Acta Scientific Pharmaceutical Sciences (ASPS)
ISSN: 2581-5423
DOI:

Review Article

Special Issue 2- 2021

Metabolism, Hepatoxicity of Psychoactive Substances and Drugs

Magdalena Gregorczyk[1], Bartłomiej Łukasz[1], Jacek Sein-Anand[2] and Iwona Rybakowska[1]*

[1]Department of Biochemistry and Clinical Physiology, Faculty of Health Sciences with Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, Poland

[2]Department of Clinical Toxicology, Faculty of Health Sciences with Subfaculty of Nursing and Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, Poland

***Corresponding Author:** Iwona Rybakowska, Department of Biochemistry and Clinical Physiology, Faculty of Health Sciences with Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, Poland.

Received: March 19, 2021
Published: April 23, 2021

Abstract

We apply different opioids include codeine, morphine and heroin. Understanding the metabolism of these drugs allows for their proper use in testing in children or in people taking other drugs. Interpretation of toxicological analysis allows to assess the period of substance ingestion and the time since opioids were used last by addicted individual. Glycoprotein transporter is one of the suspects linked to regulating morphine and its metabolites concentrations in the brain. First step in acute intoxication identification is a full understanding of drug/narcotic elimination kinetics or its metabolism and organism response. Members of cytochrome P450 family 2, subfamily D peptide 6 (CYP2D6) together with CYP1 and CYP3 are responsible for majority of organism biotransformations. Treatments inhibiting CYP3A activity may alter drugs pharmacokinetics by intestinal metabolism reduction. Pharmacogenetic alterations in CYP 450 genes, including duplications, deletions and SNPs, may dramatically change individual susceptibility to opioids and lead to over- or underexposure to delivered treatment.

Keywords: Metabolism; Drugs; Morphine; Codeine

Introduction

The pharmacological use of opiates is faced with many problems, ranging from correct dosage to overdosing. Opioids play important role in acute and chronic pain management [1]. Clinical and forensic toxicologists are confronted with establishing heroin use on a daily basis. 0.27-0.49% of the adult population report having used opiates [2-4]. In blood we may mark for example product of heroine metabolism 6-acetylmorphine (6AM), morphine (MOR), morphine-6-glucuronide (M6G) and morphine-3-glucuronide (M3G) [5]. Presence of poppy opium ingredients, e.g. codeine, noscapine, papaverine or their metabolites in the suspect-derived samples suggest heroin use. Morphine is absorbed in the intestines with a help of ATP-binding transporters in small intestine epithelial cells. Following absorption alkaloid molecules are captured by

the liver and metabolized to M3G (40%) and M6G (10%) removed later with urine. The ratio between morphine and its metabolites is dependent on the delivery route. Patients receiving oral doses show higher concentration of morphine glucuronides in plasma, comparing to parenteral administration [6]. Liver dysfunction may lead to enhanced morphine bioavailability and, in consequence, shift the balance toward its metabolites [6]. Total morphine clearance is relatively high and ranges from 75 to 118 L/h, in relation to 100 L/h liver flow rate, for a 70 kg person [7]. Morphine can produce elevated clinical response in patients suffering from nephrological disorders, leading to acute toxic effects [8]. Next substance mephedrone named street drug has a psychoactive effect and can cause serious side effects, especially when taken frequently, in large quantities and combined with alcohol and other stimulants [9,10]. It belongs to the group of empatogens that cause characteristic emotional and social effects similar to those caused by ecstasy (MDMA). Another synthetic cathinone used is metaphedrone with its strong effect – psychostimulant properties [11]. Also worth mentioning about etazen, compound from opioid group, which analgestic effect is 70 times stronger than morphine in studies on mice [12].

Mathematical detoxication time model

Toxicological analysis is one of the basic forensic medicine methods. Forensic toxicology includes substance detection, identification and quantification as well as results interpretation. First step in acute intoxication identification is a full understanding of drug/narcotic elimination kinetics or its metabolism and organism response. In a daily routine of forensic-toxicological interpretations of drug effects multiple aspects of toxicological analytics, pharmacokinetics and pharmacodynamics have to be considered (Figure 1). The time after which a drug can be detected in different biological materials is shown on figure 2. Substance/drug concentration is being compared to therapeutic or toxic reference values. The ratio of initial drug and metabolite concentrations (P/M) may be useful to discern between recent ingestion (high P/M) and chronic use [13,14]. The usual initial data obtained is blood concentration of a given substance at the time of sampling. Shape of the elimination curve, leading to that result, though, may assume different forms, depending on the initial dose, substance distribution patterns and metabolism. For the majority of pharmaceuticals, elimination time is correlated with a concentration at given time and a proportional elimination constant. Under those conditions elimination curve is exponential and described by equation:

Figure 1: Response to the drug/opiate dependent on him pharmacokinetics and pharmacodynamics, which is affected by numerous variables [18].

