Clinical Biochemistry 67 (2019) 48–53

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Consumption of Movantik™ (Naloxegol) results in detection of naloxone in the patient's urine evaluated by confirmatory urine drug testing

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A B S T R A C T

Background: Many patients on chronic opioid therapy suffer from constipation, one of the most common side effect of opioids. Movantik™ (naloxegol) is an opioid antagonist that is recently introduced in the market to treat opioid-induced constipation and contains naloxegol as the active ingredient. Naloxegol is a pegylated (polyethylene glycol-modified) derivative of α-naloxol. Detection of naloxone in the patients urine after consumption of naloxegol was not reported by the manufacturer and may mislead the prescribing clinicians. This study was conducted to investigate the presence of naloxone in the urine of patients that consume movnatik in pain management clinics.

Methods: The presence of naloxone and naloxol in the urine of 45 patients that consumed naloxegol and 25 patients that consumed suboxone™ were investigated using a liquid chromatography mass spectrometry (LCMS) method. The urinary concentration of naloxone, naloxol, and their glucuronide conjugates were evaluated in five volunteers that took one pill of naloxegol for one day and one volunteer who took the pill for three days.

Results: Naloxone was detected in the urine of 45 individuals that were prescribed naloxegol. Urinary concentration of naloxone showed a distribution with a mean of 25 ± 18 ng/ml. Consumption of one pill of 25 mg naloxegol resulted in the detection of naloxol and naloxone in the urine of 5 volunteers 1 h after taking the pill. Evaluation of urine specimens from 25 patients that consumed suboxone™, resulted in the detection of naloxone (180 ± 187 ng/ml) and naloxol (6.3 ± 7.2 ng/ml).

Conclusions: This study demonstrated that consumption of naloxegol leads to appearance of naloxone in the urine of patients receiving opioid therapy in pain management clinics.

1. Introduction

Various strategies are available to monitor adherence to therapy provided in pain management clinics. Urine drug testing can help track patient compliance and expose possible drug misuse and abuse. Several studies support the notion that combining self-report and urine drug testing improves the accuracy of drug assessment [1–7]. Many patients that receive chronic opioid therapy suffer from constipation, one of the most common side effect of opioids [8,9].

Movantik™ (naloxegol, AstraZeneca Pharmaceuticals LP, Wilmington, DE) is an opioidantagonist that was recently introduced in the market to treat opioid-induced constipation. Naloxegol contains naloxegol, which is a pegylated (polyethylene glycol-modified) derivative of α-naloxol. Previous studies demonstrated that naloxegol is metabolized by cytochrome P450 (CYP)3A4 and is a substrate of P-glycoprotein (P-gp) [10].

Pegylation makes naloxegol a substrate for the P-gp transporter. The central nervous system penetration of naloxegol is negligible due to reduced permeability and its increased efflux across the blood brain barrier, related to P-gp transporter. Naloxegol antagonizes μ- and κ-opioid receptors and displays low affinity to δ-opioid receptors in the GI tract, thereby decreasing opioids-induced constipation symptoms without reversing central analgesic effects [11,12].

Demethylation, oxidation, dealkylation, and shortening of the polyethylene glycol chain has been mentioned as naloxegol metabolism [13–15]. Due to use of chromatography and mass spectrometry techniques, confirmatory methods of urine drug testing is known to have high specificity. Compared to immunoassay methods of urine drug testing (screening methods) confirmatory urine drug testing is expected to produce minimal false positive results. Our clinical toxicology laboratory serves patients from pain management clinics that are mostly on chronic opioid therapy. We received calls from physicians who were puzzled by the detection of naloxone in their patient's urine since naloxone was not prescribed. We realized that for all these patients naloxegol were prescribed for treatment of opioid-induced constipation. In pain management clinics appearance of naloxone in the confirmatory urine drug testing report of patients that are prescribed naloxegol may mislead the clinicians. Because the clinicians are not informed by the manufacturer that naloxone can be detected in the urine of patients that consume naloxegol [16]. This study was conducted to investigate the presence of naloxone in the urine of patients that consume naloxegol in pain management clinics.

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https://doi.org/10.1016/j.clinbiochem.2019.03.006
Received 14 January 2019; Received in revised form 12 March 2019; Accepted 16 March 2019
Available online 16 March 2019

2. Methods

2.1. Materials

All certified reference standards and isotope-labeled internal standard solutions were purchased from Cerilliant Corporation (Round Rock, TX). HPLC grade methanol and water were purchased from EMD Millipore Corporation (Darmstadt, Germany). HPLC grade isopropanol and liquid chromatography mass spectrometry (LCMS) grade formic acid was purchased from Fisher Scientific (Waltham, MA). Urine toxicology negative control (human drug free urine) was purchased from UTAK (Valencia, CA). β-glucuronidase enzyme and buffer for sample preparation was purchased from Integrated Micro-Chromatography Systems, IMCS® (Irmo, SC).

