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Uniform assessment and ranking of opioid Mu receptor binding constants for selected opioid drugs ☆

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ABSTRACT

The safe disposal of unused opioid drugs is an area of regulatory concern. While toilet flushing is recommended for some drugs to prevent accidental exposure, there is a need for data that can support a more consistent disposal policy based on an assessment of relative risk. For drugs acting at the Mu-opioid receptor (MOR), published measurements of binding affinity (K_i) are incomplete and inconsistent due to differences in methodology and assay system, leading to a wide range of values for the same drug thus precluding a simple and meaningful relative ranking of drug potency. Experiments were conducted to obtain K_i's for 19 approved opioid drugs using a single binding assay in a cell membrane preparation expressing recombinant human MOR. The K_i values obtained ranged from 0.1380 (sufentanil) to 12.486 μM (tramadol). The drugs were separated into three categories based upon their K_i values: $K_i > 100$ nM (tramadol, codeine, meperidine, propoxyphene and pentazocine), $K_i = 1 - 100$ nM (hydrocodone, oxycodone, diphenoxylate, alfentanil, methadone, nalbuphine, fentanyl and morphine) and $K_i < 1$ nM (butorphanol, levorphanol, oxymorphone, hydromorphone, buprenorphine and sufentanil). These data add to the understanding of the pharmacology of opioid drugs and support the development of a more consistent labeling policies regarding safe disposal.

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1. Introduction

When patients have extra prescription drug products remaining at the end of a treatment regimen, there are questions regarding their proper disposal. The Food and Drug Administration (FDA) recommends that patients seeking to dispose of unneeded drugs follow recommendations in the Federal Guidelines: Proper Disposal of Prescription Drug (Office of National Drug Control Policy, 2009). While these guidelines recommend disposing of medicines in the household waste and community take back programs for the vast majority of drug products, toilet flushing is recommended as a means of disposal for a limited number of products, some of which contain opioid drugs (FDA, 2010). This method renders the opioid drug product immediately and permanently unavailable for accidental exposures, thus eliminating the risk of overdose and death from severe respiratory depression. However, the practice of toilet flushing as a disposal method has become a subject of debate due to public health concerns about pharmaceuticals in the water and the environment (Boleda et al., 2009; Postigo et al., 2008; Zuccato et al., 2008). Alternative methods for disposal of these substances that prevent accidental exposures would be welcome, such as drug take-back programs for opioid drugs.

With any drug, potential benefits are balanced against observed risks that must be determined prior to drug approval and also evaluated post-marketing. Additional information collected in post-marketing can be used to develop strategies that are needed to mitigate risks and ensure that the benefit of approved drugs continue to outweigh the known risk. Since there is extensive interest in encouraging the appropriate use of opioid drugs to treat pain

Abbreviations: MOR, Mu opioid receptor; DOR, delta opioid receptor; KOR, kappa opioid receptor; DAMGO, (D-Ala2, *N*-MePhe4, Gly-ol)-enkephalin; GPCR, G-protein coupled receptors; FDA, Food and Drug Administration; K_i, equilibrium dissociation constant for the test compound; K_d, equilibrium dissociation constant for the ligand; B_{max}, total number of receptors in the membranes; IC_{50}, drug concentration resulting in 50% of the maximal radioligand binding to receptor; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethane-sulfonic acid; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; PEI, polyethyleneimine; IM, intramuscular; logP, octanol:water partition coefficient; GTPγS, guanosine-5′-*O*-[γ-thio(triphosphate)].

☆ *Disclaimer:* The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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and in minimizing their misuse and abuse, the FDA continues to work to understand their pharmacology as well as their patterns of use.

