the number of reported users grew by over 50%, and in 2011 over 4.2 million Americans 12 years of age and over had tried heroin, with an estimated 23% of users becoming dependent (191). Furthermore, over 258,000 ED visits were reported in 2011 due to heroin use, representing 20.6% of all ED visits due to illicit drug use (12).

Presently there is very limited research into the drug transporters which interact with diacetylmorphine or 6-MAM. One *in situ* mouse brain perfusion study demonstrated that diacetylmorphine significantly inhibited the proton-coupled antiporter transport of clonidine at the BBB (47% reduction in the clonidine transport rate at a concentration of 10 mM) (19). Another *in situ* mouse brain perfusion study demonstrated that P-gp inhibition results in significantly higher brain uptake of morphine but not of diacetylmorphine or 6-MAM (49). One animal study found that in the post-natal development of mice, the BBB penetration of morphine decreased while that of diacetylmorphine did not, indicating a possible greater passive diffusion capacity of diacetylmorphine (192). Although morphine is often assumed to be the active metabolite responsible for heroin's pharmacodynamic effects, a number of animal studies have proposed that 6-MAM may in fact be more important (193, 194).

More research into the transporters involved in the disposition of diacetylmorphine and its active metabolites, particularly 6-MAM, are necessary to develop a clearer understanding of

their potential DDIs, as well as in developing possible interventions into heroin addiction and overdoses.

Oxycodone

Oxycodone is among the most frequently prescribed opioids in the US (195). Orally administered oxycodone has also been shown to have the highest abuse liability of the commonly prescribed opioids (196). Though first synthesized from a minor component of opium (thebaine) in 1917, full investigation of oxycodone's pharmacological properties has only occurred recently (116). Structurally akin to codeine, oxycodone shares a similar clinical profile to morphine, though estimated to exhibit a 5–40 fold lower affinity for the μ -opioid receptor (116, 197). Microdialysis studies in rats have also shown oxycodone to have the highest CL_{in} , $K_{p,uu}$ and $V_{u,brain}$ of all opioids. Further, oxycodone also demonstrated a rather unusual 3-fold higher influx clearance than efflux at the BBB (112). Nearly 60 million oxycodone prescriptions were dispensed in 2013 in the US and the 2014 production quota is nearly 150,000 kg (195). An estimated 16 million Americans used oxycodone for non-medical purpose in 2012. In 2011 over 150,000 ED visits and 43 deaths were reported due to oxycodone abuse, 65,000 ED visits were due to adverse reactions to oxycodone-containing products, and nearly 14,000 ED visits were due to oxycodone-related suicide attempts (12, 198).

In vitro permeability studies have demonstrated that oxycodone has a high passive permeability rate, greater than the suggested cut-off value for CNS penetration (199). Oxycodone was shown to stimulate P-gp ATPase activity in a concentration dependent manner, and pre-incubation with the P-gp inhibitor verapamil (100 μ M) significantly reduced oxycodone secretory transport in Caco-2 monolayer studies (110). Abcg2 (BCRP) ATPase assay results suggest oxycodone is also an Abcg2 substrate, but only at high pharmacologically irrelevant concentrations (>500 μ M) (117). As such, it is unlikely that BCRP would affect the disposition of oxycodone. Other *in vitro* transport studies in rat brain capillary endothelial cells demonstrated that oxycodone shares a pH and energy dependent proton-coupled antiporter with radiolabeled pyrilamine, and that the BBB uptake of oxycodone (30 μ M) at a pH of 7.4 was significantly inhibited in the presence of quinidine (1 mM), verapamil (1 mM), and amantadine (1 mM) (121). This same transporter was later shown to be inhibited at supra-therapeutic concentrations by a number of antidepressants such as amitriptyline (K_i = 13 μ M) and fluvoxamine (K_i = 65 μ M), which are unlikely to have major clinical implications (122). Transport studies in different cell lines have shown that oxycodone and diphenhydramine competitively inhibited the uptake of one another (123, 124). These results suggest the two share a common proton-coupled antiporter at the BBB, possibly similar to ones involved in the transport of

MDMA (200), clonidine (19, 123), and a number of other drugs.

A sheep study showed that oxycodone has 7-fold higher BBB permeability than morphine and a greater cerebral volume of distribution (201). Similarly, a rat study demonstrated that unbound oxycodone had a much greater influx rate across the BBB and 6 times the brain concentration relative to morphine, suggesting an active uptake transporter is likely involved in oxycodone's BBB disposition (202). Another study demonstrated that rats pre-treated with a known P-gp inhibitor had no difference in their plasma pharmacokinetics or brain concentrations of oxycodone relative to controls, suggesting oxycodone is likely not a P-gp substrate (112). Conversely, the brain levels of oxycodone were significantly higher in *mdr1a* knock-out mice relative to wild type mice. Oxycodone treated rats also demonstrated an up to 4-fold induction in protein levels of P-gp in various tissues, corresponding with decreased tissue concentrations of the known P-gp substrate paclitaxel (110). Nakazawa *et al.*, demonstrated in another mouse study that coadministration of likely P-gp substrate amitriptyline at clinical doses with oxycodone increased antinociception without affecting the brain pharmacokinetics of oxycodone (122). An *in situ* rat brain perfusion study supported *in vitro* findings whereby the brain uptake of oxycodone was significantly inhibited by pyrilamine (54% reduction in oxycodone brain uptake when co-perfused with 1 mM of pyrilamine), suggesting oxycodone shares the same proton-coupled antiporter at the BBB (121). Yet another rat study demonstrated that 8 days of repeated oxycodone administration (15 mg/kg i.p.) up-regulated the expression of *Abcg2* based on microarray data, qPCR, and Western blot analyses, and that this up-regulation resulted in decreased brain accumulation of known ABCG2 substrate mitoxantrone (117).

In a clinical study of 33 participants exposed to experimental pain, a strong association was found between carriers of variant alleles C3435T and G2677T/A in the ABCB1 gene and fewer adverse drug effects following oxycodone administration (113). Conversely, another clinical study of 76 mother-infant breast feeding pairs found mothers carrying at least one copy of the ABCB1 2677T variant had an increased risk of experiencing oxycodone-induced sedation themselves (114). Yet another clinical study found that cancer patients with the C3435T polymorphism of ABCB1 had little effect on the plasma disposition of oxycodone (115). The clinical implications of these variant alleles on P-gp function need further investigations to decipher their impact on opioids that are P-gp substrates.