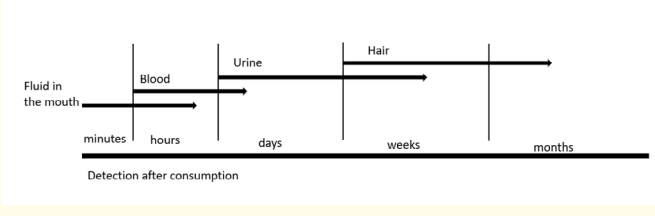

Figure 2: Detection of drugs in various biological materials.

$$C_t = C_0 e^{-kt}$$

Where: C_t – drug concentration at time t, C_0 - drug concentration at time t=0 (bloodstream introduction), k – elimination constant, e - base of the natural logarithm, Euler number (Sci Tech-17-1608, in press, „Mathematical models employed to predict the timeframe of intoxications as interpretation tools in forensic cases").

Oral fluid collection and proper storage are crucial to correctly interpret the results and the outcome of clinical and forensic cases. Samples are not always immediately tested after collection and maintaining stability during storage is essential. How often used drugs and psychoactive substances are stable in different temperatures shows figure 3. Content of oral fluid changed but for example morphine frozen after one year still contained about 70% of the substance whereas mephedrone and ketamine were hydrolysed [15].

Figure 3: Percentage of analyte change on oral fluid after storage 30 and 365 days at room temperature, 4°C, -20°C [based on 15].

Opioids metabolism

Most of the opioids undergoes extensive modifications in the liver before they enter body systems. Liver xenobiotic metabolism alleviates their renal excretion by rendering them more hydrophilic. All opioids are metabolized mainly by the cytochrome P450 (CYP450) system and, to a lesser degree, UDP-glucuronosyltransferase (UGT) resulting in both active and inactive metabolites. In some instances opioids are prodrugs, activated by metabolic modifications, in other there are more than one step required to achieve potential therapeutic activity [16,17].

Phase I of xenobiotic metabolism involves oxidation and hydrolysis reactions, which is responsible for processing 75% of all the drugs. Extensive number of CYP450 enzymes catalyze dealkylation, hydroxylation, oxidation, sulfoxidation, deamination, and dehalogenation [18]. Those enzymes are localized mainly in the liver, although found also in small intestine enterocytes and their activity reduces systemic opioids availability [19]. Combining opioids with other therapeutics also metabolized by CYP450, used for instance in pain management, may lead to interactions altering metabolism of all those substances. Examples of such drugs inhibiting or activating CYP450 are different antidepressants (fluoxetine, fluvoxamine, paroxentine, clomipramine, desipramine, imipramine), cyclooxygenase 2 inhibitors, non-steroid anti-inflammatory drugs (ibuprofen, diclofenac, naproxen, meloxicam, celeocib) or antiepileptic medications (diazepam, phenytoin). On the opposite, opioids metabolized in phase II characterize with significantly less pronounced interactions with co-administered medications. Side effects, in most cases, involve prolonged analgesia or loss of pain-relieving effects, increased risk of allergic and other unwanted reactions [20]. Members of cytochrome P450 family 2, subfamily D peptide 6 (CYP2D6) together with CYP1 and CYP3 are responsible for majority of organism biotransformations [21]. There is over 50 known CYP450 family enzymes, although the greatest significance for opioids metabolism bear CYP2D6 and CYP3A4. They are also listed among ten most encountered pharmacogenetic markers used to assess potential clinical use of opioids. Except those mentioned above, other enzymes, namely CYP3A5, UDP-glucuronosyltransferase-2B7 (UGT2B7), ATP-binding cassette sub-family B member 1 (ABCB1), canalicular multispecific organic anion transporter 2 (ABCC3), solute carrier family 22 member 1 (SLC22A1), μ-opioid receptor 1 (MOR-1), catechol-O-methyltransferase (COMT), G protein-activated inward rectifier potassium channel 2 (KCNJ6) likewise participate in opioids metabolism. CYP2D6 accounts for 2-5% of all the CYP450 isomers liver content and is responsible for metabolizing 25% of drugs. Pharmacogenetic alterations in CYP 450 genes, including duplications, deletions and SNPs, may dramatically change individual susceptibility to opioids and lead to over- or underexposure to delivered treatment [18]. In 5-10% of patients those enzymes are inactive, while the same percentage characterize with duplication, resulting in enzyme overexpression and its elevated activity. The latter case is particularly noticeable in Africans population (up to 30%), while negligible in Asians. Individuals possessing two functional copies of CYP2D6 are efficient opioids metabolizers. In case when one of the copies is nonfunctional their metabolism has an average rate, while in consequence of two inoperative alleles metabolism is reduced [17]. Striking example depicting deleterious effects of this genetic

