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Computational Systems Pharmacology-Target Mapping for Fentanyl-Laced Cocaine Overdose

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Abstract

The United States of America is fighting against one of its worst-ever drug crises. Over 900 people a week die from opioid- or heroin-related overdoses, while millions more suffer from opioid prescription addiction. Recently, drug overdoses caused by fentanyl-laced cocaine specifically are on the rise. Due to drug synergy and an increase in side effects, polydrug addiction can cause more risk than addiction to a single drug. In the present work, we systematically analyzed the overdose and addiction mechanism of cocaine and fentanyl. First, we applied our established chemogenomics knowledgebase and machine-learning-based methods to map out the potential and known proteins, transporters, and metabolic enzymes and the potential therapeutic target(s) for cocaine and fentanyl. Sequentially, we looked into the detail of (1) the addiction to cocaine and fentanyl by binding to the dopamine transporter and the μ opioid receptor (DAT and μ OR, respectively), (2) the potential drug–drug interaction of cocaine and fentanyl via p-glycoprotein (P-gp) efflux, (3) the metabolism of cocaine and fentanyl in CYP3A4, and (4) the physiologically based pharmacokinetic (PBPK) model for two drugs and their drug–drug interaction at the absorption, distribution, metabolism, and excretion (ADME) level. Finally, we looked into the detail of JWH133, an agonist of cannabinoid 2-receptor (CB2) with potential as a therapy for cocaine and fentanyl overdose. All these results provide a better understanding of fentanyl and cocaine polydrug addiction and future drug abuse prevention.

Graphical Abstract

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Author Contributions

Z.F. and X.-Q.X. designed the project; J.C., S.W., W.L., N.W., Y.W., M.C., and Z.F. performed computational work, analyzed the data, and wrote the manuscript. All the authors reviewed the manuscript.

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Keywords

fentanyl; cocaine; fentanyl-laced cocaine; overdose; polydrug addiction; computational systems pharmacology-target mapping

INTRODUCTION

Overdose deaths pose a severe health issue in the United States. The increasing opioid overdose epidemic includes not only the most commonly prescribed opiates like oxycodone, hydrocodone, and tramadol but also synthetic opioids (primarily fentanyl). Due to the difficulty of distinguishing from other drugs, synthetic opioids are often mixed with cocaine, heroin, methamphetamine, and counterfeit pills to increase their euphoric effects. Among drug overdose deaths caused by synthetic opioids in 2016, 79.7% involved the use of at least one other substance (or polydrug addiction), leading to an increase in risk over being addicted to a single drug, which can be attributed to drug synergy and an increase in side effects. “The most common co-involved substances are other synthetic opioids (~47.9%), heroin (~29.8%), cocaine (~21.6%), prescription opioids (~20.9%), benzodiazepines (~17.0%), alcohol (~11.1%), psychostimulants (~5.4%), and antidepressants (~5.2%).”¹

Fentanyl, a synthetic opioid pain reliever clinically used for anesthesia, is 50 to 100 times the potency of morphine and heroin. It is classified as a Schedule II drug by the Drug Enforcement Agency (DEA). It acts as a selective agonist of the μ opioid receptor (μ OR) in the central nervous system (CNS) by mimicking endogenous opiates. The study conducted by Yoshida et al. found that the activation of μ OR by fentanyl can result in the increased release of dopamine in freely moving rats.² Among other effects, fentanyl often causes difficulty breathing, constipation, and hallucinations. Fentanyl is associated with an increased risk of hypotension, serotonin syndrome, and adrenal insufficiency. The onset of action of fentanyl is just 5 min; in addition, less than 2 mg of fentanyl can cause overdose and even death. Moreover, fentanyl can be manufactured illicitly at very low cost, and it can also be prescribed in the form of transdermal patches or lozenges, which leads to its abuse in the United States. In 2017, fentanyl and its analogs have caused over 29 000 deaths in the

U.S. (<https://www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates>); the number of overdose death showed a greatly increasing trend over the past 4 years.

Cocaine, a benzoic acid stimulant, was used as a local anesthetic until it was discontinued for being highly addictive. Cocaine is also classified as a Schedule II drug by the DEA. It binds to transport proteins (e.g., dopamine transporter, DAT) for neurotransmitters such as dopamine, serotonin, and norepinephrine, inhibiting the reuptake of these neurotransmitters into presynaptic neurons. As a result, it may increase postsynaptic receptor activation, causing them to accumulate in the presynaptic cleft. This can lead to some of cocaine's well-known effects such as agitation and temporary happiness. "Addiction to cocaine occurs through inhibition of monoamine reuptake and/or allosteric dopamine D2 receptor agonism."³ Some literature reported that cocaine could inhibit the reuptake of dopamine via DAT; in addition, fentanyl has been shown to increase the expression and release of dopamine,⁴ which is convincing evidence that DAT could be the key in this overdose crisis.⁵ Cocaine also blocks voltage-gated sodium channels, which inhibits action potentials and thus conduction of nerve impulses through the CNS leading to the loss of sensation throughout the body. Cocaine is also associated with an increased risk of stroke, myocardial infarction, blood infections, and lung problems, among other conditions.

Replacing an amount of cocaine or heroin with illicit fentanyl could bring huge economic benefits in terms of low cost and easy transportation. Moreover, cocaine mixed with fentanyl will have a more potent effect than cocaine alone. Federal data (<https://www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates>) shows that fentanyl and its analogs have increasingly appeared in cocaine overdose deaths. The rise in deaths involving both cocaine and fentanyl is startling, with big implications for America's ongoing drug overdose crisis. For example, deaths involving both cocaine and opioids have more than tripled since 2010, while cocaine deaths not involving opioids have only increased by 1.5-fold.

