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Opioid Analgesics and P-glycoprotein Efflux Transporters: A Potential Systems-Level Contribution to Analgesic Tolerance

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Abstract

Chronic clinical pain remains poorly treated. Despite attempts to develop novel analgesic agents, opioids remain the standard analgesics of choice in the clinical management of chronic and severe pain. However, mu opioid analgesics have undesired side effects including, but not limited to, respiratory depression, physical dependence and tolerance. A growing body of evidence suggests that P-glycoprotein (P-gp), an efflux transporter, may contribute a systems-level approach to the development of opioid tolerance. Herein, we describe current *in vitro* and *in vivo* methodology available to analyze interactions between opioids and P-gp and critically analyze P-gp data associated with six commonly used mu opioids to include morphine, methadone, loperamide, meperidine, oxycodone, and fentanyl. Recent studies focused on the development of opioids lacking P-gp substrate activity are explored, concentrating on structure-activity relationship development to develop an optimal opioid analgesic lacking this systems-level contribution to tolerance development. Continued work in this area will potentially allow for delineation of the mechanism responsible for opioid-related P-gp up-regulation and provide further support for evidence based medicine supporting clinical opioid rotation.

Keywords

Opioids; analgesics; P-glycoprotein; efflux transporters; tolerance; dependence

Introduction

Chronic clinical pain remains poorly treated. A number of drug classes are currently used to relieve pain, including non-steroidal anti-inflammatory agents (NSAIDs), anesthetics, N-methyl-D-aspartate (NMDA) receptor antagonists and opioids. NSAIDs primarily have a peripheral site of action and are useful for the treatment of mild to moderate pain, while producing an anti-inflammatory effect; whereas, anesthetics (local or general) inhibit pain transmission through the inhibition of voltage-regulated sodium and potassium channels. These agents, however, are highly sedative or toxic when used in the appropriate dose needed for chronic or acute pain relief. NMDA receptor antagonists, such as ketamine, inhibit the action of the NMDA receptor producing a dissociative analgesia [1]. Current research is investigating other receptor systems which may operate by novel mechanisms resulting in analgesic effects. These include, centrally acting α -adrenergic- [2], cannabinoid- [3], GABA- [4], and nicotinic-[5] receptor agonists as well as N-type calcium channel

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blockers, such as Ziconotide [6]. Despite all these attempts, opioids remain the standard analgesics of choice in the clinical management of chronic and severe pain.

The pharmacological actions of opioids are a result of their interaction with the opioid receptors which are seven transmembrane domain, G-protein coupled receptors (GPCR) that are located in high concentrations in the brain and spinal cord [7]. Three subtypes have been cloned: mu (μ , MOR) [8], kappa (κ , KOR) [9] and delta (δ , DOR) [10, 11] and each has unique central pharmacological actions. Agonists of the μ receptor produce effects of analgesia and euphoria, respiratory depression, and are largely responsible for the physical dependence associated with opioids [12]. κ receptor agonists provide analgesia and show little dependence liability; however, they are poor therapeutic targets, as they produce intense dysphoric reactions [13]. Agonists at the δ receptor are also poor targets, as their activation produces convulsions as an undesirable side effect, at least in rodents [14]. Centrally active μ opioid agonists therefore remain the primary choice in the clinical setting and methods are urgently required to reduce side effects.

In addition to central activity, μ opioid agonists stimulate peripheral μ opioid receptors in the gastrointestinal (GI) tract inhibiting intestinal motility which is the primary cause for opioid-related constipation [15]. Opioids that do not readily enter the central nervous system (CNS), such as loperamide (Imodium®) and diphenoxylate, are used as anti-diarrheal agents [16] and in the treatment of irritable bowel syndrome [17]. Due to their lack of central activity, such compounds do not produce the centrally-mediated effect of euphoria and therefore, have low abuse potential [18].