variability is a 13 days old newborn, who died from morphine toxicity. His mother, who appeared to ultrarapid opioids metabolizer due to three functional CYP2D6 alleles, was prescribed codeine as an analgesic. Increased O-demethylation of codeine to morphine resulted in four times higher than expected levels of morphine in mother's breast milk samples [22]. Metabolism of opioids differs, as well, between sexes and women appear to characterize with its lower levels [23]. CYP3A participates in 40-60% of total isoenzymatic activity in the liver and is ubiquitous in that organ. In opposition to CYP2D6 it shows wide substrate specificity and ability to actively bind small and large molecules. CYP3A also less often shows polymorphism. It takes part in many pharmaceuticals metabolism, especially 3A3 and 3A4 isoforms. CYP3A4 present in intestines participates in pre-liver transformation, reducing absorbed drug dose, delivered to portal circulation. Treatments inhibiting CYP3A activity may alter drugs pharmacokinetics by intestinal metabolism reduction [24]. Numerous therapeutic substances is non- or competitive CYP2D6 and CYP3A3/4 inhibitors. Drugs with more affinity to isoenzyme heme complex compete with a substrate, which cannot undergo biotransformation. In respect to noncompetitive inhibition, active ingredient damages the enzyme [17]. Wide range of food products and beverages is able to influence CYP450 enzymes activity. The effects of bioactive citrus compounds, including limonoids and flavonoids on glutathione S-transferase and their inhibition of CYP enzymes is well studied. Grapefruit juice, for example, contains furanocoumarins able to suppress CYP2D6, CYP3A3/4 and CYP2C9 activity. Among furanocoumarins family, paradisin A is the strongest inhibitor, followed by dihydroxybergamottin, bergamottin, bergaptol and geranylcoumarin, which has the lowest inhibiting potential. Bergamottin, one of the main grapefruit juice ingredients, blocks CYP activity in a time- an concentration dependent manner covalently binding to CYP3A4 with a reactive furano-epoxy fragment. Co-administration of grapefruit juice with dihydropiridine calcium channel blockers, cyclosporine, midazolam, triazolam or terfenadine may increase their bioavailability, leading side effects in the form of migraines, hypo- or hypertension and facial wrinkles [25]. Enzymes may also lead to elevated activity of e.g. corticosteroids and in effect stimulate DNA transcription. Increased drug transformation result in enhance toxicity if active metabolites are being formed.

In phase II hydrophilic substrate is attached to a drug. Main reaction here is glucuronidation catalyzed by uridine 5'-diphosphoglucuronosyltransferase (UGT). This enzyme, responsible for glucuronidation, potentially affects opioids metabolism. Research on functional UGT2B7 genetic polymorphism didn't show its effects on opioids metabolism. The most often detected alteration though, namely 802C>T SNP, leads to two-fold decrease in transcriptional activity of hepatocellular carcinoma and colorectal cancer cell lines. Studies of Japanese adults population, characterizing with two functional UGTB7 alleles, revealed reduced nausea accompanying treatment of cancer pain with morphine in comparison to patients with only one allele.