Taken together, the role of efflux transporters, particularly P-gp, on the disposition of oxycodone appears to be controversial (116). The emerging role of proton-coupled antiporters in the BBB disposition of oxycodone warrants further investigation. Preclinical evidence suggests a number of transporter-mediated DDIs between oxycodone and other potential

substrates of an emerging proton-coupled antiporter of the BBB. However, these interactions occurred at supra-therapeutic concentrations and may not be clinically relevant. Further investigation is necessary to clarify these potential DDIs. Additional studies, especially clinical investigations, are needed to clarify these transporter mechanisms. The number of adverse events from oxycodone-containing pharmaceuticals is also alarming. Greater understanding of the mediators of oxycodone transport is necessary for elucidating the pathophysiology behind these reactions and ensuring the most safe and efficacious use of oxycodone-containing products.

Oxymorphone

Oxymorphone is an active primary metabolite of oxycodone that has been available on the US market since 1959 (203). It is estimated to have a 10–45 fold greater affinity for the μ -opioid receptor than oxycodone (204) and to be 9-times more potent than morphine in producing analgesia in humans (205). Oxymorphone is believed to exert its analgesic effects through both μ and δ opioid receptors (206). Over 1 million prescriptions for oxymorphone were written in 2012 and over 12,000 reported ED visits were due to oxymorphone abuse – more than twice the number in 2010 (181, 203). Thus, both oxycodone and oxymorphone are highly addictive substances showing alarming increases in abuse.

Despite the prevalence of oxymorphone use and adverse events, there appears to be limited investigation into its drug transporters at the current time. One *in vitro* study demonstrated that oxymorphone does not significantly stimulate P-gp ATPase activity (111), while a rat study demonstrated active transport is involved in the BBB uptake of oxymorphone through an unidentified transporter (125). Due to the limited transporter information available, it is not possible to predict potential transporter-mediated DDIs for oxymorphone without further studies.

Fentanyl

Fentanyl is a highly potent synthetic opioid analgesic that has been available on the US market since the 1960s. Estimated to be more than 100 times more potent than morphine, fentanyl is highly lipophilic and can rapidly cross the BBB (207). Fentanyl is a Schedule II controlled substance and is available in a number of formulations including lozenges, buccal tablets, transdermal patches, and injectable solutions. Reports of fentanyl abuse date back to the 1970s. In 2012 nearly 7 million prescriptions were written for fentanyl, and 3.4 million were written in the first half of 2013. The Centers for Disease Control and Prevention (CDC) reported over 1000 deaths from non-pharmaceutical fentanyl-related abuse between 2005 and 2007 (208). In 2011 over 20,000 ED visits were

reportedly due to fentanyl abuse in the US, more than twice the amount reported in 2004 (12).

Pulmonary endothelial transport studies demonstrated that fentanyl likely undergoes both passive and saturable active transport during pulmonary uptake (209). To investigate the active transport mechanisms further, another uptake study using radiolabeled fentanyl and primary cultured bovine brain microvessel endothelial cell (BBMEC) monolayers was conducted. Results indicated that fentanyl is a likely P-gp substrate, but that a yet to be identified fentanyl uptake transporter has a prominent role in fentanyl's BBB uptake (126). Fentanyl was also shown to significantly increase P-gp ATPase activity in mouse brain capillary endothelial cells (127). However, results from another study demonstrated that fentanyl does not behave as a P-gp substrate in LLC-PKI monolayers but does inhibit P-gp mediated transport of digoxin in Caco-2 cells (33). *In vitro* transport studies in HEK293 cells overexpressing OATP1B1 found no difference in fentanyl uptake relative to controls, suggesting it is not an OATP1B1 substrate (135).

Pre-treatment of mice with the P-gp substrate and inhibitor cyclosporine A was found to significantly increase the analgesic effects of fentanyl, without affecting its plasma pharmacokinetics (128). Similarly, another mice study demonstrated that coadministration of fentanyl with a cyclosporine A analog increased sensitivity to fentanyl (129). P-gp deficient mice were found to have significantly increased fentanyl antinociceptive and analgesic effects (51, 127), as well as significantly increased (24%) fentanyl brain uptake (130), relative to wild-type mice. The coadministration of known P-gp substrate verapamil with fentanyl in rats had only modest effects on fentanyl pharmacokinetics. However, the brain/plasma and lung/plasma ratios of fentanyl in rats were reduced 4-fold and 6-fold, respectively, when coadministered with known Oatp substrates pravastatin (1 mg/kg) and naloxone (0.1 mg/kg) (136).

Two randomized, double-blind studies in healthy volunteers found that pre-treatment with known P-gp inhibitor quinidine resulted in increased oral fentanyl plasma concentrations, but did not affect fentanyl pharmacodynamics. These results suggest quinidine may inhibit P-gp efflux during intestinal absorption but does not have much effect on fentanyl's BBB disposition (131). Another clinical study of 126 patients undergoing spinal anesthesia found certain ABCB1 genotypes were linked with increased fentanyl-induced respiratory depression. Patients with the genotypes 1236TT, 2677TT, and 3435TT were found to have early and profound respiratory depression (65–73% of initial respiratory rate) following intravenous fentanyl administration, suggesting ABCB1 polymorphisms may have important clinical implications in fentanyl safety (132). However, another clinical study of 83 patients also undergoing intravenous fentanyl treatment for spinal anesthesia concluded that ABCB1 polymorphisms had no effect on fentanyl-induced sedation or respiratory depression (133).

ABCB1 and ABCG2 polymorphisms were also found to have no relation with the clinical manifestations of fentanyl-induced delirium or coma (134). One randomized cross-over study found no significant differences in fentanyl pharmacokinetics between carriers of SLCO1B1*1a or SLCO1B1*15 (OATP1B1) haplotypes. Further, coadministration with known OATP1B1 inhibitor rifampicin also had no effect on fentanyl pharmacokinetics in either haplotype, supporting the observation that fentanyl is likely not an OATP1B1 substrate (135).