After repeated administration of morphine, however, patients become tolerant to the central effects and require greater doses to maintain the same level of analgesia, a phenomenon known as central tolerance [19]. The development of peripheral tolerance, however, does not occur as rapidly [20]. Consequently, when the dose of morphine is increased to reach a stable level of analgesia, the constipatory effect is increased. The disparity between the developmental rates of central and peripheral tolerance is a significant problem in the management of chronic pain, as the constipation experienced itself can add to the pain experienced by the patient [21].

Several theories exist regarding the mechanisms behind the development of tolerance, including the classical views of receptor desensitization and internalization and adaptations in downstream signaling pathways [19]. The mechanism behind receptor-mediated tolerance is complicated by the finding that not all opioids activate the arrestin-mediated downstream signaling pathway and promotes opioid receptor internalization; morphine, in fact, does not activate this pathway [22, 23]. Receptor oligomerization has been hypothesized as an alternative mechanism for opioid receptor desensitization [24]. However, receptor-mediated tolerance alone would not cause the disparity between the developmental rates of central and peripheral tolerance, as similar receptor desensitization events would occur both in the brain and in the GI tract. Additional factors at the systems level must therefore be involved in the development of central tolerance to opioid analgesia.

A growing body of evidence suggests that efflux transporters in the Blood-Brain Barrier (BBB), specifically P-glycoprotein (P-gp) may contribute to the development of central tolerance to opioids. P-gp is a member of the ATP-binding cassette (ABC) super-family of transport proteins, and is involved in various functions including the extrusion of xenobiotics, uptake of nutrients, transport of ions and peptides, and cell signaling [25]. Forty-eight ABC transporters have been identified in humans and classified on the basis of phylogenetic analysis into 7 subfamilies [26] as shown in Table (1). P-gp (ABCB1) is a member of the ABCB (MDR/TAP) subfamily and is one of the most characterized efflux

transporters to date. A number of excellent reviews are available which discuss the secondary and tertiary structures of P-gp as well as the substrate-binding pocket [25, 27–29]. The exact mechanism of P-gp function has not been fully delineated to date; however, two models, the “hydrophobic vacuum cleaner” and the “flippase” are readily accepted. A brief description of the pump function of each model follows. In the “hydrophobic vacuum cleaner” model P-gp extracts its hydrophobic substrates from the lipid bilayer and expels them directly to the external aqueous medium [30], whereas in the “flippase” model, substrates are flipped from the inner leaflet of the lipid bilayer to the outer leaflet of the plasma membrane or directly into the extracellular environment [31].

The substrate specificity for P-gp remains broad despite various efforts to establish a structure-activity relationship (SAR) for P-gp. In general, P-gp substrates contain a high number of hydrogen bonds, a basic nitrogen and are lipophilic with a molecular weight below 500 [25]. Interestingly, a correlation between P-gp and cytochrome P450 3A4 (CYP 3A4) substrate specificity exists [32, 33] and numerous studies have demonstrated clinically relevant drug-drug interactions when a P-gp inhibitor is co-administered with a CYP 3A4 substrate. Contrary to cytochrome P450 enzymes, which are only involved in drug metabolism, P-gp participates in the absorption, distribution, and elimination phases with regard to the pharmacokinetic law (ADME: absorption, distribution, metabolism, and elimination) [34] and therefore effect the bioavailability of drugs [35]. This review briefly discusses current *in vitro* and *in vivo* P-gp analyses, while focusing on reporting the P-gp substrate activity of clinically relevant mu opioid analgesics. The importance of opioid and P-gp interactions at the BBB and the GI will be discussed along with future research directions.

Assessment of P-gp function: *In vitro* and *in vivo* systems

Various *in vitro* and *in vivo* techniques have been developed in order to evaluate the correlation between test compounds and P-gp. It is readily understood that a combination of techniques should be employed in order to fully delineate the effects of a compound. A brief description of available techniques used for P-gp and opioid analysis follows along with references to current literature for a more thorough explanation of each technique including their advantages and disadvantages.