Recently, many different chemicals have been successfully used for the *in vivo* treatment of drug addiction. For example, dopamine transporter (DAT) inhibitors, D3 receptor (D3R) antagonists, and μ OR antagonists all have been reported to be effective in the therapeutic treatment of cocaine and opioid addiction.^{6–11} Of note, D3R and μ OR belong to class A of G-protein coupled receptors (GPCRs), which is the largest family of trans-membrane proteins, targeted by 30–40% of marketed drugs.¹² Moreover, ligands of other GPCRs are also reported to show potential therapeutic effects regarding drug abuse, including muscarinic acetylcholine receptor M4,¹³ γ -amino-butyric acid type B receptor (GABAB),^{14–17} metabotropic glutamate receptor 2 (mGluR2),^{18–20} metabotropic glutamate receptor 5 (mGlu5),^{21–25} trace amine-associated receptor 1 (TAAR1),^{26–29} adenosine A2A receptor (A2aR),^{30–32} cannabinoid 1-receptor (CB1R),^{33,34} and cannabinoid 2-receptor (CB2R).^{3,35–37} Especially, CB2R, a member of GPCRs that is expressed in both peripheral tissues and brain, has been reported to produce a large therapeutic effect when treated with its agonists. For example, activating CB2R is supposed to protect against various peripheral disorders, such as atherosclerosis, renal fibrosis, and liver cirrhosis.^{38,39} When CB2R that is expressed in brain neurons is targeted, it can be a potential strategy in medication development for the potential treatment of pain, neuroinflammation, neurodegenerative diseases, and drug abuse.^{39–42} This assertion is supported by the fact that activation of

CB2Rs by JWH133 and other CB2R agonists can directly inhibit these neurons and decrease NAc dopamine release.^{35,36,43–45} Recently, Adamczyk et al.³ reported that “systemic administration of the CB2 antagonist SR144528 did not show any effect on intravenous cocaine self-administration but attenuated cocaine-induced relapse of drug-seeking behavior in rats, demonstrating the therapeutic effects of CB2 antagonist on DA.”

In the present work, we focused on the targets of both cocaine and fentanyl to explore the mechanism behind the increase in deaths when combining cocaine and fentanyl from a systems pharmacology and pharmacometrics point of view. We proposed potential targets, like CB2R, as effective pharmacotherapy for the cocaine–fentanyl dual agent addiction issue.

RESULTS AND DISCUSSION

Overview of Target Proteins, Transporters, Metabolic Enzymes, and Potential Therapeutic Targets for Cocaine and Fentanyl by Computational Systems Pharmacology-Target Mapping.

As shown in Figure 1, we first conducted computational systems pharmacology-target mapping for cocaine and fentanyl. The green circles and solid lines represent the known targets and interaction for the drugs, while the purple circles and dashed lines represent the predicted targets and interaction. In addition, the red circle and solid line represent the known therapeutic target and reported therapeutic effect, while the red dashed line represents the predicted therapeutic effect. In addition, the orange solid line represents a known target that has an indirect effect.

As shown in Figure 1, we found that both cocaine and fentanyl could bind to several known target proteins by our computational systems pharmacology-target mapping analysis, including cytochrome P450 3A4 (CYP3A4), P-glycoprotein 1 (P-gp), dopamine transporter (DAT), muscarinic acetylcholine receptor M1 (CHRM1), and muscarinic acetylcholine receptor M3 (CHRM3).

First, cocaine is mainly metabolized by cholinesterase enzymes that are primarily distributed in the liver and plasma but can also be metabolized via CYP3A4 into a minor metabolite, norcocaine.⁴⁶ CYP3A4 is an important hepatic metabolic enzyme and its inhibitors and inducers have been reported to modulate cocaine toxicity.⁴⁷ Moreover, some studies suggest that cocaine can be transported by P-gp.^{48,49} P-gp or multidrug resistance protein 1 (MDR1) is an important transmembrane protein that exports many foreign compounds or substances out of cells. Importantly, according to some reported animal experiments, fentanyl inhibits both CYP3A4 activity and P-gp transport activity in mice.⁵⁰ Many research works have described that clinically relevant drug–drug interactions (DDIs) can be observed at the metabolism level, largely affected by P-gp and CYP3A4.⁵¹ Therefore, coadministration of cocaine and fentanyl could decrease the metabolism of the cocaine thus increase its adverse effects: (1) cocaine addiction is mainly attributed to the increased release and accumulation of dopamine in the brain, extending from the ventral tegmental area (VTA) of the midbrain to the nucleus accumbens (NAc); (2) therefore, exposure to fentanyl, which can inhibit the transport of cocaine out of the brain via P-gp, can also increase the risk of cocaine addiction.

Second, DAT is a membrane-spanning protein playing the role of recycling dopamine after being released by pumping dopamine out of the synapse back into the cytosol. Cocaine could inhibit DAT thus decreasing the reuptake and storage of dopamine, causing more dopamine to accumulate in the synapse.^{52–54} Fentanyl was reported to decrease the inhibition of the release of dopamine, serotonin, acetylcholine, and norepinephrine neurotransmitters, but there is no direct evidence showing that this effect can be contributed by the inhibition of DAT. In addition, fentanyl was shown to decrease the binding of 2- β -carbomethoxy-3- β -(4-iodophenyl)-tropane (β -CIT) to DAT in the basal ganglia of humans and rats, which suggests that fentanyl may affect DA reuptake directly.⁵