In vitro study results appear to conflict on the role of P-gp in fentanyl's disposition. A number of *in vivo* studies have concluded that fentanyl is a P-gp substrate while one suggested it is rather an Oatp substrate (136). Clinical results are not in consensus either, with some suggesting P-gp function has a significant role in the pharmacodynamics of fentanyl (131, 132), while others suggest otherwise (133, 134). Clinical evidence also suggests fentanyl is not an OATP substrate, implying fentanyl may be safely concomitantly administered with OATP substrates or inhibitors (135). In conclusion, there appears to be some controversy over the role of P-gp in fentanyl's transport.

Methadone

Methadone is the oldest of the synthetic opioids and has been used for decades in the detoxification and treatment of opioid addiction (210, 211). In recent years, its demonstrated clinical efficacy and cost-effectiveness have made methadone an increasingly prescribed opioid, particularly in treating moderate-to-severe pain (211, 212). Methadone consists of a racemic mix of R- and S-methadone (213), though the R-isomer is believed to account for its opioid effects (214). Methadone is also lipophilic, highly protein bound (85%), and known for its relatively long half-life (215) and often unpredictable pharmacokinetics (216). Methadone has unique analgesic properties in both its full μ -opioid receptor agonism and NMDA-receptor antagonism.

The drug raised concerns after reports of patients experiencing greater incidences of QT prolongation and Torsades de Pointes (217, 218). A sharp increase in methadone related deaths and poisonings led the FDA to issue a black box warning and public health advisory recommending methadone doses in pain relief be carefully chosen and monitored by the prescriber. As well as being used in opioid detoxification, methadone has an extensive history of abuse and dependence. According to the 2012 National Survey on Drug Use and Health (NSDUH), nearly 2.5 million Americans aged 12 and over had used methadone for non-medical purposes in their lifetime (190). In 2011 nearly 67,000 ED visits were due to non-medical methadone use, representing an 82% increase from 2004 (12). The American Association for Poison Control Centers (AAPCC) also reported 51 methadone related deaths

in 2012 (219). The unpredictable pharmacokinetics and potentially fatal consequences of methadone use has led to a number of studies investigating its transport mechanisms.

Methadone was found to increase the accumulation of known P-gp substrate vinblastine in multidrug resistant Chinese hamster ovary cells (B30) (32). An *in vitro* gut sac model confirmed that methadone was a P-gp substrate and that its transport was increased in the presence of P-gp inhibitors verapamil and quinidine (137). Caco-2 monolayer transport of P-gp substrate rhodamine 123 was found to be potently inhibited by methadone ($IC_{50}=7.5 \mu\text{M}$) at intraluminal concentrations (138), and methadone was also found to increase rhodamine 123 accumulation in human trophoblasts (139). The intra/extra cellular ratio of methadone was significantly decreased in human ABCB1 transfected cells relative to controls (140). An *in vitro* model of transplacental transport found that P-gp inhibitor GF120918 increased the transfer of methadone by 30%, and that uptake of methadone in the BeWo cell line was increased in the presence of P-gp inhibitor cyclosporine A (141). The same group demonstrated that methadone transfer was significantly higher in the fetal-to-maternal direction, likely due to the unidirectional activity of P-gp (142). Methadone was also shown to inhibit the transfer of paclitaxel in human placental inside-out vesicles, demonstrating a greater affinity for P-gp than the classic inhibitor verapamil (143). Similarly, methadone was shown to stimulate P-gp activity at higher concentrations (100 μM), and Caco-2 monolayer transport studies demonstrated that verapamil and GF120918 significantly reduced methadone efflux (111). Methadone (25–100 μM) was also found to significantly inhibit P-gp in HEK293 cells transfected with hMDR1, and demonstrated P-gp mediated transport in hMDR-MDCKII bidirectional transport studies (36). Equine intestinal mucosa transport studies similarly indicated that the intestinal transport of methadone is likely mediated by P-gp (144). A more recent *in vitro* study utilizing cells stably transfected with various genotypes of ABCB1 (P-gp) found that methadone most potently inhibited wild-type P-gp ($IC_{50}=2.17 \mu\text{M}$), while the genotypes 1236T-2677T-3435T ($IC_{50}=2.97 \mu\text{M}$) and 1236T-2677A-3435T ($IC_{50}=4.43 \mu\text{M}$) exhibited much less inhibition. The study also confirmed that methadone stimulated P-gp ATPase activity and inhibited verapamil (145).

Methadone-induced analgesia was higher in P-gp knock-out mice relative to wild types, and pretreatment with P-gp inhibitor cyclosporine markedly increased the analgesic effects of methadone in wild-type mice, but not in P-gp knockout mice (51). Brain concentrations of both R- and S-methadone were found to be 15 to 23-fold higher in P-gp knock-out mice relative to wild-types (146) and P-gp knock-out mice were shown to have a 2.6-fold increase in brain uptake of methadone relative to wild types (130). Similarly, P-gp knock-out mice were found to have significantly increased methadone brain uptake and methadone-induced antinociceptive effects

(111). Exposure of P-gp inhibitor rifampin to transgenic mice expressing human pregnane X receptor (hPXR) resulted in increased expression of P-gp in the brain endothelium and correspondingly attenuated methadone antinociceptive effects by ~70% (220). Pre-treatment of rats with the P-gp inhibitor valsopodar (10 mg/kg i.v.) was found to increase the brain concentrations of methadone 6-fold and 4-fold following oral (6 mg/kg) and intravenous (0.35 mg/kg) administration relative to controls, respectively (147). Valsopodar was also found to increase the bioavailability and analgesic effects of methadone in rats (148). Conversely, the pharmacokinetics of methadone in dogs was unaffected by P-gp inhibition (221).