Currently, three different categories exist for *in vitro* methods which evaluate drug efflux transporter activity; a) accumulation/efflux, b) transport studies, and c) ATPase activity studies. Accumulation/efflux studies are performed using cell suspensions, cell monolayers, or membrane vesicle preparations in which the uptake of a probe, typically either a fluorescent or radiolabeled compound, is examined under controlled conditions in the presence of a P-gp inhibitor. Transfected or drug induced cells which overexpress P-gp are also used and the accumulation studies are compared to the wild-type (WT) or parental cell line [36]. P-gp transport studies are performed using confluent cell monolayers in which the test compound is applied to either the apical or basolateral side of the cell and the resulting flux of the compound across the confluent cell monolayer is measured. Examples of cell lines used in P-gp transport studies include CaCo-2 [37], LLC-PK1 [38] and MDR1 transfected MDCK [39] cells. Lastly, ATPase activity studies monitor the stimulation of ATPase activity in cell membrane preparations or purified membrane proteins in order to identify compounds which increase ATPase activity over basal ATPase activity. The Promega® P-gp-GLO kit [27] is an example of an ATPase activity study. Further details and limitations of the *in vitro* techniques can be found in the following literature [36, 40].

In vitro assays are useful in the characterization of interactions between compounds and P-gp, however, the ultimate determination of the impact of P-gp on drug absorption,

distribution, and elimination requires *in vivo* examination. Current *in vivo* P-gp techniques include the use of transgenic (genetically engineered) and mutant (naturally P-gp deficient) animal models, as well as P-gp anti-sense and inhibitors. A popular transgenic animal model is the P-gp knock-out (KO) animal, *mdr1a/b* (–/–), in which both rodent drug-transporting P-gp genes are deleted; due to the fact that the rodent *mdr1a* and *mdr1b* genes perform the same function as the MDR1 P-gp gene in humans [41, 42]. One naturally occurring mutant animal model exists; CF-1 is a mouse model deficient of P-gp [43]. Lastly, P-gp anti-sense [44] and inhibitors [36], such as GF120918 and PSC833, have also been used to evaluate interactions between opioids and P-gp in WT [*mdr1a/b* (+/+)] animals by effectively blocking the protein translation or transporter action, respectively. Further details and limitations of the *in vivo* techniques can be found in the following literature [29, 36].

Opioids and P-gp

Callahan and Riordan first discovered a correlation between synthetic and natural opioids with P-gp in MDR cells in 1993 [45]. Since then, many opioids have been identified as P-gp substrates using *in vitro* and *in vivo* techniques as previously described. In general, for a compound to be considered a P-gp substrate, it should exhibit one or more of the following characteristics [46]: a) have an efflux ratio more than 1.5 that can be decreased to 1 by P-gp inhibitors [40], b) show significantly higher accumulation in brain or other tissues of *mdr1a/b* (–/–) mice in comparison to *mdr1a/b* (+/+) WT mice [44, 47, 48], or c) results in up-regulation of P-gp upon multiple administration [49, 50]. We hypothesize that P-gp contributes to analgesic tolerance through a systems-level approach; as P-gp is located in both the BBB and the GI tract, contributing to central and peripheral tolerance, respectively. Opioids investigated within this review include morphine, methadone, loperamide, meperidine, oxycodone, and fentanyl (Figure 1).

Morphine

The most thoroughly investigated interaction between opioids and P-gp is that involving morphine, the prototypical μ opioid analgesic. Morphine was first identified as a P-gp *in vitro* substrate in cell culture systems, through the use of radiolabeled morphine across MDR cells [45, 51] and later found to be an *in vivo* P-gp substrate, as it was the first opioid analyzed in P-gp KO mice [52]. Subsequent *in vivo* studies investigated the inhibition of P-gp in rats with GF120918 followed by morphine administration, resulting in significantly elevated antinociceptive effects [53]. Later, a microdialysis study was performed using KO and WT mice to evaluate the role of P-gp in the transport of morphine across the BBB, concluding that morphine is transported across the BBB by P-gp [54]. In 2000, Aquilante *et al.* showed that chronic administration of morphine to rats resulted in decreased antinociceptive response and a 2-fold increase of brain P-gp. They hypothesized that the P-gp up-regulation enhanced morphine efflux from the brain, reducing the pharmacological activity of morphine and ultimately purported that P-gp up-regulation may be one mechanism involved in the development of morphine tolerance [49].