Third, it is certain that there exists a close relationship between M1, M2, and M3 receptors and cocaine and opioid addiction. M1 receptor knockout rat models showed decreased behavioral effects of cocaine and morphine.^{55,56} Selective blockades of M2 receptors can attenuate cocaine self-administration in rats.⁵⁷ Moreover, there are some pieces of evidence showing that fentanyl could bind with muscarinic receptors in rat brain.⁵⁸

In addition, fentanyl and cocaine were predicted to bind to their own specific targets. For example, fentanyl was predicted to target three known opioid receptors, including δ OR (δ opioid receptor), μ OR, and κ OR (κ -opioid receptor).⁵⁹ Cocaine showed potential binding to serotonin (5-HT) receptor 2B and TAAR1.^{26,60,61}

Lastly, CB2Rs are reported to be expressed in mouse and rat brain VTA dopamine neurons and their ligands have shown potential therapeutic effects for drug abuse. For example, JWH133, a selective CB2 agonist, suppresses intravenous or intra-accumbens cocaine self-administration and cocaine-enhanced locomotion in mice.^{33,34} Moreover, two other CB2 agonists, AM1710⁶² and LY2828360,³⁷ have been reported to attenuate morphine tolerance and withdrawal. Since morphine and fentanyl both bind to opioid receptors and share similar pharmacological effect, we suggest that CB2 ligands may have effects on fentanyl self-administration.

Addiction Analyses of Cocaine and Fentanyl.

Cocaine is known to exert its neurological effect and addictive properties by acting as an inhibitor to the dopamine transporter (DAT). Inhibition of DAT results in the decrease in reuptake of dopamine for degradation, therefore causing an accumulation of dopamine. Moreover, fentanyl, a potent agonist of the opioid receptors, can increase the expression of dopamine. The concurrent use of cocaine and fentanyl could enhance the accumulation of dopamine, resulting in a higher risk of overdose and addiction. Aside from the ability to increase expression of dopamine, fentanyl also interacts with the μ OR, which contributes to its addictive properties. In the present study, we performed docking studies to provide insight into the interactions between ligands and targets.

As shown in Figure 2a, the orthosteric ligand-binding pocket of DAT is mainly formed by residues on the transmembrane domains (TMDs) 1, 3, 6, and 8. These key residues include Phe76, Asp79, Ser149, Val152, Tyr156, Phe320, and Ser422, which are consistent with the reported data. The binding of cocaine to DAT resulted in a conformation where the azabicyclic structure faced TMD1 and TMD6, while the benzene ring faced TMD3. Two

strong hydrogen bonds were observed, one between the nitrogen on the azabicyclic structure and one of the oxygens on Asp79 with a distance of 3.6 Å and the other between the oxygen on the ester group of cocaine and the oxygen on the phenyl ring of Tyr156 with a distance of 4.7 Å. Aside from the hydrogen bonds, several hydrophobic interactions were also observed in the binding pocket, which further secured cocaine in the binding pocket, involving Phe76 (2.9 Å), Ser149 (4.0 Å), Val152 (3.1 Å), Phe320 (4.5 Å), and Ser422 (3.8 Å).

Figure 2b illustrates the detailed binding interactions between fentanyl and μ OR (mu opioid receptor). The orthosteric binding site of μ OR is mainly composed of residues on TMDs 3, 5, 6, and 7. The key residues include Asp149, Tyr150, Trp295, Trp320, Ile324, and Tyr328. The binding of fentanyl to μ OR resulted in a conformation where the benzene ring of fentanyl faced TMD7, while the propenamide group faced TMD5. Two hydrogen bonds were observed. One was found between the nitrogen on the propenamide group of fentanyl and the oxygen on the phenyl ring of Tyr150 with a distance of 4.0 Å; another was observed between the nitrogen on the six-member ring of fentanyl and the oxygen on Asp149 with a distance of 5.3 Å. Aside from the hydrogen bonds, four hydrophobic interactions were also observed in the binding pocket, including Trp295 (3.1 Å), Trp320 (3.7 Å), Ile324 (5.7 Å), and Tyr328 (3.0 Å).

Drug–Drug Interaction of Cocaine and Fentanyl in P-Glycoprotein (P-gp).

P-gp is a part of the endogenous blood–brain barrier efflux transport system and is responsible for the transport of many substances out of the brain. So far, there are no available structure delineations for its substrate specificity, but a number of drugs have been reported to interact with this transporter. Fentanyl acts as both a substrate and an inhibitor of P-gp.⁶³ The efflux effect of P-gp is overpowered by the active inward transport of fentanyl by transports that are yet to be identified. Even though the change in P-gp does not impact the effect of fentanyl significantly, the inhibition of P-gp due to fentanyl can cause potentially detrimental events such as overdose when used with other agents. Cocaine, a potential substrate for P-gp, can be affected when used concurrently with fentanyl. In this section, molecular docking between P-gp and fentanyl as well as cocaine were performed to explore the drug interactions of these two substances.

As shown in Figure 3, the ligand-binding site of P-gp is mainly composed of Tyr271, Tyr274, Phe300, Gln632, Phe635, Tyr860, and Phe885. Interestingly, cocaine and fentanyl shared very similar binding modes, resulting in almost the same interactions. A total of two strong hydrogen bonds and five hydrophobic interactions were observed in the binding pocket with both ligands with the involvement of the same residues. During the binding of cocaine and P-gp, a hydrogen bond was observed between the ester oxygen on the methyl ester-group of cocaine and the oxygen on the amide group of Gln632 with a distance of 3.7 Å; another was observed between the nitrogen on the azabicyclic structure of cocaine and the oxygen on the phenyl ring of Tyr271 with a distance of 3.8 Å. Similarly, the oxygen on the propenamide group of fentanyl was also observed to form a hydrogen bond with the oxygen on the amide group of Gln632 with a distance of 3.5 Å; the nitrogen on the propenamide group formed a hydrogen bond with the oxygen on the phenol ring of Tyr271 as well with a distance of 4.0 Å. Hydrophobic interactions were observed between both

agents (cocaine and fentanyl) and Tyr274 (4.4 and 3.6 Å), Phe300 (3.6 and 4.0 Å), Phe635 (3.1 and 4.0 Å), Tyr860 (3.4 and 3.5 Å), as well as Phe885 (3.0 and 3.1 Å) at similar atoms.