Opioid drugs elicit their pharmacological effects through activation of one or more membrane-bound receptors that are part of the G coupled-protein receptor (GPCR) family. Opioid receptors have been classified as μ (MOR), κ (KOR), δ (DOR), and nociceptin (Waldhoer et al., 2004). Mu opioid receptors are responsible for supraspinal analgesia, respiratory depression, euphoria, sedation, decreased gastrointestinal motility, and physical dependence (Waldhoer et al., 2004; Gutstein and Akil, 2006; Trescott et al., 2008). The majority of the clinical opioid analgesic and anesthetic drugs have significant agonist activity at the MOR.

Competitive receptor binding studies provide a means of measuring the interaction between a given drug and its receptor (Leslie, 1987; Trescott et al., 2008). Determinations of receptor binding affinities for different families of GPCRs are subject to significant variability across laboratories and model systems. The differences in K_i values (equilibrium dissociation constant) are due to the ligand selectivity, species/strain, tissue or cell source for the receptor, and assay methodology (e.g., pre-incubation, ligand and drug concentration) (de Jong et al., 2005; Leslie, 1987; Simantov et al., 1976; Thomasy et al., 2007; Robson et al., 1985; Selley et al., 2003; Nielsen et al., 2007; Titeler et al., 1989; Yoburn et al., 1991). As a result, available data sets are incomplete and often inconsistent due to differences in receptor source and analytical methods, which confounds comparisons of relative binding affinities within this pharmacologic class. A compendium of uniformly derived binding constants for drugs interacting with the MOR would be considered an important contribution to the basic understanding of the comparative pharmacology of this important GPCR family.

The objective of this study was to generate a single, well controlled set of MOR binding data for currently prescribed opioid drugs using a single competitive receptor binding assay in a cell membrane preparation expressing recombinant human MOR. The opioids tested included MOR agonists (alfentanil, codeine, diphenoxylate, fentanyl, hydrocodone, hydromorphone, levorphanol, meperidine, methadone, morphine, oxycodone, oxymorphone, propoxyphene, sufentanil and tramadol) and mixed agonists–antagonists (buprenorphine, butorphanol, nalbuphine, pentazocine). Naloxone, a MOR antagonist, served to monitor assay quality and reproducibility for the radioligand, DAMGO ([D-Ala2, *N*-MePhe4, Gly-ol]-enkephalin), which was chosen as it is a stable synthetic opioid peptide agonist with high MOR specificity and is routinely used in MOR binding studies.

2. Methods

2.1. Materials

Trizma-HCl, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethane-sulfonic acid (HEPES), dimethyl sulfoxide (DMSO), magnesium chloride, calcium chloride, bovine serum albumin (BSA), and polyethyleneimine (PEI) were purchased from Sigma Chemical Company (St. Louis, MO). The opioid drugs, DAMGO and naloxone were from Sigma, USP (Rockville, MD), RBI (St. Louis, MO) or Fluka (St. Louis, MO). Tramadol metabolites ±M1, +M1 and −M1 were from Toronto Research Chemicals (North York, Ontario, Canada). [^{3}H]-DAMGO was from Perkin Elmer (Waltham, MA). The Chemiscreen™ membrane preparation (Millipore, Billerica, MA) contained a full length OPRM1 cDNA encoding the human MOR in an adherent Chem-5 cell line. In order to avoid the adverse effect of freezing and thawing, the membranes were thawed and aliquoted into single use preparations and stored at −80 °C. Corning 3641 non-binding polystyrene 96-well plates (Corning, NY) and MultiScreen® GF/C 96-well plates with glass fiber filters (Millipore) were used in the binding assays. For measuring the bound radioligand, scintillation cocktail (Complete Counting Cocktail 3a70B™, Research Products International, Mount Prospect, IL) and glass vials (Wheaton Science Products, Millville, NJ) were utilized.

2.2. Drug stock solutions

All drugs were prepared as 10, 100 or 1000 mM stock solutions depending upon final concentrations in the competitive assays (Table 1). Drugs were resuspended at the required concentration in purified distilled water (Barnstead NANOpure, Dubuque, IA), except for those resuspended in DMSO (codeine, buprenorphine, diphenoxylate, oxymorphone and pentazocine) or methanol (butorphanol, ±*O*-desmethyltramadol (±M1), and its enantiomers +M1, and −M1).