Extensive clinical research has been performed aiming to elucidate the role of P-gp in the pharmacokinetics (PK) and pharmacodynamics (PD) of methadone with often inconsistent results. There have been case reports of DDIs between P-gp inducers rifampicin (149, 150) and St John's Wort (151) with methadone, resulting in increased opioid withdrawal symptoms. A double-blind, placebo controlled crossover study in healthy volunteers showed that the P-gp inhibitor quinidine did not alter the PK or PD of intravenous methadone, but did increase plasma concentrations following oral methadone ingestion. These results may suggest that P-gp has a large effect on intestinal methadone absorption but not its BBB transport (131). However, a number of consequent clinical studies demonstrated that methadone bioavailability was largely unchanged in the presence of a number of antiretrovirals believed to inhibit (ritonavir, indinavir, ritonavir-lopinavir) and induce (nelfinavir, and efavirenz) P-gp activity. Together these results suggest that P-gp may not play a prominent role in intestinal methadone absorption or in the mechanism(s) behind well-noted methadone-antiretroviral drug interactions (95, 131, 152–154).

A number of clinical studies have investigated the potential role of ABCB1 polymorphisms on methadone pharmacokinetics. An Australian study comparing opioid-dependent and non-dependent patients found ABCB1 polymorphisms had no influence on the development of opioid dependence, but may have an influence on daily methadone dose requirements. Patients carrying 2 copies of the wild-type haplotype (AGCGC) were found to require significantly higher maintenance methadone doses relative to those carrying 1 or 0 copies. Additionally, carriers of the AGCTT haplotype (SNPs at 2677 and 3435 loci) were found to require significantly lower (approximately 50%) daily doses of methadone (155). However, the same group later performed an expanded study of methadone maintenance treatment (MMT) patients (doses ranging from 15 to 300 mg/day) and found no significant associations between any one ABCB1 haplotype and methadone dosing. When controlling for ORMP1 (encodes μ -opioid receptor) variation, AGCTT carriers had significantly lower methadone doses and trough concentrations (C_{trough}) relative to wild-type subjects. These results suggest a number

of genetic factors besides ABCB1 are likely to contribute towards the PK/PD of methadone (160).

The ABCB1 polymorphisms 2677G>T and 3435C>T were found to have no influence on the plasma pharmacokinetics of levomethadone following a single dose (163). The same 2 polymorphisms as well as the 61A>G polymorphism were associated with lower methadone C_{trough} , but had no effect on peak concentrations in another study of patients undergoing MMT (164). A follow up study found no significant associations between ABCB1 haplotypes and methadone dose requirements (161). Similarly, a Spanish study of opioid dependent patients on methadone maintenance found no relationships between ABCB1 allelic variations and the use of illicit opioid substances or methadone dosages (162), and a Danish postmortem study of drug users found no associations between ABCB1 genotypes and methadone concentrations (165).

Alternatively, significant associations were found between certain ABCB1 polymorphisms and methadone maintenance dose requirements in a clinical study of formerly severe heroin-dependent Israeli patients. Patients homozygous for the 'T' allele in SNP 1236C>T and carriers of the 3-locus genotype pattern of 'TT-TT-TT' (3435C>T, 2677G>T and 1236C>T polymorphisms) were estimated to have an approximately 7-fold and 5-fold increased chance of requiring higher (>150 mg/day) maintenance methadone doses relative to non-carriers, respectively (156). A Swiss study of 14 MMT subjects found the ABCB1 genotypes 3435 TT, CT, and CC resulted in 3, 23 and 33% increases in (R)-methadone concentration/dose ratios following administration of P-gp substrate quetiapine, respectively (158). Carriers of the variant ABCB1 3435C>T were found to require higher doses of methadone than non-carriers among a population of Han Chinese patients (157), and a pilot study of 178 Taiwanese patients in a MMT program found the ABCB1 2677T allele had positive effects on methadone plasma concentration (159).

In conclusion, *in vitro* and *in vivo* studies appear to overwhelmingly suggest that methadone is a P-gp substrate, while clinical studies investigating the effects of ABCB1 polymorphisms are inconsistent on the matter. Some clinical studies have suggested there is a likely relationship (155–159), while others have suggested there is likely not (161–163, 165), and still others propose that ABCB1 polymorphisms are only one of a number of interacting pharmacogenetic determinants influencing methadone pharmacokinetics (160). Despite extensive research, there remains considerable controversy over the functional consequences of various ABCB1 polymorphisms (222–225). There currently does not appear to be any investigations (preclinical or clinical) into the role other transporters besides P-gp may play in the disposition of methadone. Thus, methadone is a highly understudied drug when it comes to its interactions with other transporters. Its response is characterized by a large inter-patient variability and thus

further research into the transporter(s) involved in its disposition may assist in elucidating its often unpredictable pharmacokinetics.

Buprenorphine

Like methadone, buprenorphine has been marketed as an effective maintenance medication for treating opioid addiction (226). Buprenorphine is a semi-synthetic partial μ -opioid receptor agonist and κ -opioid receptor antagonist (227). Buprenorphine has an estimated 20–30 times greater analgesic potency than morphine (228) and a half-life greater than 24 h (229). Buprenorphine's unique pharmacological properties among μ -opioid agonists include an apparent "ceiling effect" at higher doses, reducing the risk of respiratory depression and opioid withdrawal symptoms (227, 230, 231). Such characteristics have made buprenorphine an attractive alternative to methadone in treating opioid addiction and moderate pain (228, 229). The primary metabolite of buprenorphine in humans is norbuprenorphine, which exhibits plasma concentrations similar to those of buprenorphine (232). Norbuprenorphine is pharmacologically active and demonstrates a high affinity for all 3 opioid receptors (227, 233). However, norbuprenorphine has minimal antinociceptive effects (234) and causes greater respiratory depression than buprenorphine (227).

As of July 2013, it is estimated that around 15,700 U.S. physicians are approved by the Substance Abuse and Mental Health Services Administration (SAMSHA) and the Drug Enforcement Administration (DEA) to prescribe buprenorphine treatments in the US. In 2012 over 9 million buprenorphine prescriptions were dispensed in the US (228). An estimated 21,483 ED visits were related to buprenorphine abuse in 2011, representing a nearly 5-fold increase from 2006 (12). The 2011 AAPCC annual report estimated that nearly 4000 Poison Control Center cases and 3 deaths related to buprenorphine abuse (228). Although intended to help in treating opioid addiction, buprenorphine, like many prescription opioids, is showing an alarming trend of increasing abuse.