Various *in vivo* experiments with morphine and its metabolites, specifically morphine-6-glucuronide (M6G) [55, 56] have since been performed. The first dose response study with KO and WT mice by tail flick analysis showed that morphine antinociception was significantly increased in KO mice, specifically, the ED₅₀ for morphine was >2-fold lower in KO mice (3.8 mg/kg) compared to WT mice (8.8 mg/kg) [47]. Similarly, in the first time course study (%MPE vs. time) conducted with KO and WT mice by hot plate analysis, greater antinociceptive effects and morphine brain concentrations resulted in the KO mice [57]. Morphine was also evaluated in mice with P-gp antisense; the decreased P-gp expression resulted in significantly enhanced systemic morphine antinociception and

prevented tolerance, but diminished the antinociception of centrally administered morphine [44].

With these new findings, studies were underway to determine the extent of morphine transport out of the brain by P-gp. Morphine was found to be a weak P-gp substrate in Caco-2 [58] and L-MDR1 [59] cells with a efflux:influx ratio of 1.5. Whereas, *in situ* animal studies resulted in decreased clearance uptake (Cl_{up}) of morphine [48] and confirmed that morphine is a weak P-gp substrate with a P-gp effect of 1.24 (Table 2) [60]. Studies have also been performed in healthy human volunteer subjects, examining the CNS effects of morphine after pretreatment with quinidine, a brain and intestinal P-gp inhibitor. Human volunteers which received morphine intravenously did not experience enhanced CNS opioid effects [61] whereas, human volunteers which received morphine orally sustained increased plasma concentrations, hence a clinically relevant effect, but no influence on morphine pharmacodynamics [62]. These human results suggest that P-gp plays a role in orally administered morphine and intestinal disposition,

Most recent studies have determined the relationship between the P-gp ATPase activating effect of morphine and its P-gp dependent antinociceptive effects evaluated by dose-response and time course studies. The results between the *in vitro* and *in vivo* systems correlated well, resulting in increased ATPase activity and 2.8-fold greater antinociception in KO mice [63, 64]. Lastly, pharmacokinetic modeling has been employed and the finding show that morphine brain distribution is determined by three factors: limited passive diffusion; active efflux, reduced 42% by P-gp inhibition; and low capacity active uptake [65].

Methadone

Methadone is a synthetic opioid agonist used in the treatment of pain and/or opioid addiction. The racemic mixture is administered during treatment although the (R)-enantiomer accounts for the analgesic effects [66]. The interaction of methadone and P-gp has been studied with the use of different *in vitro* models, showing that methadone is a P-gp substrate [45, 51, 67–69] with a P-gp effect of 2.61 from *in situ* evaluation (Table 2) [60]. *In vivo* studies performed with P-gp KO mice and rats treated with a specific P-gp inhibitor have shown that the analgesic effect of methadone was greater and its brain concentrations were markedly higher when P-gp was absent or inhibited [57, 70, 71]. Investigations of the stereoselectivity of P-gp transport are currently being investigated, and results suggest only a weak stereoselectivity for P-gp transport of methadone; the (S)-enantiomer is transported 10% more than the (R)-enantiomer [72].

Loperamide

The synthetic opioid loperamide is commonly used as an over-the-counter anti-diarrheal drug due to its peripheral opioid-like effects on the GI tract leading to constipation, whereas CNS effects are not observed. The interaction of loperamide and P-gp has been studied with the use of different *in vitro* models, showing that loperamide is a P-gp substrate [45, 51]. Loperamide was found to a good P-gp substrate in L-MDR1 transport studies having an efflux:influx ratio of 10 [59], and by *in situ* animal studies resulting in a P-gp effect of 10.4 (Table 2) [60]. Furthermore, Caco-2 transport studies confirm that loperamide is a good P-gp substrate, however diphenoxylate, a loperamide analog used as an anti-diarrheal agent, is not a P-gp substrate by *in vitro* transport studies [73]. Interestingly, when loperamide was administered to KO mice, they displayed opioid-mediated CNS effects and accumulated 13-fold higher levels of radioactivity in the brain and 2-fold higher levels of radioactivity in the plasma compared to WT mice [52]. These results suggest that loperamide would be a

centrally active opioid without P-gp in the BBB and would therefore not be obtained as an over-the-counter drug.