ABCB1, also known as P-glycoprotein or multidrug resistance protein 1 (MDR1), is an ATP-dependent membrane efflux pump compatible with broad substrate range. In the intestinal epithelium it transports xenobiotics back to intestinal lumen, while in the capillary endothelial cells of blood-brain or blood-testis barrier divert them back into the capillaries. *In vitro* studies have shown that morphine, fentanyl and oxycodone are ABCB1 transporter substrates. Approximately 8000 SNPs (single nucleotide polymorphism) mutations were found for ABCB1 gene, although only 4% among them have allele frequency above 5% level. 3435C>T polymorphism is the most researched one. Healthy individuals with 3435TT variant demonstrated 50% lower *ABCB1* expression in duodenum. Drop in protein expression was observed also in the cells building blood-brain barrier, correlating with a higher cerebrospinal fluid morphine concentrations following intravenous administration. Higher risk of morphine-induced nausea and vomiting in patients after large intestine surgery was also noted. 3435TT genotype bearers more frequently demonstrated excessive sweating and sedation after opioids, more specifically remifentanil, treatment during spinal fusion surgery procedure. Interestingly, similar studies did not confirm these negative effects in children patients. Work involving *ABCC3* knockout mice revealed its product's role in transporting M3G and M6G heroin metabolites from the liver to the blood stream. Among 51 mutations only 211C>T was associated with a significant decrease in *ABCC3* gene mRNA levels and alterations in transcription factors binding. This specific polymorphism is linked with reduced M3G and M6G serum concentrations following drug delivery [1].

Solute carrier family 22 member 1 (SLC22A1) is present on the hepatocytes membrane and responsible for capturing positively charged molecules, including morphine or active tramadol metabolites (O-desmethyltramadol), in physiological pH. The influence of SLC22A1 polymorphism on tramadol transformation was elevation

of O-desmethyltramadol concentration in serum.

Analgesic effects of opioids, like morphine and fentanyl, are induced mainly *via* μ-opioid receptors (MOR) encoded by the *OPRM1* gene. Predominantly observed mutation in this gene (15% in white and 40% in Asian population) is 118A>G transition. *In vivo* studies unveiled that its presence reduced signal transduction from the affected receptor as well as receptor affinity to morphine and its M6G metabolite. Lower affinity of 118G MOR was likewise observed for alfentanil and oxycodone. The effects of codeine, tramadol an sufentanil in the affected individuals appear to differ less explicitly. Children, characterizing with 118G mutation, born with a withdrawal syndrome presented less opioids use-related symptoms and needed shorter hospitalization.

Another element of opioids pharmacogenetic is catechol-O-methyltransferase (COMT), which, by regulating MOR expression, is connected with several physiological functions, pain perception included. Val158Met substitution (472G>A SNP) resulted in increased MOR protein production and dropped met-enkephalin concentrations. Patients with above mutation required smaller opioids doses to relive cancer-associated and postoperative pain.

G protein released in the process of μ-opioid receptor activation activates the product of *KCNJ6* gene, i.e. G protein-activated inward potassium (GRIK) channel 2. Reduced expression of *KCNJ6* mRNA was found in subjects with 1032A/A genotype [1] and this polymorphism is able to affect organism response to administered opioids.

Hepatotoxicity

Psychoactive substances are responsible for fatal and non–fatal intoxications. Toxicological symptoms occurs also 3-MMC (metaphedrone) analogue of cathinone. Hepatocyte damage increased with concentration of 3-MMC. Toxicological effects were noticed in lysosomes at lower concentrations, followed by mitochondria and cytoplasmic membrane in primary rat hepatocytes. 3-MMC damages less mitochondria than most cathinones and other substituted amphetamines [26].

Drug induced rise in reactive species removing *in vivo* causes the decrease of antioxidant protection. The formation of ROS/RNS is one of the mechanism responsible for the toxicity of amphetamines, cathinones in high concentrations in primary rat hepatocytes. 4-methylethcathinones induce increase in oxidative stress at concentration higher than 400μM, and 3,4-methylenodioksymethamfetamine at 1mM concentration and also in liver of animals exposed for 1h at doses higher than 5mg/kg (where we can observed increase of malondialdehyde the main product of lipid peroxidation). Oxidative stress also increased depending on concentration of 3-MMC, starting with 10 μM concentration. N-acetyl-L-cysteine and vitamin C partially may revert cell death induced by cathinone derivatives in primary rat hepatocytes [27].

Glutathione as important non-enzymatic antioxidant cell defense gives us also information about redox status. Decrease in GSH was observed for all concentrations of 3-MMC, with exception of 10 μM. It is possible that at this concentration, the cell is trying activate pro survival mechanisms. Enzymes GSH-Px, superoxide dismutase and catalase were overexpressed in the mice following administration of mephedrone [26].