2.3. Binding assay

The Chemiscreen™ MOR membrane preparations (Millipore, 2008) were rapidly thawed and diluted in binding buffer (50 mM HEPES, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, pH 7.4) to a concentration of 0.1 mg/mL. The radioligand and unlabeled compounds were diluted in binding buffer to achieve the desired final concentration in each well. The assays were performed in microtiter plates with 40 μL of binding buffer or unlabeled ligand, 10 μL of radioligand, and 50 μL of diluted membranes with three wells per group. The plates were then incubated at room temperature for various time points. The binding incubation was terminated by the addition of 100 μL cold binding buffer to each well. The glass fiber filter plates were presoaked for 30–45 min with 0.33% PEI buffer. The PEI solution was removed from the filter plate with a vacuum manifold (Millipore) and the filters washed with 200 μL priming buffer (50 mM HEPES, 0.5% BSA, pH 7.4) per well. The binding reaction was transferred to the filter plate and washed with 200 μL washing buffer (50 mM HEPES with 500 mM NaCl and 0.1% BSA, pH 7.4). The plate was dried and the filters removed in a cell harvester and punch assembly (MultiScreen® HTS, Millipore) for analysis in a scintillation counter (Beckman Coulter, Fullerton, CA).

2.4. Competition assays

For the competitive binding experiments, assays were conducted as above with 2 nM (^{3}H)-DAMGO and an incubation time of 2 h. The unlabeled opioid drugs were added at one third-log increments with 5 log separation between highest and lowest concentrations (Table 1). Naloxone inhibition of (^{3}H)-DAMGO binding was evaluated (0.01–1000 nM) in the same plate in separate wells to monitor assay quality and reproducibility.

Table 1

Assay concentration (nM)	Drug stock	Drugs
0.001–100	10 mM	Butorphanol, levorphanol, sufentanil
0.01–1000	10 mM	Buprenorphine, fentanyl, hydromorphone, methadone, morphine, nalbuphine, oxymorphone, ±M1, +M1
0.1–10,000	10 mM	Alfentanil, diphenoxylate
1–100,000	10 mM	Hydrocodone, oxycodone, pentazocine, propoxyphene
10–1000,000	1000 mM	Codeine, meperidine
100–10,000,000	1000 mM	Tramadol, −M1

2.5. Data analysis

The data sets were analyzed by GraphPad Prism® (version 5.02, La Jolla, CA) to calculate B_{max} and K_d values for (^{3}H)-DAMGO for one-site specific binding.

$$\text{Specific Binding} = \frac{B_{max} \times [L]}{K_d + [L]}$$

where [L] is the concentration of free radioligand ((^{3}H)-DAMGO), B_{max} is the total number of receptors (pmol/mg protein) and K_d is the equilibrium dissociation constant (nM).

For the competitive binding experiments with the opioid drugs, the K_i value was calculated from the IC_{50} value by GraphPad Prism®, using the equation of Cheng and Prusoff (1973):

$$K_i = \frac{IC_{50}}{1 + [L]/K_d}$$

where [L] is the concentration of (^{3}H)-DAMGO, K_d is the equilibrium dissociation constant for DAMGO, and IC_{50} is the concentration of opioid that results in 50% of maximal activity.

3. Results

3.1. DAMGO and naloxone

Based upon preliminary experiments with the Chemiscreen™ human MOR membrane preparations (data not shown) with (^{3}H)-DAMGO, it was determined that a 2 h incubation would allow the system to achieve equilibrium for ligand binding to the receptors. The B_{max} for DAMGO was 1.59 ± 0.035 pmol/mg protein and the K_d was 0.6887 ± 0.06157 nM (mean ± SE, R^2 = 0.9937) (Fig. 1). A concentration representative of 50% the B_{max} value equating to approximately 2 nM (^{3}H)-DAMGO for the competitive binding assays was selected. Naloxone was evaluated along with each of the opioid drugs (n = 19) and its IC_{50} and K_i values were 5.926 ± 0.253 nM and 1.518 ± 0.065 nM, respectively, with R^2 values greater than 0.97. A representative data set for a naloxone experiment is shown in Fig. 2.