Meperidine

Meperidine is a moderately potent, short acting μ opioid analgesic and was found to be a P-gp substrate in various *in vitro* cell culture systems [45, 51]. Interestingly, the first *in vivo* experiment with meperidine using KO mice in a time course experiment (%MPE vs. time) resulted in the finding that antinociceptive effects were not greater in KO mice compared to WT mice [57]. Further *in situ* perfusion studies in KO mice by Dagenais *et al.* confirmed the previous finding, where meperidine was determined to have a P-gp effect of 0.98 (Table 2) [60]. Furthermore, meperidine was found to not increase ATPase activity *in vitro* and the antinociceptive effects were the same in KO and WT mice in both time course (%MPE vs. time) and dose-response *in vivo* studies [63]. These key experiments led to the finding that meperidine is not a P-gp substrate *in vivo*, although it is a P-gp substrate *in vitro*.

Oxycodone

Another commonly used opioid, oxycodone, has only recently been studied for its interactions with P-gp. Initial *in vivo* studies in which rats were preadministered PSC833 and then treated with oxycodone indicated that coadministration of the inhibitor and oxycodone did not alter the plasma pharmacokinetics, brain concentrations, or the associated tail flick latency of oxycodone, suggesting that oxycodone may not be a P-gp substrate in rats [74]. Whereas, hydrocodone, an oxycodone analog was reported to be a P-gp substrate in WT animals using P-gp inhibitors [75]. However, a more recent publication reported a Caco-2 transport efflux ratio of 2.06 and that P-gp was up-regulated in oxycodone tolerant mice (5 mg/kg oxycodone, i.p., 2x daily for 8 days) [46]. These studies conclude that oxycodone is a P-gp substrate *in vivo* [46]. Additionally, the oxycodone induced P-gp up-regulation had a true physiological effect as it was found to effect paclitaxel tissue distribution definitely influencing the pharmacokinetic parameters [46].

Fentanyl

Fentanyl and its analogs alfentanil and sufentanil are potent short acting synthetic opioid analgesics. *In vivo* analysis of fentanyl in KO animals resulted in 2-fold antinociceptive effects in KO versus WT mice [57]. In L-MDR1 *in vitro* cell transport studies, fentanyl, alfentanil and sufentanil did not behave as P-gp substrates [59]. However, fentanyl was shown to be a P-gp substrate by *in situ* perfusion in KO mice having a P-gp effect of 1.24 (Table 2) [60] and alfentanil was shown to be an *in vivo* P-gp substrate using KO animals [76]. Most recent studies have determined the relationship between the P-gp ATPase activating effect of fentanyl and its P-gp dependent antinociceptive effects evaluated by dose-response and time course studies. The results between the *in vitro* and *in vivo* systems correlated well, resulting in increased ATPase activity and 2.2-fold greater antinociception in KO versus WT animals [63]. Lastly, human studies with quinidine, an *in vivo* inhibitor for intestinal and brain P-gp, have also been performed resulting in increased oral fentanyl absorption, suggesting that P-gp plays a role in the intestinal disposition of fentanyl, whereas the role of P-gp in brain fentanyl access requires further investigation [77].