The caspase-3, caspase-8, caspase-9 which may activate apoptotic cascade were maximal when cell were treated with 10 μM 3-MMC, a much lower concentration than that observed for 4-MMC which was 1.6mM [26].

Mechanism responsible for hepatotoxicity were conducted at 3-MMC concentrations ranging between 1 and 500 μM. Respectively, a mean 3-MMC concentration in blood cases was 9.03 μM [28]. That kind levels are described in clinical and forensic cases.

Psychoactive substances and alcohol

Amphetamines, 3,4-methylenedioxymethamphetaminen and mephedrone in interaction with alcohol increase cardiovascular effects, induce more intensive feeling of euphoria. Mephedrone reduce the sedative effects produced by alcohol. Mephedrone produced increase in blood pressure, heart rate, pupil diameter, extraocular musculature contraction. All this effects were rapid and there duration were short. After mephedrone and alcohol co-administration cardiovascular and subjective effects started at 0.25—5h whereas return to pre-dose values at 4-8h after administration. Using only mephedrone had an effect observed between 0.5-1h. Higher increase in plasma cortisol (cortisol - an organic chemical compound, a natural steroid hormone produced by the band layer of the adrenal cortex, the main representative of glucocorticosteroids; it has a wide impact on metabolism and is sometimes called the stress hormone along with adrenaline) concentration were found after the mephedrone-alcohol administration [29]. The same is observed after cocaine [30], MDMA [31] and alcohol co-administration.

Conclusion

Opioids play important role in acute and chronic pain management. Used outside of the treatment of ailments, it can lead to severe addiction, which is a potentially fatal disease. First step in acute intoxication identification is a full understanding of drug/narcotic elimination kinetics or its metabolism and organism response. Most of the opioids undergoes extensive modifications in the liver before they enter body systems. All opioids are metabolized mainly by the cytochrome P450 (CYP450) system and, to a lesser degree, UDP-glucuronosyltransferase (UGT) resulting in both active and inactive metabolites. For example toxicological effects of metaphedrone were noticed in lysosomes at lower concentrations, followed by mitochondria and cytoplasmic membrane in primary rat hepatocytes. It is worth paying attention to the interaction of drugs with other stimulants such as alcohol, where, for example, an increase in the response of the cardiovascular system or cortisol can be observed.

Summing up, due to the way opioids and other substances from the stimulant group are metabolized, it is worth paying attention to the proper functioning of the liver in people undergoing treatment, especially knowing that these compounds also have hepatotoxic properties. Additionally, it is also worth noting that frequent consumption of alcohol weakens the functioning of the liver in people who use the stimulant.

Bibliography

1. Matic M., *et al*. "Analgesia and opioids: a pharmacogenetics shortlist for implementation in clinical practice". *Clinical Chemistry* 63 (2017): 1204-1213.

2. Rummans TA., *et al*. "How Good Intentions Contributed to Bad Outcomes: The Opioid Crisis". *Mayo Clinic Proceedings* 93 (2018): 344-350.

3. Goodwin AJ. "Critical Care Outcomes Among Opioid Users: Hidden Sequelae of a Growing Crisis?" *Critical Care Medicine* 46 (2018): 1005-1006.

4. Koenig KL. "The Opioid Crisis in America: Too much, too little, too late". *Western Journal of Emergency Medicine* 19 (2018): 557-558.

5. Dubois N., *et al*. "Estimation of the Time Interval between the Administration of Heroin and Sampling of Blood in Chronic Inhalers". *Journal of Analytical Toxicology* 39 (2015): 300-305.

6. Fujita K., *et al*. "Association of UGTB7 and ABCB1 genotypes with morphine-induced adverse effect drug reactions in Japanese patients with cancer". *Cancer Chemotherapy and Pharmacology* 65 (2010): 151-158.

7. Sverrisdottir E., *et al*. "A review of morphine and morphine-6-glucuronide's pharmacokinetic- pharmacodynamics relationship in experimental and clinical pain". *European Journal of Pharmaceutical Sciences* 74 (2015): 45-62.

8. King S., *et al*. "A systematic review of the use of opiod medication for those with moderate to severe cancer pain and renal impairment: a European Palliative Care Research Collaborative opioid guidelines project". *Palliative Medicine* 25 (2011): 525-535.