3.2. Competitive assays

The competitive assays with the opioid drugs demonstrate their range of binding affinity for the human MOR (Fig. 3). Inhibitor concentrations in the assays ranged from 10^{-3} to 10^{7} nM for the drugs

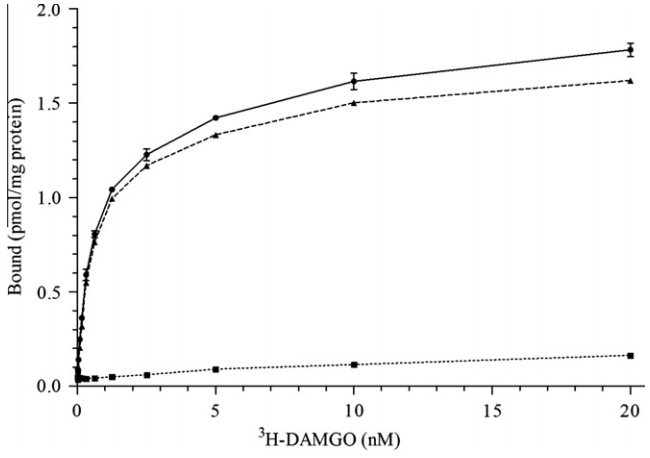

Fig. 1. Binding curve for DAMGO showing total (●—●), non-specific (■···■) and specific binding (▲—–▲). Mean ± SE of three wells. Incubation was for 2 h with 25 μM cold DAMGO.

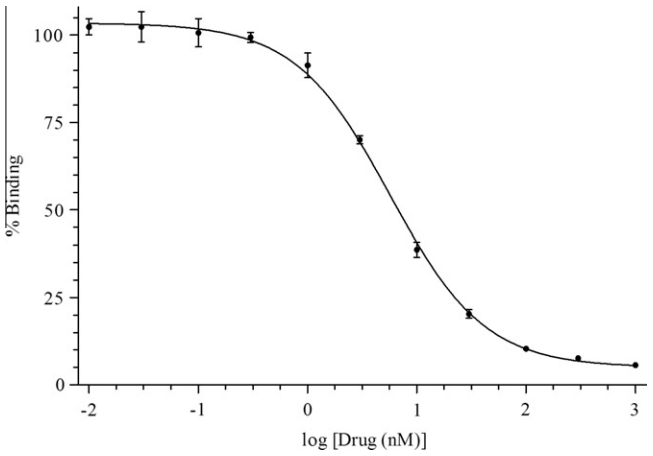

Fig. 2. Representative binding curve for naloxone (R^2 = 0.9922). Symbols represent mean ± SE of three wells. Solid line is the nonlinear fit of the binding data.

reflecting the variable affinity of these clinically relevant drugs to the MOR. Calculation of the K_i values for the drugs allowed for the ranking of the opioid drugs based upon binding affinity (Table 2). The opioid drugs separated into three categories based upon binding affinity as measured by K_i (R^2 > 0.98): K_i > 100 nM (tramadol, codeine, meperidine, propoxyphene and pentazocine), K_i = 1–100 nM (hydrocodone, oxycodone, diphenoxylate, methadone, nalbuphine, fentanyl and morphine), and K_i < 1 nM (butorphanol, alfentanil, levorphanol, oxymorphone, hydromorphone, buprenorphine and sufentanil).