Metabolism of Cocaine and Fentanyl in CYP3A4.

CYP3A4 is one of the major isozymes in human liver that is known to metabolize various xenobiotic and endogenous biochemicals. Both cocaine and fentanyl have been reported to undergo metabolism via CYP3A4. Cocaine, a strong psychic stimulant with addiction, has two major metabolic pathways. It could be metabolized into norcocaine primarily by CYP3A4.⁶⁴ The other one is metabolism mediated by serum and hepatic esterases, which include pseudocholinesterase, carboxylesterase-1,⁶⁵ and carboxylesterase-2. The main metabolites from this pathway are ecgonine methyl ester (29%–54%), benzoylecgonine (26%–60%) and cocaethylene. Based on the first pathway, the CYP3A4 inhibitors, such as nefazodone, ketoconazole, and erythromycin, can eliminate the metabolism of cocaine, causing an increase in toxicity. However, CYP3A4 inducers (nevirapine and rifampin) can shift the metabolism from hydroxylation to *N*-demethylation and produce high levels of toxic metabolites. In addition, CYP3A4 predominately dealkylates fentanyl into norfentanyl, a more potent active metabolite. Fentanyl can also be metabolized into active despropionyl-fentanyl via amide hydrolysis and active hydroxyfentanyl via alkyl hydroxylation. To explore the interaction of cocaine and fentanyl at the metabolic level, docking studies were performed and are reported in this section.

As shown in Figure 4, the key residues in the ligand-binding pocket of CYP3A4 included Arg105, Phe108, Phe215, Phe304, Thr309, and the heme structure. The binding pose between cocaine and CYP3A4 resulted in a conformation where the benzene ring of cocaine faced the heme structure of CYP3A4, while the azabicyclic structure faced away from the heme structure. Two strong hydrogen bonds were observed during the binding of cocaine and CYP3A4, one of which was observed between the single bond on the methyl ester structure of cocaine and the oxygen on Ser119 with a distance of 2.5 Å; another was observed between the double bonded oxygen on the benzene ester structure of cocaine and one of the terminal nitrogens on Arg105 with a distance of 3.2 Å. A total of five hydrophobic interactions were observed between cocaine and Phe108 (4.7 Å), Phe215 (3.9 Å), Phe304 (4.3 Å), Thr309 (3.6 Å), and the heme (3.1 Å). The hydrophobic interaction between the benzene ring structure and the heme structure is crucial for the metabolic mechanism of CYP3A4.

Similar to the binding pose of cocaine in CYP3A4, the binding conformation of fentanyl in CYP3A4 showed the benzene ring structure facing toward the heme structure. This allowed the close vicinity for the formation of hydrophobic interaction between the benzene ring on fentanyl and the heme structure (3.0 Å). Four additional hydrophobic interactions were also observed between fentanyl and Phe108 (3.6 Å), Phe215 (3.2 Å), Phe304 (3.9 Å), and Thr309 (3.6 Å). Two hydrogen bonds were found; one was between the nitrogen in the six-membered ring of fentanyl and the oxygen on Ser119 with a binding distance of 4.3 Å, and the other was observed between the oxygen on fentanyl and one of the terminal nitrogens on the Arg105 with a binding distance of 3.2 Å.

Virtual Drug–Drug Interaction (DDI) Studies between Cocaine and Fentanyl.

In order to better understand the quantitative changes in the two drugs' metabolism when used simultaneously, we built a physiologically based pharmacokinetic (PBPK) model for both drugs to see their drug–drug interactions. The profile of simulated systemic concentration in plasma of cocaine over time after a single 20.5 mg iv bolus dose in healthy volunteers, using the optimized cocaine PBPK model, generated a consistent representation of the observed plasma concentration–time profiles⁶⁶ (Figure 5a). The simulated systemic concentration in plasma over time profile produced by fentanyl PBPK model after a single 7.0 mg iv bolus dose in healthy volunteers was also consistent with the observed plasma concentration–time⁶⁷ (Figure 5b). Our compound models were further validated by other clinical pharmacokinetic data after a single dose. For cocaine, median values of predicted AUC over the dosing time (AUC) were within 2-fold of the observed values (22200 vs 14640 ng/(mL·min);⁶⁸ 24520.2 vs 28628 ng/(mL·min)⁶⁹), meanwhile, the AUC difference between the prediction of our fentanyl model and the observed data is also acceptable (73.2 vs 48.5 ng/(mL·min)⁷⁰). In general, the simulation predicted by our models is similar to clinical data, which supports the further utilization of our models for the DDI studies at PBPK level.

Virtual DDI studies between cocaine and fentanyl were conducted using the cocaine or fentanyl optimized PBPK models with inhibitors as fentanyl or cocaine, respectively. Considering that the objects of our study are drug addicts, who have a high possibility of developing drug tolerance, approximate lethal dose was applied for all the DDI simulations (cocaine 96 mg/kg; fentanyl 2 mg).