Both human P-gp and BCRP-mediated transport in stably transfected HEK293 cells were significantly inhibited in a concentration dependent manner by norbuprenorphine and buprenorphine (25–100 μM), while only norbuprenorphine demonstrated P-gp mediated transport (36). MDCK cells stably transfected with human P-gp demonstrated significantly increased net efflux of norbuprenorphine, but not of buprenorphine nor two of its glucuronidated metabolites, relative to untransfected cells (166). Buprenorphine did not undergo P-gp mediated transport in human placental lobules (167) or Caco-2 cells, nor did it stimulate P-gp ATPase activity (111).

Buprenorphine rapidly crosses the BBB of rats (234). P-gp inhibitors increased brain uptake and decreased brain efflux

of radiolabeled buprenorphine in rats (168). Conversely, no changes were found between brain distribution and antinociceptive effects of buprenorphine in P-gp knock-out mice relative to wild-types (111). Another P-gp knock-out mice study demonstrated that the brain/plasma ratio of norbuprenorphine was significantly greater, as was the magnitude and duration of its antinociceptive effects, relative to wild-types. These results suggest that P-gp plays a major role in limiting the BBB access of norbuprenorphine, potentially explaining its minimal antinociceptive effects (166). Administration of the P-gp inhibitor PSC833 in mice was found to significantly increase the respiratory depressive effects of buprenorphine and norbuprenorphine, as well as significantly increase plasma concentrations and reduce brain efflux of norbuprenorphine. Similar effects were also seen in P-gp knock-out mice, suggesting that P-gp plays an important role in the BBB disposition of norbuprenorphine, and that its inhibition can lead to major respiratory side effects (169). A follow up study by the same group found no differences in P-gp mediated BBB transport of buprenorphine or norbuprenorphine among different strains and genders of mice, despite their variable buprenorphine-induced respiratory toxicities (235).

One clinical study compared the analgesic effects of buprenorphine and morphine in the palliative care of patients with confirmed P-gp⁺ or P-gp⁻ malignant tumors, based on immunohistochemistry (IHC) results of pretreatment tumor biopsies. The results demonstrated that P-gp expression had no influence on buprenorphine's analgesic effects, while P-gp⁺ patients required higher doses of morphine for analgesic effects (59). However, other groups have questioned these findings, citing the 10% IHC cut-off value for P-gp +/- categorization as arbitrary and the unknown correlation between P-gp expression in tumor cells and that of the BBB, as potential shortcomings (170). A retrospective study analyzing the co-use of illicit and non-prescribed drug use in buprenorphine patients found that cocaine and marijuana were the most commonly coadministered illicit drugs, while benzodiazepines were the most commonly coadministered non-prescription drugs (229). These results are concerning as other studies have shown that frequent and heavy cocaine use inhibits the pharmacokinetics of buprenorphine, triggering opioid withdrawal symptoms possibly by P-gp induction (236). Further, there have been numerous reports of severe respiratory depression and death from concomitant use of buprenorphine with CNS depressants, particularly benzodiazepines (216, 228, 237–239). However, the role of drug transporters in these dangerous DDIs has yet to be investigated.

In vitro studies appear in agreement that buprenorphine is most likely not a P-gp substrate (111, 166, 167), while nobuprenorphine is (36, 166). The apparent P-gp mediated efflux of norbuprenorphine at the BBB may explain its minimal antinociceptive effects, despite its opioid receptor

affinities. *In vivo* studies largely demonstrated the same findings, except one mouse study which suggested that buprenorphine BBB transport may be P-gp-dependent (168). There appears to be only one clinical study to date addressing P-gp mediated transport of buprenorphine (59), and its findings have been contested (170). Most concerning is the fact that buprenorphine has been shown to be commonly co-abused with illicit drugs and prescription medications, often leading to untoward DDIs (229). Of particular concern is the apparent prevalent co-abuse of buprenorphine with benzodiazepines, potentially causing fatal DDIs through a yet established mechanism. The only other transporter besides P-gp investigated to date appears to be BCRP, which suggests that both buprenorphine and norbuprenorphine may be inhibitors of (36).

Tramadol

Tramadol is a Schedule IV controlled substance (240) that is generally administered as a racemic mixture of (+) and (-) enantiomers, both of which have analgesic effects by inhibiting neuronal monoamine uptake. However, *in vitro* evidence suggests it is only the active CYP2D6 metabolite (+)-O-desmethyltramadol that is a μ -opioid receptor agonist (80). Tramadol is well absorbed orally and generally administered every 4 to 6 h for moderate to moderately-severe pain management. *In vivo* tramadol has been found to have a $K_{p,uu}$ of greater than 1, suggesting it is actively transported across the BBB (176). An estimated 43.8 million tramadol prescriptions were dispensed in the U.S. in 2013 and over 13,000 tramadol exposures were reported to the AAPCC in 2012, 9 of which resulted in deaths (240). In 2011 an estimated 20,000 emergency room visits were due to non-medical use of tramadol (12) and in 2012, an estimated 3.2 million Americans used tramadol for non-medical purposes (190).

Caco-2 monolayer transport studies of (+)-tramadol, (-)-tramadol and (+)-O-desmethyltramadol reported that none appeared to be P-gp substrates, while a proton-based efflux transporter was likely involved in restricting their GI absorption and enhancing their renal excretion (171). Transport studies in immortalized brain capillary endothelial cells (hCMEC/D3) demonstrated that tramadol (5 μ M) uptake was significantly inhibited at non-clinically relevant concentrations (1 mM) by other opioids that are cationic at physiological pH such as morphine, codeine, and oxycodone, as well as other organic cations including apomorphine, clonidine, diphenhydramine, quinidine, and pyrilamine. However, tramadol uptake was not significantly affected by substrates of OCT or OCTN2 but was altered by changes in transmembrane pH gradients. Together these results suggest that tramadol is a substrate of a proton-coupled antiporter at the BBB (176). Tramadol uptake was found to be unaffected by OCT1 overexpression in HEK293 cells while that of

O-desmethyltramadol was increased 2.4-fold. This increased uptake was reversed in the presence of OCT1 inhibitors and in cells overexpressing loss-of-function OCT1 variants (80).