Tramadol is a racemic mixture of (+) and (−) enantiomers which undergoes N- and O-demethylation. The ±M1, +M1 and −M1 metabolites of tramadol (K_i = 12.486 μM) were also evaluated in the competitive assays since +M1 has a higher affinity for the MOR than tramadol in receptor binding assays (Gillen et al., 2000). The metabolites' K_i values were significantly lower than that of the parent drug with 3.359 nM for +M1, 18.59 nM for ±M1, and 674.3 nM for −M1. This confirms that the metabolites of tramadol have a greater affinity for the MOR than the parent compound.

4. Discussion

Binding affinity is a widely used measure of a drug's relative potency. However, published data for MOR binding affinity of clinically relevant opioid drugs are incomplete and often inconsistent, precluding the systematic ranking of binding affinity to this

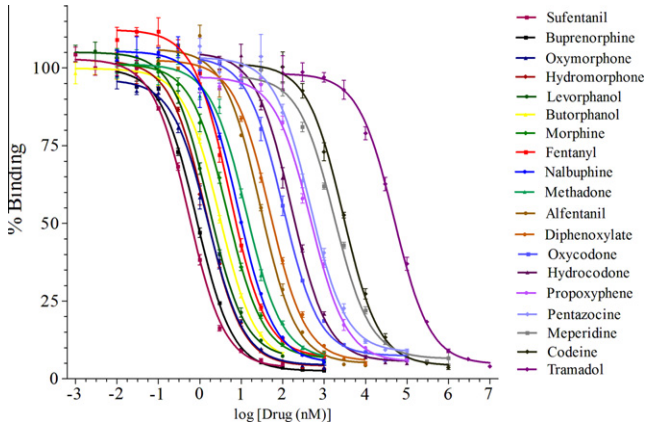

Fig. 3. Competitive binding data for opioid drugs. Symbols represent mean ± SE of three wells. Solid line is the nonlinear fit of the binding data.

Table 2

Drug	K_i (nM)	Drug	K_i (nM)	Drug	K_i (nM)
Tramadol	12,486	Hydrocodone	41.58	Butorphanol	0.7622
Codeine	734.2	Oxycodone	25.87	Levorphanol	0.4194
Meperidine	450.1	Diphenoxylate	12.37	Oxymorphone	0.4055
Propoxyphene	120.2	Alfentanil	7.391	Hydromorphone	0.3654
Pentazocine	117.8	Methadone	3.378	Buprenorphine	0.2157
		Nalbuphine	2.118	Sufentanil	0.1380
		Fentanyl	1.346		
		Morphine	1.168		

receptor. A review of the literature shows that membrane preparations ranged from brain homogenates from multiple species, human neuronal cell lines, and cell lines transfected with human, rat or mouse MOR. Ranges of K_i values were as much as 10- to 100,000-fold different for some drugs (Fig. 4). For example, literature K_i values for the widely used reference drug morphine ranged from 0.26 (Chen et al., 1993) to 611 nM (Brasel et al., 2008). The range for fentanyl was even more dramatic, from 0.007 to 214 nM (Chen et al., 1993; Traynor and Nahorski, 1995). Variability in the measured K_i values can be due to the radioligand, tissue source, animal species and strain, and assay methodology. Numerous articles have shown that the radioligand used in the competitive binding assays can result in different K_i values for the same drug (Spetea et al., 2003; Chen et al., 1993; Emmerson et al., 1996; Toll et al., 1998; Ilien et al., 1988; Childers et al., 1979; Nielsen et al., 2007).

Because of the variability of the reported binding affinity data for narcotic drugs, our study was designed to develop a compendium of uniformly derived binding constants using commercially available cell membranes expressing human MOR. The results of the assays allowed for the ranking of the opioid drugs based upon binding affinity measured as K_i values from micromolar to nanomolar values. The ranking was similar to a smaller set presented by Chen et al. (1991) in rat brain homogenates with (^{3}H)-DAMGO as the radioligand. With only a two exceptions (fentanyl, hydromorphone), the binding affinity for 13 drugs ranked similarly to their intramuscular equianalgesic dose (Inturrisi, 2002).