Development of opioid analgesics lacking P-gp substrate activity

The data reported here leads to the generalized conclusion that the up-regulation of P-gp contributes to the development of opioid tolerance, specifically in morphine [49] and oxycodone [46] tolerant animals resulting in less opioid in the general circulation. Recent studies by our group have investigated the P-gp effects of various opioid analogs in order to ultimately develop a clinically useful opioid analgesic which does not exhibit P-gp substrate

activity. Meperidine was primarily chosen as a lead compound due to the fact that it is not an *in vivo* P-gp substrate. Initial studies investigated the effects of *N*-substitution on meperidine and results showed that *N*-phenylbutylnormeperidine was not a P-gp substrate *in vitro* [78]. Additionally, this compound was previously reported as twice the potency of meperidine [79] making it a good lead compound to test our hypothesis that opioids which lack P-gp substrate activity would not induce opioid tolerance. A series of 3- and 6-desoxymorphine analogs was also investigated, resulting in the finding that removal of the 3- and/or 6-OH group generally decreased *in vitro* P-gp substrate activity; 6-desoxymorphine was chosen as the lead compound from this series since it has about 10x the potency of morphine and it lacked *in vitro* P-gp substrate activity [80]. The *N*-phenylbutylnormeperidine and the 6-desoxymorphine synthetic analogs were further evaluated in KO and WT animals. Both analogs were not P-gp substrates *in vivo*, however the *N*-phenylbutylnormeperidine analog was not twice as potent as meperidine as previously reported and eventually led to toxicity issues and subsequent discontinuation of further studies [81]. Lastly, the effects of *m*-OH addition to meperidine was investigated and the results were consistent with the morphine analog series; the *m*-OH addition dramatically increased the P-gp substrate activity [82].

Conclusion

The development of opioids which lack P-gp substrate activity both *in vitro* and *in vivo* are necessary for the development of an opioid and P-gp structure activity relationships as well as development of a quantitative structure activity relationship (QSAR). Further investigation will lead to an optimal opioid analgesic lacking this systems-level contribution to tolerance development and allow for delineation of the mechanism responsible for opioid-related P-gp up-regulation. Analgesics lacking P-gp substrate activity will provide further support for evidence based medicine supporting clinical opioid rotation.

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Abbreviations

NSAIDs	non-steroidal anti-inflammatory agents
NMDA	N-methyl-D-aspartate
GPCR	G-protein coupled receptor
μ	mu opioid receptor
MOR	mu opioid receptor
κ	kappa opioid receptor
KOR	kappa opioid receptor
δ	delta opioid receptor
DOR	delta opioid receptor
GI	gastrointestinal
CNS	Central Nervous System
P-gp	P-glycoprotein

BBB	Blood Brain Barrier
SAR	structure-activity relationship
CYP 3A4	cytochrome P450 3A4
Caco-2	human colon carcinoma cell line
LLC-PK1	pig kidney epithelial cells
MDCK	Manine-Darby canine kidney epithelial cells
QSAR	quantitative structure activity relationship

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Figure 1.
Structures of investigated opioids and analogs.

Table 1

Phylogenetic analysis of ABC transporters

Subfamily	Number of Members	Previous Subfamily Name
ABCA	12	ABC1
ABCB	11	MDR/TAP
ABCC	12	MRP/CFTR
ABCD	4	ALD
ABCE	1	OABP
ABCF	3	GCN20
ABCG	5	White

Information derived from references [25, 26]

Table 2Initial brain uptake clearance of opioids during *in situ* perfusion in mice

Compound	Receptor	WT Mice	KO Mice	P-gp Effect
Morphine	μ	1.04 ± 0.03	1.29 ± 0.08 ^{**}	1.24 ± 0.08
Methadone	μ	41.7 ± 5.8	109 ± 17 ^{***}	2.61 ± 0.55
Loperamide	μ	9.86 ± 1.73	103 ± 6 ^{***}	10.4 ± 1.9
Meperidine	μ	185 ± 38	180 ± 33	0.98 ± 0.27
Fentanyl	μ	184 ± 24	228 ± 9 [*]	1.24 ± 0.17

P-gp effect is defined by the ratio between the Cl_{up} in *mdr1a* (–/–) P-gp deficient (KO) and wild-type (WT) mice. Data are presented as mean ± SD of four individual experiments at a single time point or from multiple time point experiments. (N = 4 per point at three time points)

^{*} P < 0.05;

^{**} P < 0.01;

^{***} P < 0.001. Table modified from Dagenais *et al.* [60].