In line with their *in vitro* findings, rat brain microdialysis studies by Kitamura *et al.*, reported that tramadol had a 2.3-fold higher concentration in the brain than plasma, suggesting an active tramadol uptake transporter at the BBB (176). *In situ* rat brain perfusion studies by Cisternino *et al.*, showed that tramadol likely shared the same hypothetical proton-coupled antiporter as nicotine and diphenhydramine at the BBB (20). Another rat study found that P-gp inhibition through verapamil pre-treatment had no effect on the brain concentration of tramadol, suggesting it is not a likely P-gp substrate (172).

Initial studies by Slanar *et al.*, in 21 healthy volunteers reported that increasing numbers of MDR1 C3435T alleles were associated with slight increases in the maximum concentration of tramadol (C_{max}) and its AUC_{0-24} , irrespective of patient CYP2D6 status (173). However, a following study by the same group in 156 post-operative patients investigated the possible impact of MDR1 C3435T polymorphisms on tramadol-induced analgesia found no significant differences among subgroups (174). Another clinical study investigated the effects that coadministration of the potent P-gp inhibitor ketoconazole may have on the DDI between tramadol and the CYP2B6 inhibitor ticlopidine. Results found that ketoconazole had no effect on the PK of tramadol or ticlopidine nor did it modify their interaction (175). In line with their *in vitro* findings, Tzvetkov *et al.*, demonstrated in healthy volunteers that OCT1 polymorphisms had no effect on the PK or PD of tramadol, while loss-of-function OCT1 polymorphisms correlated with elevated plasma concentrations of O-desmethyltramadol and prolonged miosis; a surrogate PD marker of opioids (80).

In conclusion, neither enantiomer of tramadol nor the active metabolite O-desmethyltramadol appear to be substrates of P-gp. All three do appear to be substrates of pH-dependent active transporters during their gastric efflux and renal excretion. Similarly, tramadol also appears to be a substrate of an emerging proton-coupled antiporter at the BBB. Moreover, *in vitro* and clinical studies suggest that tramadol is a likely OCT1 substrate which could result in a number of DDIs with fellow substrates or inhibitors (Table I). Such interactions could result in impaired hepatic clearance of tramadol and/or the interacting drug, leading to prolonged exposure and adverse events (240).

Hydrocodone

Hydrocodone is the most frequently prescribed opioid as well as the most associated with drug abuse and diversion (241). In the

U.S. hydrocodone has been in the spotlight recently as congress changed its status from a Schedule III to Schedule II controlled substance in 2014. Hydrocodone is a semi-synthetic opioid structurally similar to codeine. Hydrocodone is believed to be at least as potent as codeine as an antitussive, and nearly as potent as morphine in producing analgesia. In 2012 an estimated 143 million hydrocodone containing prescriptions were dispensed in the US (241). Reports of hydrocodone abuse and addiction date back to the 1960s, and in 2011 an estimated 23.2 million people in the US aged 12 or older had used hydrocodone products for non-medical purposes (190). In the same year, over 80,000 ED visits (representing a 107% increase in the number from 2004) and 37 deaths were reported from non-medical use of hydrocodone (190). Additionally over 12,000 ED visits were reportedly due to hydrocodone-related suicide attempts, and over 74,000 ED visits were due to adverse reactions to hydrocodone products (12). Hydrocodone abuse shows an alarming increase in abuse trends and accounts for the most ED visits due to adverse reactions of all narcotic analgesics.

There appears to be a number of CYP450-based DDIs regarding hydrocodone (242–244). However, to the best of our knowledge there have been no investigations into the transporters involved in the disposition of hydrocodone. Considering hydrocodone is addictive, frequently prescribed and abused, and recently available on the U.S. market in a new sole formulation, greater understanding of its transport mechanisms is necessary.

Hydromorphone

Hydromorphone is a potent Schedule II semi-synthetic opioid which is derived from and displays greater analgesic potency than morphine. In 2012 nearly 4 million hydromorphone prescriptions were dispensed in the U.S. and the aggregate production quota of hydromorphone in 2013 was almost 6000 kg. Known to be highly addictive, hydromorphone abuse has been a continuing problem in the US for decades. Hydromorphone IR formulations were once among the leading opioid products of abuse and diversion (245). The 2011 NSDUH reported over 1 million Americans aged 12 or over had tried hydromorphone for non-medical purposes, and over 18,000 ED visits related to hydromorphone abuse were reported in the US, representing an over 400% increase from 2004 (12, 190). Hydromorphone remains a major prescription opioid of abuse with increasing trends of hospital visits from its misuse (245).

Similar to hydrocodone, there does appear to be evidence of CYP450 but not transporter-mediated DDIs with hydromorphone (242). Despite the increasing prevalence of hydromorphone licit and illicit use, there currently does not appear to be any literature available on its transport mechanisms.

EMERGING TRANSPORTERS AT THE BBB

Proton-Coupled Antiporters

There appears an emerging class of pH dependent proton-coupled antiporters that have yet to be fully identified at the molecular level but could play prominent roles in the uptake of a number of drugs at the BBB (Fig. 1). Okura *et al.*, demonstrated both *in vitro* and *in vivo* that oxycodone BBB influx was mediated via a pH and energy dependent proton-coupled antiporter shared with pyrilamine. *In vitro* findings have suggested that a number of medications, including quinidine, verapamil, amantadine (121), the antidepressants amitriptyline, imipramine, clomipramine, amoxapine, and fluvoxamine, as well as the antiarrhythmics mexiletine, lidocaine, and flecainide, and ketamine (122) may also be substrates of this transporter. A proton-coupled antiporter was also reported to be responsible for the luminal BBB transport of clonidine, diphenhydramine, tramadol, and nicotine. Further, a number of secondary and tertiary amine drugs of abuse including oxycodone, codeine, morphine, diacetylmorphine, cocaine, and MDMA appear to inhibit or interact with this transporter (19, 20). Fischer *et al.*, suggested that codeine may also be a substrate of a proton-coupled antiporter during intestinal and brain uptake with strong similarities to the pyrilamine transporter described by Okura *et al.* The cellular uptake of codeine was potently inhibited by pyrilamine, clonidine, diphenhydramine, propranolol, verapamil, as well as a number of drugs of abuse, including D- and L-amphetamine, cocaine, and methamphetamine (109). However, Sadiq *et al.*, have suggested that any clinical DDI between oxycodone and diphenhydramine was unlikely at therapeutic doses (123).