K_i values have been found to correlate with *in vitro* measurements of potency and efficacy. Lalovic et al. (2006) found that oxycodone and its metabolites, oxymorphone and its metabolite, morphine and DAMGO exhibited the same rank order of potency for the activation of [^{35}S]-guanosine-5′-*O*-[γ-thio(triphosphate) ([^{35}S]-GTPγS) binding to CHO cell membrane expressing human μ-opioid receptor (EC_{50}) as the receptor binding affinity constant (K_i). Similarly, Alt et al. (1998) compared the binding affinity of endogenous opioids (enkephalins, endorphins and endomorphins) and exogenous drugs (sufentanil, morphine and meperidine) closely matched (R^2 = 0.972) the potency (EC_{50} value) determined in the [^{35}S]GTPγS binding assay. Kalvass et al. (2007) found that the *in vitro* K_i of seven opioids and their GTPγS EC_{50} values were strongly correlated (R^2 = 0.9). The *in vitro–in vivo* correlation using K_i was stronger than the corresponding correlation using GTPγS EC_{50}, with the strongest between K_i and unbound brain $EC_{50,u}$ (R^2 = 0.7992) used as a measure to express opioid potency (Kalvass et al., 2007).

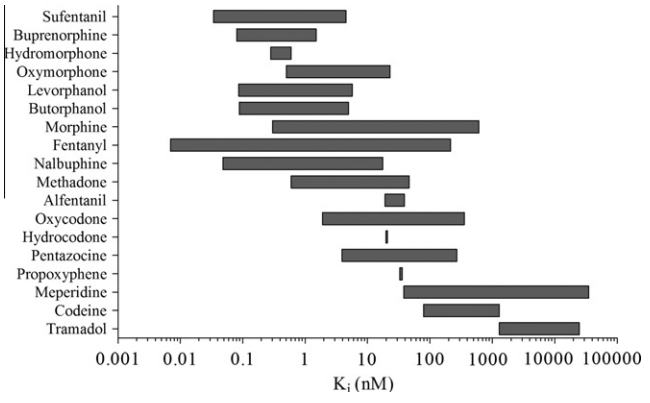

Fig. 4. Range of literature K_i values for opioid drugs in MOR inhibition assays. (Alt et al., 1998; Bot et al., 1998; Brasel et al., 2008; Carroll et al., 1988; Chang et al., 1980; Chen et al., 1991; Chen et al., 1993; Childers et al., 1979; de Jong et al., 2005; Emmerson et al., 1996; Leysen et al., 1983; Nielsen et al., 2007; Raffa et al., 1992; Raffa et al., 1993; Raynor et al., 1994; Toll et al., 1998; Traynor and Nahorski, 1995; Tzschentke et al., 2007; Wentland et al., 2009; Yeadon and Kitchen, 1988).

However, other factors contribute to the potencies of the opioid drugs when used clinically, including their ability to act as full or partial agonists, their secondary pharmacology, and their relative ability to partition into the brain. For example, based on the binding data alone, the affinity of fentanyl and morphine are similar. However, a typical intramuscular (IM) dose of fentanyl is 50–100 μg compared to 10 mg of IM morphine; that is, fentanyl is ~100 times more potent than morphine. The difference in potency can in part be attributed to the differential lipophilicity of these drugs. Specifically, the calculated logP (octanol:water partition coefficient) for fentanyl is 4.28 compared to morphine at 1.07 (Peckham and Traynor, 2006). As a result, compared to phenanthrene drugs (*e.g.*, morphine, oxycodone), phenypiperidine drugs (*e.g.*, alfentanil, fentanyl, sufentanil) have greater lipophilicity and rapidly cross the blood brain barrier resulting in greater analgesic potency. Likewise, the partition coefficient for hydromorphone is almost twice that of morphine (Roy and Flynn, 1988), which explains why hydromorphone is approximately 6–8 times more potent than morphine (Inturrisi, 2002) whereas the binding affinity reported here is only ~3 times greater. In another case, pentazocine and propoxyphene show similar binding affinity to the MOR (Table 2). Although the typical initial oral analgesic dose of both drugs are similar (propoxyphene is 65 mg and pentazocine is 50 mg), the oral LD_{50} for these drugs in rats differ (135 and 1110 mg/kg, respectively) by 8-fold due to the fact that pentazocine is a partial agonist at the MOR (Funderburk et al. 1969; Lewis 1996). This limits the respiratory depressant effects of pentazocine, while propoxyphene is a full agonist at the MOR and can also block sodium and potassium channels, which contributes to the potential toxicity of the compound.