Plasma cocaine concentration was simulated with and without the presence of fentanyl. The predicted profiles and AUC of cocaine are very similar (Figure 6a,b), which indicated that the fentanyl, in our case, may impact less on the systemic concentration in plasma of cocaine. Nevertheless, these results are reasonable since not only is the proportion of fentanyl extremely low compared with cocaine in the polydrug formulation, but also the cocaine is mainly metabolized by cholinesterase enzymes, which are unaffected by fentanyl.

Next, we study the role of fentanyl in the death from polydrug overdose. After running the simulation of fentanyl with or without cocaine as inhibitor, we found out that the profile of fentanyl with cocaine is obviously higher among the whole absorption, distribution, metabolism, and excretion (ADME) process, and the AUC ratio is 1.39 (Figure 6c,d), which indicated that fentanyl rests longer in the organism in combination use compared with being used alone. In addition, the concentrations of both the cocaine and fentanyl in the brain are positively corrected with their plasma concentration,⁷¹ combining their potential inhibitory effect on P-gp, which reduces the efflux of fentanyl; this combined use may provoke a cascade of drug accumulation in the brain. If the dosage is not adjusted, drug addicts bear a greater risk of overdose. In addition, the lethal dose of fentanyl is very low (from 3 to 58 $\mu\text{g/L}$); once the accumulation occurs in the organism, it may endanger life.

CB2 Agonists, Potential Therapy for Cocaine and Fentanyl.

When it comes to the therapy for cocaine and fentanyl addiction, the blockade of μ OR should be the first choice for fentanyl addiction therapy because of the direct effect targeting three known opioid receptors (μ OR, σ OR, and κ OR).⁵⁹ The D3R is also a potential therapeutic target for cocaine addiction.^{72–74} Xi et al. reported that acute administration of D3R selective antagonist SB-277011A can decrease conditioned place preference induced by cocaine or heroin in the rat model.⁷³ However, considering its short half-life, clinical research regarding SB-277011A has been terminated. NGB 2904, a novel D3-selective antagonist, significantly reduced cocaine self-administration under progressive-ratio (PR) reinforcement.⁷⁴ Recently, CB2 agonist treatment provides a potential therapy for drug abuse due to promising efficacies and minimal side effects. For example, JWH133 (a selective CB2 agonist) can suppress intravenous or intra-accumbens cocaine self-administration and cocaine-enhanced locomotion in mice.^{33,34} Moreover, Xi et al.⁷⁵ have reported that “intraperitoneal administration of JWH133 (10 or 20 mg/kg/body weight) can produce a significant and dose-dependent reduction in cocaine self-administration and cocaine intake in mice.” As mentioned previously, AM1710⁶² and LY2828360,³⁷ two other selective CB2 agonists, have been successfully used to attenuate morphine tolerance and withdrawal. In terms of the toxicity, only a limited number of side effects are reported about CB2 agonism in animal models. However, the CB2 selective agonists may produce mild psychotropic side effects following long-term treatment since CB2 selective agonists still have some affinity on CB1.^{42,76} Owing to the lack of clinical study on CB2 agonist treatment, further side effects are still unclear. Recently, the crystal structure of CB2 bound with AM10257 (antagonist, PDB 5ZTY, resolution 2.8 Å)⁷⁷ was released, which provides a structural basis for rational drug development toward CB2. In our present work, we docked JWH133 into the crystal structure of CB2 to explore its binding interactions.

The binding pocket of CB2 is shown in Figure 7. The important residues in CB2 orthosteric binding pocket included Phe87, Phe94 (not shown in the figure), Ile110, Val113, Thr114 (not shown in the figure), Phe117, Phe183, Trp194, Trp258, Phe281, and Ser285. Based on the binding results, we found that the residues in the binding pocket of CB2 formed strong hydrophobic interactions with JWH133, including Phe87 (3.3 Å), Phe94 (3.4 Å, not shown in the figure), Ile110 (4.0 Å), Val113 (4.2 Å), Thr114 (3.4 Å, not shown in the figure), Phe117 (3.3 Å), Phe183 (3.4 Å), Trp194 (3.8 Å), and Phe281 (4.2 Å). All these residues are consistent with the structural findings of CB2–AM10257 complex.

In order to further explore the role of these binding residues, we carried out a 100 ns molecular dynamics (MD) simulation for the complex of CB2–JWH133. As shown in Figure 8a, the root mean squared deviation (RMSD) of the CB2 and JWH133 fluctuated around 2.5 (black line) and 1.5 Å (red line) from 20 to 100 ns, indicating the system remained stable during the MD simulation. Sequentially, 500 snapshots were extracted from 50 to 100 ns to calculate the mean residual binding energy, as shown in Table 1 and Figure 8b. According to the binding energy, we found that several key hydrophobic residues contributed greatly to the binding of JWH133 in CB2 via the total energy contribution (green line in Figure 8b), including Phe87 (–2.96 kcal/mol), Phe94 (–2.19 kcal/mol), Ile110 (–3.04 kcal/mol), Val113 (–3.21 kcal/mol), Thr114 (–2.22 kcal/mol), Phe183 (–3.25 kcal/

mol), Trp194 (−2.63 kcal/mol), and Phe281 (−1.98 kcal/mol) (Table 1 and Figure 8b). All our MD results were consistent with the docking results.