The functional expression of a proton-coupled antiporter was demonstrated in an *in vitro* human BBB model, but its molecular identity remains unknown (124). Due to the lack of molecular information it is presently unclear whether these findings refer to the same transporter or multiple possibly related transporters of an emerging class. A host of potentially harmful transporter-based clinical DDIs could result from a number of combinations, thus further research into proton-coupled antiporters is necessary. Greater understanding of these transporters could improve the clinical safety and efficacy of the many possible substrates, as well as possibly aid in the development of new CNS acting drugs and anti-addiction therapies (20, 124).

Other Emerging BBB Transporters

In vivo data has suggested the glucose transporter 1 (GLUT-1) is involved in the BBB transport of M6G (56, 81). GLUTs have established roles as glucose and/or fructose transporters, but their other major substrates have likely not yet been

identified, making predicting potential transporter-mediated DDIs difficult (246). There also appear to be unidentified active transporters involved in the BBB transport of oxycodone (125) and fentanyl (126). Other active transporters which have yet to be fully elucidated include the probenecid-like transporter of M6G (putative OAT) (57), the digoxin-sensitive BBB transporter (putative OATP1B1) (56) of morphine, and pyrilamine BBB transporter of oxycodone (putative OCT) (121).

TRANSPORTER POLYMORPHISMS

Pharmacogenomics appears to be playing an increasingly important role in transporter research and drug development. A host of genetic polymorphisms in a number of drug transporters have previously been linked to certain diseases, chemotherapy resistance, and immune deficiencies (17). There have also been a number of preclinical and clinical investigations into drug transporter polymorphisms and opioids.

The role of ABCB1 polymorphisms in the PK and PD of morphine remains currently unclear despite a number of clinical investigations (62–64). However, there does appear to be a present consensus that OCT1 loss-of-function polymorphisms have significant impacts on morphine PK, elevating morphine_{AUC} (38) and decreasing its clearance (65). OCT1 loss-of-function variants were also found to significantly affect the PK and PD of O-desmethytramadol (80).

As with morphine, there does not appear to be a clear consensus on the role of ABCB1 polymorphisms in the PK and PD of oxycodone (113–115), fentanyl (132–134), or methadone (155–162, 164, 165). The reasons behind this apparent lack of clarity may be complex, and possibly involve the geographic distribution of patients enrolled as well as the polymorphisms selected for investigation. A more standardized approach including more transporters of interest may help in elucidating the impact these polymorphisms have on the PK and PD of opioids. These findings could potentially provide a platform towards personalized opioid pain-management in the future.

POTENTIAL IMPLICATIONS FOR CANCER THERAPY

Tumor expression of drug transporters such as P-gp, BCRP, and a number of MRPs have been linked to chemotherapy resistance in a number of different cancers, and in some cases even serve as indicators of poor prognosis and survival (247–250). Given the apparent prominent role of drug transporters in the PD/PD of a number of anti-cancer agents, and the frequent use of opioids in cancer-pain management, there

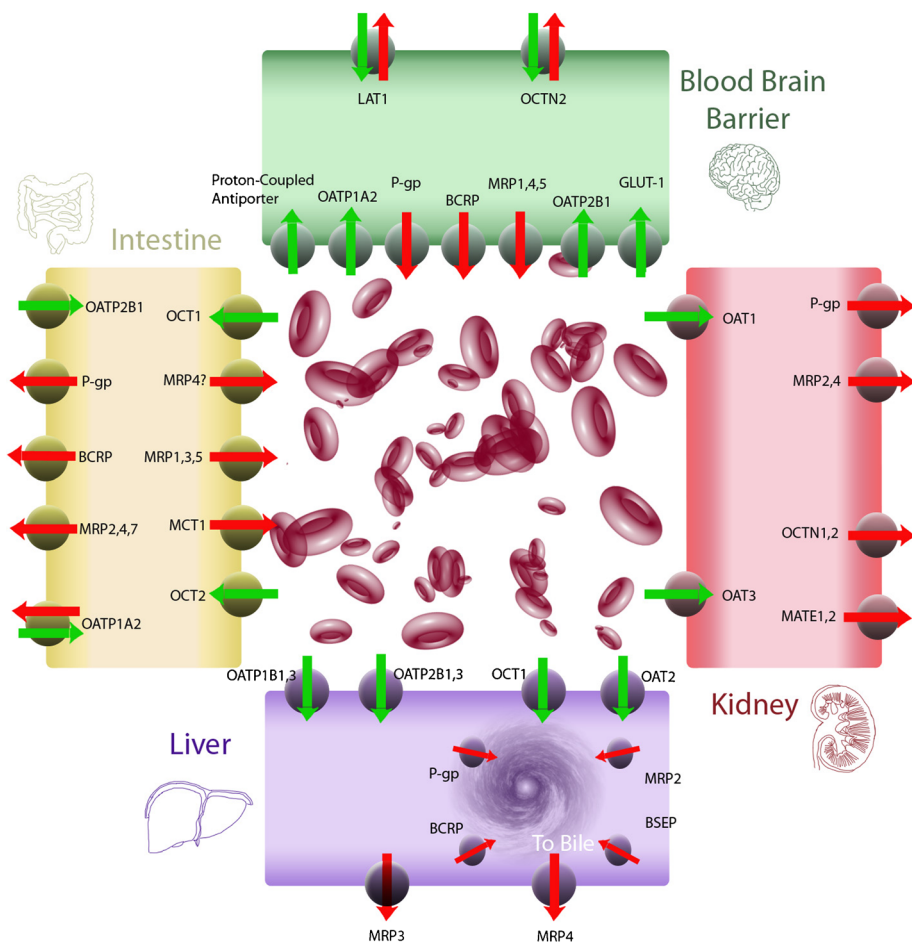
may be an increased likelihood of transporter-mediated DDIs between the two (251–253).