In a final comparison, the greater clinical analgesic potency of oral oxycodone (1.8-fold) compared to morphine (Curtis et al. 1999) is not readily explained by either binding affinity or lipophilicity. Recent studies in rats have provided a potential explanation for this discrepancy. Boström et al. (2006) have demonstrated that the concentration of unbound oxycodone in the rat brain is 3 times higher than that of the blood at steady state. In contrast, unbound morphine in the rat brain is 2–3 times lower than that in the blood

(Boström et al. 2008), which may explain why oral oxycodone shows greater analgesic activity than oral morphine even though the affinity at the MOR is far lower, suggesting differential transport across the blood brain barrier for these two drugs. These examples clearly delineate that while relative binding affinity significantly impacts the clinical utility and safety of this class of drugs, binding affinity alone can not always be used to compare the relative safety and efficacy of drugs.

Given the above considerations, it can be argued that binding affinity (K_i) for the opioid drugs provides only a limited indication of clinical potency and risk. However, the utility of other potential metrics for comparing these drugs (*e.g.*, clinically effective plasma levels) may also be limited because of data gaps, variability in clinical response and a lack uniformity in how data were obtained. The examples of fentanyl and oxymorphone demonstrate the challenges of using labeled dosing information to rank opioid drug potency. Fentanyl is administered intramuscularly or intravenously (0.05–0.1 mg/60 kg), buccally (0.002 mg/60 kg), and transdermally (0.025 mg/60 kg). Doses for oxymorphone range from 0.5 to 20 mg/60 kg depending on whether it is administered by the subcutaneous, intramuscular, intravenous, rectal, or oral route. Reported plasma concentrations for drugs also vary greatly among patients, as well as based on dosage form and route of administration. For example, in a review of over 60 clinical studies with information on more than 2000 subjects, the maximal plasma concentration of morphine differed whether is was an immediate release (1.8–38 nM), controlled release (0.6–25 nM) or once daily form (0.4–0.7 nM) (Collins et al., 1998). For these reasons, the measurement of MOR binding affinity in a well controlled single cell-based test system, as presented in this study, can be viewed as providing a reasonable set of relative values to help inform decisions on the development of labeling recommendations for the disposal of opioid drugs.

5. Conclusions

The comparative opioid pharmacology at the MOR lies at the base of hazard knowledge for opioids and is an important part of identifying risk mitigation strategies to help support the most appropriate uses of opioids and their safe disposal. Considering the wide range of binding affinities found in the study, this information can help delineate what other factors are important in driving risk. For instance, a drug that has a high affinity for MOR with a comparatively low incidence and severity of adverse events can be compared to a drug with opposite findings to determine what factors (*e.g.*, drug formulation, labeling, packaging and disposal directions) might enhance the safe use of opioid drugs. In addition to the MOR binding data, specific recommendations for disposal may be improved by also weighing the contributions of drug pharmacokinetics, pharmacodynamics, patterns of use, emergency room admissions, and the potential for abuse. This class of drugs provides important therapeutic benefits for millions, and it is essential that FDA continues to work to understand the scientific basis for their appropriate use.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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