CONCLUSION

The recreational use of opioids has been a growing problem in the United States and has contributed to numerous cases of overdose death in the past decades. Fentanyl is one of the most potent opioid substances and has been found to be used as an additive to recreational drugs such as cocaine and heroin to increase their hallucinogen effect in recent years. This phenomenon poses a serious problem on top of the opioid epidemic since the use of only a small amount of fentanyl is required to cause detrimental symptoms such as respiratory depression. In addition, the concordant use of fentanyl and other substance can potentially increase the risk of overdose and other adverse events. In the present study, we carried out computational systems pharmacology-target mapping, molecular docking, PBPK modeling, and MD simulation to systematically analyze the potential mechanism of fentanyl/cocaine polydrug addiction. Additionally, JWH133, an agonist of CB2, can be used as a treatment for cocaine and morphine overdose. Based on our studies, we suggest that CB2 agonist(s), such as JWH133, may have potential therapeutic effect for both cocaine and fentanyl addiction.

MATERIALS AND METHODS

Genes and Proteins.

Genes or proteins that associated with the specific disease (e.g., drug abuse) were retrieved from public databases to construct our domain-specific knowledgebase, including Ensembl,⁷⁸ UniProt,⁷⁹ KEGG,⁸⁰ GPCRdb,⁸¹ and NCBI Protein Database.⁸² Reported cryo-EM structures and crystal structures of target proteins were downloaded from the Protein Data Bank (<https://www.rcsb.org/>).

Homology Models.

For some important receptors without available crystal structures, we built their homology models, including dopamine 5 receptor (D5R, uniprot ID P21918), 5-hydroxytrypt-amine receptor 7 (5-HT7, uniprot ID P34969), trace amine-associated receptor 1 (TAAR1, uniprot ID Q96RJ0), and muscarinic acetylcholine receptor M3 (CHRM3, uniprot ID P20309). The sequences of these proteins were received from UniProtKB (<https://www.uniprot.org/uniprot/>). Then, Modeler 9.18⁸³ was applied to construct their 3D homology models using the reported protocol. Once we obtained their 3D models, SYBYL-X 1.3⁸⁴ was used to conduct the energy minimization.

Chemogenomics Knowledgebase and Computational Systems Pharmacology Analyses.

In the present work, our established knowledgebase of drug abuse (<https://www.cbiligand.org/CDAR/>) (DA-KB) and the drug abuse-related GPCRs database (DAKB-GPCRs) (<https://www.cbiligand.org/dakb-gpcrs/>)⁸⁵ were utilized for off-target prediction and systems pharmacology analysis. Several in-house tools and algorithms including HTDocking, TargetHunter, and Spider Plot were used in the present work.^{86,87} Briefly,

cocaine and fentanyl were docked into the collected target proteins, followed by the ranking of predicted proteins based on the docking score to the chemical agents. Generally, target protein with a higher docking score may have a higher binding affinity and therefore a greater chance of interacting with the query compound(s). Finally, we mapped out a network between cocaine and fentanyl and their targets^{88,89} using Cytoscape 3.4.0⁹⁰ as described previously.⁸⁹

Database Infrastructure.

A query compound can be drawn using JSME Molecular Editor v2017-03-01.⁹¹ (DAKB-GPCRs) (<https://www.cbligand.org/dakb-gpcrs/>) was built upon an internal docking service using SQLite database management system (<https://sqlite.org/>) and an open source HTTP server Kestrel (<https://github.com/aspnet/KestrelHttpServer>).

HTDocking.

Each knowledgebase adopts our established high-throughput protein–ligand docking technique, HTDocking,^{87,92,93} for the identification of possible interactions between ligands and predicted protein targets. HTDocking generates docking scores by automatically docking each query compound into a 3D structure. The likelihood of a protein target being a potential candidate of the queried small molecule is indicated by a high docking score. For each query compound in the binding pocket of potential targets, the top 9 predicted values of binding energy (G values) can be generated by iDock⁹⁴ from different docking poses. Our docking program only considers the best binding energy and further transforms it to the docking score using the formula $\text{docking score} = -\log_{10}(e^{-G \times 4184/8.314/310.15})$.

Detailed Docking Study of Ligand–Receptor.

Surflex-Dock GeomX (SFXC) implemented in SYBYL-X 1.3 was used to build the ligand–receptor complexes, in which the docking scores were expressed in $-\log_{10}(K_d)$.⁹⁵ The main protocols or parameters of docking were addressed in our previous publications:^{96–99}

“Briefly, we set the ‘number of starting conformations per ligand’ to 10, and the ‘number of max conformations per fragment’ to 20; (b) we set the value of ‘maximum number of rotatable bonds per molecule’ to 100; (c) we also turned on flags including ‘pre-dock minimization’, ‘post-dock minimization’, ‘molecule fragmentation’, and ‘soft grid treatment’; (d) then we set the value of ‘activate spin alignment method with density of search’ to 9.0; and (e) finally, we set the value of ‘number of spins per alignment’ to 12.”

Simcyp Simulations.

PBPK models for cocaine and fentanyl were performed using Simcyp Population-based ADME Simulator (version 17.0, Simcyp Limited, <https://www.certara.com/software/pbpbk-modeling-and-simulation/?ap%5B0%5D=PBPK>). Physicochemical and PK characteristics of the model were obtained from published in vitro and in vivo data or predicted by ADMET predictor 9.0 in GastroPlus (<https://www.simulations-plus.com/software/admetpredictor/>) based on the structure (Tables 2 and 3). Both the cocaine and fentanyl are inhibitors of CYP3A4 enzyme; therefore the inhibitory effects have been considered in our PBPK model.