Although it appears unclear based on clinical evidence, pre-clinical evidence strongly suggests that morphine is a P-gp substrate. The concomitant administration of morphine with the apparent P-gp substrate paclitaxel may result in a transported-mediated DDI with unpredictable consequences (34). *In vivo* results have demonstrated that co-administration of oral morphine with the P-gp substrate etoposide resulted in significantly increased acute morphine-induced analgesia, likely as a result of impaired P-gp-mediated efflux at the intestinal epithelium (48)(Fig. 1). Patients with P-gp⁺ tumors ($\geq 10\%$ IHC staining) were found to have significantly decreased morphine-induced analgesia, requiring higher doses compared to P-gp⁻ patients, though both groups of patients had similar levels of plasma morphine (59). However, the findings of this study have been contested (170). The mechanism(s) by which the centrally acting analgesic effects of morphine were modulated is unclear as there does not appear to be any correlation between tumoral and BBB P-gp expression (254). The morphine phase II metabolite M6G may also be a weak OATP1B1 substrate at the BBB based on *in vivo* evidence (56). This could signal potential transporter-mediated DDIs with other substrates/inhibitors

which may include docetaxel (93) and paclitaxel ($IC_{50} = 50 \mu M$) (94), warranting further investigation. *In vitro* evidence has demonstrated that morphine's OCT1-mediated hepatic uptake (Fig. 1) can be inhibited at clinically relevant concentrations of irinotecan ($IC_{50} = 1.5 \mu M$) and ondansetron ($IC_{50} = 1.17 \mu M$). Given that either of these agents may be concomitantly administered with morphine in cancer patients, there is a distinct possibility of OCT1-mediated DDIs that could lead to serious adverse events associated with prolonged morphine exposure (38). *In vitro* evidence also suggests that oxaliplatin is an OCT1 substrate (74).

Preclinical evidence has suggested M3G and M6G are likely MRP2 and MRP3 substrates (39, 40), potentially incurring transporter-mediated DDIs with other substrates or inhibitors during hepatic clearance (Fig. 1). Therefore, concomitant administration of morphine with other potential MRP2 substrates such as etoposide (84), irinotecan (85), methotrexate (86), vinblastine (87), or inhibitors such as daunorubicin, etoposide ($K_i = 756 \mu M$), vincristine ($K_i = 802 \mu M$) (87), and possible MRP3 substrates such as methotrexate, folic acid, and leucovorin (90) may potentially result in adverse clinical DDIs associated with reduced clearance of these various anti-cancer agents and/or M6G and M3G.

Fig. 1 Distribution of key opioid transporters. This figure illustrates the location and function of the key transporters responsible for the disposition of many drug substrates. The central compartment with red blood cells is representative of systemic circulation throughout the body. Each surrounding compartment is representative of a single cell of its respective tissue type with specific transporters located at the apical or basolateral membranes of each, as well as, in the case of hepatocytes, at the canicular membrane. *Green arrows* indicate that a protein functions as an uptake transporter from circulation into the cell while *red arrows* represent efflux from the cell either back into circulation or to the bile. The few transporters with *bidirectional arrows* have been shown to function as shuttles for drug both into and out of cells.



Tournier *et al.* demonstrated that buprenorphine and its metabolite norbuprenorphine inhibit the activity of BCRP *in vitro*. If clinically accurate, there may be a dual benefit of buprenorphine treatment in the pain management of cancer patients, providing pain relief while also reducing the efflux of BCRP substrate anti-cancer agents from cancerous cells. . Conversely, Hassan *et al.* demonstrated *in vivo* evidence that oxycodone is a BCRP inducer after 8 days of repeated administration, increasing the efflux of mitoxantrone from tissues. Moreover, oxycodone appeared to be a BCRP substrate but only at a supra-therapeutic concentrations. These findings may have implications for more long-term concomitant use of oxycodone-containing products with BCRP substrates such as daunorubicin and doxorubicin (117). Oxycodone was also shown to induce P-gp expression in rats, corresponding with decreased tissue concentrations of paclitaxel (110). Although preliminary, these preclinical results suggest buprenorphine may have advantages over oxycodone in cancer pain management due to more favorable transporter interactions.

In vitro studies have also shown that methadone increases the accumulation of vinblastine in multi-drug resistant cells while also inhibiting P-gp mediated uptake of paclitaxel (32, 143). In addition, neither tramadol nor its active metabolite O-desmethyltramadol appear to be P-gp substrates, potentially avoiding transporter-mediated interactions with a number of anti-cancer molecules (172, 175)

CONCLUSIONS

The US is currently facing a severe epidemic in opioid-related deaths as well as dramatic increases in reports of opioid abuse and adverse events. Despite these alarming trends, relatively little is known about major components of opioid pharmacokinetics – membrane drug transporters. A lack of clarity and even controversy exist regarding the role certain drug transporters play in the disposition of particular opioids, such as in the case of P-gp and the disposition of oxycodone and methadone. P-gp appears to be by far the most investigated transporter, yet its role in the disposition of many opioids discussed here remains unclear. Further, there appears to be a complete absence of transporter information for some of the most commonly used and abused opioids, such as hydrocodone and hydromorphone. There also appears to be an emergence of a number of yet to be fully identified transporters, such as the proton-coupled antiporters which may play important roles in the BBB transport of opioids and a number of other drugs of abuse. In addition, drug transporter genomics is also emerging as an area of interest in predicting individualized patient responses to various opioids. This review highlights, explains and predicts a number of transporter-mediated opioid DDIs, as well as points to the potential clinical manifestations

of these interactions, particularly in the management of pain in patients receiving anti-cancer therapies. It is important to note that a number of the reported preclinical transporter-mediated DDIs occurred at supra-therapeutic or non-clinically relevant doses. As such, caution must be exercised while interpreting these data and predicting their clinical implications. By and large, the field of transporters is expanding rapidly and new transporters are being identified. Indeed, our understanding of the role of the known and new transporters in the disposition of many drugs is improving. However, investigating such interactions with opioids is progressing relatively slowly despite the alarming number of opioid-mediated DDIs that may be related to transporters. The FDA (16), Japan's Pharmaceutical and Medical Devices Agency (PMDA) (14), the European Medicines Agency (EMA) (15), and the International Transporters Consortium (ITC) (255) have all released guidelines highlighting the importance of studying drug-transporters interactions. A lack of information and clarity on the role of drug transporters in the disposition of opioids underscores the need for further research to improve their safety and efficacy, as well as aid in the development of new anti-addiction therapies.

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