9. Nordqvist C. "What is mephedrone? What are the effects of mephedrone?" *Medical News Today* (2014): 9/09/2014.

10. Skowronek R., *et al*. "Mephedrone and its new derivatives - toxicological, medical and legal aspects". *Problems of Forensics* 275.1 (2012).

11. Backberg M., *et al*. "Characteristics of analytically confirmed 3-MMC-related intoxications from the Swedish STRIDA project". *Clinical Toxicology* 53 (2015): 46-53.

12. Hunger A., *et al*. "Benzimidazol-Derivate und verwandte Heterocyclen. II. Synthese von 1-Aminoalkyl-2-benzyl-benzimidazolen". *Helvetica Chimica Acta* 43 (1960): 800-809.

13. Holmgren P and Ahlner J. "Pharmacogenomics for forensic toxicology: Swedish experience". in: S.H Wong, M. Linder, R. Valdes Jr, Pharmacogenomics and Proteomics, AACC Press, Washington (2006): 295-299.

14. Musshoff F., *et al*. "Pharmacogenetics and forensic toxicology". *Forensic Science International* 203 (2010): 53-62.

15. Marchei E., *et al*. "Stability and degradation pathways of different psychoactive drugs in neat and buffered oral fluid". *Journal of Analytical Toxicology* (2020): 1-10.

16. Hutchinson MR., *et al*. "CYP2D6 and CYP3A4 involvement in the primary oxidative metabolism of hydrocodone by human liver microsome". *British Journal of Clinical Pharmacology* 57 (2004): 287-297.

17. Mercadante S. "Opioid metabolism and clinical aspects". *European Journal of Pharmacology* 769 (2015): 71-78.

18. Gudin J. "Opioid therapies and cytochrome P450 interactions". *Journal of Pain and Symptom Management* 44 (2012): S4-S14.

19. Wilkinson GR. "Drug metabolism and variability among patients in drug response". *The New England Journal of Medicine* 353 (2005): 2211-2221.

20. Holmquist G. "Opioid metabolism and effects of cytochrome P450". *Pain Medicine* 10 (2009): S20-S29.

21. Virani A., *et al*. "Drug interactions in human neurophatic pain pharmacotherapy". *Pain* 73 (1997): 3-13.

22. Madadi P., *et al*. "Safety of codeine during breastfeeding. Fatal morphine poisoning in the breastfed neonate of a mother prescribed codeine". *Canadian Family Physician* 53 (2007): 33-35.

23. Smith HS. "Opioid metabolism". *Mayo Clinic* 84 (2009): 613-624.

24. Davis MP and Homsi J. "The importance of cytochrome P450 monooxygenase CYP2D6 in palliative medicine". *Support Care Cancer* 9 (2001): 442-451.

25. Girennavar B., *et al*. "Potent inhibition of human cytochrome P450 3A4, 2D6 and 2C9 isozymes by grapefruit juice and its furocoumarins". *Journal of Food Science* 72 (2007): C417-C421.

26. Da Silva DD., *et al*. "The new psychoactive substance 3-methylmethcathione (3-MMC or metaphedrone) induces oxidative stress, apoptosis, and autophagy in primary rat hepatocytes at human-relevant concentrations". *Archives of Toxicology* 93 (2019): 2617-2634.

27. Valente MJ., *et al*. "Editor´s high-light: characterization of hepatotoxicity mechanisms triggered by designer cathinone drugs (beta-keto amphetamines)". *Toxicological Science* 153 (2016): 89-102.

28. Adamowicz P., *et al*. "3-Methylmethcathionone-interpretation of blood concentrations based on analysis of 95 cases". *Journal of Analytical Toxicology* 40 (2016): 272-276.

29. Papaseit E., *et al*. "Mephedrone and Alcohol Interactions in Humans". *Frontiers in Pharmacology* 10 (2020): 1588.

30. Farre M., *et al*. "Cocaine and alcohol interactions in humans: neuroendocrine effects and cocaethylene metabolism". *Journal of Pharmacology and Experimental Therapeutics* 283 (1997): 164-176.

31. Hernandez-Lopez C., *et al*. "3,4-Methylynedioxymethamphetamine (ecstasy) and alcohol interactions in humans: psychomotor performance, subjective effects, and pharmacokinetics". *Journal of Pharmacology and Experimental Therapeutics* 300 (2002): 236-244.