A minimal PBPK model including four compartments was chosen for cocaine: central, liver, gut, and single adjusting compartment (SAC). The SAC is a nonphysiological compartment that permits adjustment to the drug concentration profile in the systemic compartment, where the latter represents a lump of all tissues excluding the liver and portal vein. This model can be used for compounds with a relatively small volume of distribution (<4 L/kg) like cocaine (~ 1.96 L/kg). The total clearance of cocaine in this model is 100 L/h, and the metabolism through CYP3A4 is responsible for more than 5%, which is consistent with other studies.^{100,101}

The fentanyl model was build based on the full PBPK model of Simcyp. The full PBPK distribution model simulates the concentrations in various organ compartments utilizing a number of time-based differential equations. Organ compartments simulated include the blood (plasma), bone, adipose, brain, heart, kidney, liver, gut, lung, muscle, skin, pancreas, and spleen. Interindividual variability is introduced through tissue volume prediction considering factors such as weight, height, sex, and age. Based on the literature, the elimination of fentanyl was constituted of metabolism by CYP3A4 in a predominant way (about 90%), the renal clearance in a minor way (less than 10%), and additional clearance is negligible so that was neglected in this model.

Observed plasma concentration profiles of cocaine and fentanyl were obtained from the literature and digitized using GetData Graph Digitizer version 2.26 (<http://getdata-graph-digitizer.com/>) and were used for optimizing the initial cocaine and fentanyl models. Virtual studies were simulated using 10 trials of 10 virtual individuals in a population representative of healthy volunteers. Predicted arithmetic mean values and 95th/5th percentile values of exposure parameters were compared to the corresponding observed values. The DDI simulations for cocaine and fentanyl were performed using 10 trials of 10 virtual populations with the same parameters. All the simulations administered drug using iv bolus route, and thereby the absorption phase can be ignored.

Molecular Dynamics (MD) Simulations.

CB2R complexed with JWH133 was used for MD simulation. This system was put in 0.15 M NaCl solution with a cubic water box, which included 20820 water molecules, 56 Na^+ ions, 67 Cl^- ions, and POPC lipid molecules. The initial conformations of JWH133 and CB2 were obtained from the previous docking study. The size of the starting simulation box was set to $\sim 93 \text{ \AA} \times 93 \text{ \AA} \times 93 \text{ \AA}$.

AMBER ff14SB force field¹⁰² was applied to CB2, and the water molecules were treated with the TIP3P water model.¹⁰³ “The partial atomic charges of JWH133 was derived by the semi-empirical with bond charge correction (AM1-BCC) method.”^{104,105} “The other force field parameters came from GAFF in AMBER16.”¹⁰⁵ The residue topologies for JWH133 were prepared with ANTECHAMBER.

Then the MD simulation was performed with the PMEMD.mpi and PMEMD.cuda modules in the AMBER16^{106–108} package. In order to avoid the possible steric crashes, several minimization steps were conducted for the system of CB2–JWH133 complex. This system was then gradually heated from 0 to 300 K during the heating stage followed by equilibrium

and production stages maintained at 300 K with a time step of 2 fs. Constant temperature and pressure (NPT) ensembles were maintained by utilization of periodic boundary conditions. The pressure was set to 1 atm and controlled by the anisotropic (x , y , z) pressure scaling protocol with a pressure relaxation time of 1 ps. The temperature was kept constant using Langevin dynamics with a collision frequency of 2 ps^{-1} .^{109,110} Long-range electrostatics were handled by particle mesh Ewald (PME) method,^{111,112} and the value of real-space interactions was set to $10 \text{ \AA}$. A SHAKE algorithm¹¹³ was used to constrain all covalent bonds involving hydrogen atoms. Finally, 100 ns MD simulation was performed for the CB2–JWH133 system, and the trajectory of MD simulation was recorded in a 100 ps interval.

Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) Calculation.

For the recorded trajectory of the CB2–JWH133 simulation, molecular mechanics/generalized Born surface area (MM/GBSA)^{114–120} method was applied to calculate the binding energies of CB2 with the binding of JWH133. In order to calculate the mean binding energy, 500 snapshots were extracted from the MD trajectory in a 100 ps interval from 50 to 100 ns using the following formula:

$$\Delta E_{\text{bind}} = \Delta E_{\text{MM}} + \Delta E_{\text{SOL}} = \Delta E_{\text{MM}} + \Delta E_{\text{GB}} + \Delta E_{\text{SA}}$$

where E_{bind} represents the binding energy. E_{MM} is the sum of molecular mechanical energies in vacuo, which can be further divided into the contributions from van der Waals, electrostatic, and internal energies. Generally, E_{MM} can be computed by molecular mechanics method. E_{SOL} represents the solvation energy, which included the polar solvation energy (E_{GB}) and the nonpolar part (E_{SA}). The E_{GB} can be calculated by the generalized Born (GB) approximation model,^{121,122} while E_{SA} can be obtained by fitting solvent accessible surface area (SASA)¹²³ with the LCPO model.^{124,125} In addition, the energies of each residue will be decomposed into the side-chain and backbone atoms, which can be used for the analyses of contributions of the key residues.¹²⁶

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Figure 1.

Computational systems pharmacology-target mapping for target proteins, transporters, metabolic enzymes, and potential therapeutic targets of cocaine and fentanyl. The green circles and solid lines represented the known targets and interactions for the drugs, while the purple circles and dashed lines represented the predicted targets and interactions. In addition, the red circle and solid line represents the known therapeutic target and reported therapeutic effect, while the red dashed line represents the predicted therapeutic effect. Cytochrome P450 3A4 (CYP3A4), P-glycoprotein 1 (P-gp), dopamine transporter (DAT), muscarinic acetylcholine receptor M1 (CHRM1), muscarinic acetylcholine receptor M2 (CHRM2), muscarinic acetylcholine receptor M3 (CHRM3), μ opioid receptor (OPRM1), δ opioid receptor (OPRD1), κ opioid receptor (OPRK1), trace amine-associated receptor 1 (TAAR1), cannabinoid 2 receptor (CB2), dopamine receptor D2 (DRD2), dopamine receptor D5 (DRD5), serotonin (5HT) receptor 7 (HTR7), and serotonin (5HT) receptor 2 (HTR2B).

Figure 2.
Detailed interactions between (a) cocaine and dopamine transporter (DAT) and (b) fentanyl and mu opioid receptor (μ OR) for addiction analyses.

Figure 3.
Detailed interactions of (a) cocaine and (b) fentanyl in P-glycoprotein (P-gp) for their potential drug–drug interaction.

Figure 4.
Detailed interactions of (a) cocaine and (b) fentanyl in CYP3A4 for their metabolism.

Figure 5.
Observed and simulated concentration–time profiles of (a) cocaine and (b) fentanyl.

Figure 6.

Virtual DDI studies between cocaine and fentanyl. (a) Systemic plasma concentration of cocaine over time with and without fentanyl. (b) Trial arithmetic mean and standard deviation for 10 groups of 10 individuals out of a population of 100 for a cocaine PK profile simulation. (c) Systemic plasma concentration of fentanyl over time with and without cocaine. (d) Trial arithmetic mean and standard deviation for 10 groups of 10 individuals out of a population of 100 for a fentanyl PK profile simulation.

Figure 7.
JWH133, a CB2 agonist, as potential therapy for cocaine and fentanyl polydrug addiction.
CB2 3D model is from the crystal structure of CB2 complexed with AM10257 (antagonist),
PDB ID 5ZTY.

Figure 8.

MD simulation (100 ns) for JWH133 (agonist) and CB2. (a) RMSD of CB2 and JWH133 during 100 ns MD. (b) Free energy decomposition of key residues in CB2 complexed with JWH133 during 100 ns MD simulation.

Table 1.

Energy (kcal/mol) Decomposition for Key Residues by MM/GBSA Method

residues	van der Waals	electrostatic	polar solvation	nonpolar solvation	total
Phe87	-1.68	-0.45	0.38	-1.21	-2.96
Phe94	-1.21	0.00	0.04	-1.02	-2.19
Ile110	-1.69	0.25	-0.10	-1.49	-3.04
Val113	-1.63	-0.41	-0.03	-1.14	-3.21
Thr114	-1.63	0.17	0.37	-1.13	-2.22
Phe183	-1.80	-0.26	0.29	-1.49	-3.25
Trp194	-1.49	-0.23	0.25	-1.16	-2.63
Phe281	-1.16	-0.02	0.14	-0.94	-1.98

Table 2.

Input Parameters for Cocaine Model

parameter ^a	value	source
PhysChem and Blood Binding		
MW (g/mol)	303.353	<i>b</i>
log <i>P</i>	2.3	<i>b</i>
p <i>K</i> _a	8.61	<i>b</i>
B/P	1	ref 66, <i>c</i>
fu	0.48	<i>d</i>
Distribution		
SAC Q (L/h)	500	<i>e</i>
<i>V</i> _{sac} (L/kg)	1.61	<i>e</i>
<i>V</i> _{ss} (L/kg)	1.96	ref 127, <i>c</i>
Elimination		
Cl _{iv} (L/h)	100	refs 66 and 128–130, <i>c</i>
Cl _{int} 3A4 (μL/min/pmol)	0.0345	ref 101, <i>c</i>
Cl _R (L/h)	1.86	refs 66 and 128–130, <i>c</i>
Cl _{add} (L/h)	90	<i>e</i>

^aAbbreviations: MW, molecule weight; log *P*, log of the octanol–water partition coefficient for the neutral compound; B/P, blood/plasma concentration ratio; fu, fraction of drug unbound in plasma; SAC Q, equivalent to the intercompartment clearance in compartmental modeling; *V*_{sac}, apparent volume [L/kg] associated with the SAC; *V*_{ss}, steady-state volume of distribution; Cl_{iv}, systemic plasma clearance; Cl_{int} 3A4, intrinsic clearance of CYP 3A4; Cl_R, renal clearance; Cl_{add}, additional systemic clearance.

^bFrom PubChem (PubChem CID 446220) (<https://pubchem.ncbi.nlm.nih.gov>).

^cDerived from published data and then optimized based on observed data.

^dPredicted by Simcyp.

^eAdjusted based on observed data.

Table 3.**Input Parameters for Fentanyl Model**

parameter ^a	value	source
PhysChem and Blood Binding		
MW (g/mol)	336.47	<i>b</i>
log <i>P</i>	2.8	<i>b</i>
p <i>K</i> _a	8.06	<i>b</i>
B/P	0.963	ref 131, <i>c</i>
fu	0.297	<i>d</i>
Distribution		
<i>V</i> _{ss} (L/kg)	4.089	<i>d</i>
Elimination		
Cl _{int} 3A4 (μL/min/pmol)	0.496	ref 132, <i>c</i>
Cl _R (L/h)	4.6	ref 133, <i>c</i>
Interaction		
<i>K</i> _i	24.2	ref 134

^aAbbreviations: MW, molecule weight; log *P*, log of the octanol–water partition coefficient for the neutral compound; B/P, blood/plasma concentration ratio; fu, fraction of drug unbound in plasma; *V*_{ss}, steady-state volume of distribution; Cl_{int} 3A4, intrinsic clearance of CYP 3A4; Cl_R, renal clearance.

^bFrom PubChem (PubChem CID: 446220) (<https://pubchem.ncbi.nlm.nih.gov>).

^cDerived from published data and then optimized based on observed data.

^dPredicted by Simcyp.

^eAdjusted based on observed data.