2.2. Instrumentation and conditions

LC–MS-MS analysis was performed on a Sciex 4500 mass spectrometer (AB Sciex, Framingham, MA,) coupled with a Shimadzu Nexera XR high-pressure liquid chromatography (HPLC) system (Shimadzu Corporation, Kyoto, Japan). Naloxone, naloxol, and naloxone glucuronide were measured in the positive mode of mass spectrometry. The chromatography separation was performed on a Raptor™ Biphenyl column, 2.7 μm, 50 × 3.0 mm (Restek, Bellefonte, PA) using gradient elution comprising of 0.1% formic acid and 0.1% ammonium formate in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B). A Raptor Biphenyl EXP Guard Column Cartridge (2.7 μm, 5 × 3.0 mm) was installed preceding the bi-phenyl analytical column for cleaning up the samples. Analytes were detected by mass spectrometry using scheduled multiple reaction monitoring (MRM) positive electrospray ionization (ESI) modes. Two characteristic MRM transitions were monitored for each analyte. The MRM ratios, which are defined as the peak area ratios between primary and secondary ion transitions, were only acceptable if ≤30% for all analytes. The data was collected using the AB Sciex Analyst, 1.7 software and quantified with the MultiQuant, 2.1 software.

2.3. Sample preparation

In a 1.5 ml microcentrifuge tube add 100 μl of each urine specimen, calibrator, or control. Add 40 μl of IMCS rapid hydrolysis buffer, 30 μl of β-glucuronidase enzyme (activity > 50 Ku/Ml), and 10 μl of internal standard mix. Vortex for 10 s and incubate at 55 °C for 30 min. Add 500 μl of sample diluent to all samples (0.1% formic acid in 75:25 water/methanol), vortex for 10 s, and centrifuge at 3700 rpm for 15 min. Transfer 500 μl of supernatant into a vial for LCMS analysis.

2.4. Assay validation

A dilute and shoot confirmatory LCMS method measured naloxol, naloxone, and naloxone glucuronide. The methods were developed and validated using Food and Drug Administration recommendations and acceptance criteria for validation of mass spectrometry methods [17]. Precision, accuracy, analytical measurement range, sensitivity (limit of detection and lower limit of quantification, LLOQ), and specificity were assessed as a part of method validation. Coefficient of variation (CV%) for naloxol, naloxone, and naloxone glucuronide assays for within-run precision were 6.1%, 3.8%, and 5.5% and for between-run precision were 7.1%, 7.6%, and 5.5%, respectively. The average accuracy of naloxol, naloxone and naloxone glucuronide assays were ± 9.3%, ± 6.4%, and ± 11.5% of nominal concentration of calibrators, respectively. Limit of detection for naloxol, naloxone, and naloxone glucuronide assays were 0.5 ng/ml. LLOQ and upper limit of quantification for naloxol, naloxone and naloxone glucuronide were 1 ng/ml and 200 ng/ml, respectively. Naloxol, naloxone, and naloxone glucuronide assays had a linear calibration curve within the analytical measurement ranges and concentration-response relationship fitted with a regression model ($r > 0.98$ for all assays). No interferences between naloxol, naloxone. and naloxone glucuronide measurement were detected.

2.5. Subjects

Eligible participants in the case studies were healthy employees of Elite Medical Laboratory, Tomball, Texas, 22–54 years of age. The study protocol, amendments, and informed consent forms were reviewed and approved by an independent ethics committee. The study was conducted in accordance with the Declaration of Helsinki, and all subjects provided written informed consent before study participation. Use of medications or herbal products with enzyme-inducing properties was prohibited within 3 weeks prior to study. Use of products containing grapefruit or seville oranges was prohibited within 1 week of study. The retrospective case control studies were performed on urine specimens from the patients of pain management clinics who were prescribed and consumed naloxegol or suboxone™. The patients that consumed both naloxegol and suboxone™ were excluded from the retrospective studies because identification of source of naloxone in these patients was not possible.

2.6. Study protocols

In a retrospective study the presence of naloxone in the urine of 45 patients that had consumed naloxegol in pain management clinics of Houston, Texas was investigated. In concurrence the urinary concentration of naloxone, naloxol, and naloxone glucuronide were evaluated in five volunteers that took one pill of 25 mg naloxegol. The presence of naloxone and naloxol in the urine of a volunteer following consumption of one pill of 25 mg naloxegol per day for 3 days was evaluated.

Urine of 25 patients that consumed a medication that contains naloxone, suboxone™ were assessed for the presence of naloxone and naloxol. Urinary creatinine were measured for all specimens by a chemistry auto analyzer, AU 680 (Beckman Coulter, Brea, CA) and naloxone and naloxol concentrations were normalized for creatinine concentration for all urine specimens.

3. Results

3.1. Naloxone was detected in the patients that consumed naloxegol

Within a period of 18 months (April 2017 to December 2018) 38,000 confirmatory urine drug testing reports that were analyzed in Elite Medical Laboratory, Tomball, Texas by a LCMS method were evaluated. Naloxone was detected in urine of 45 individuals (female, $n = 35$ male, $n = 10$) that were prescribed naloxegol but not suboxone™ for treatment of opioid-induced constipation. Fig. 1 shows the urinary concentration of naloxone in the patients with a mean of 25 ± 18 ng/ml.

3.2. Consumption of naloxegol leads to appearance of naloxol and naloxone in urine

Consumption of one pill of 25 mg naloxegol resulted in the detection of naloxol and naloxone in the urine of five volunteers 30 min to one hour after taking the pill. Both naloxone and naloxol disappeared 24 h after the pill consumption. There was a significant inter-individual variability in the urinary concentrations of naloxone and naloxol (Figs. 2–3).

When naloxegol was taken by a volunteer for three days a steady urinary concentration of 10–15 ng/ml was achieved 24 h after the first pill (see supplementary figure-1). Supplementary figure-2 indicates the ratio of naloxone to naloxol concentration after consumption of 25 mg of naloxegol by a volunteer. Urine specimens were collected at 0, 1, 2,

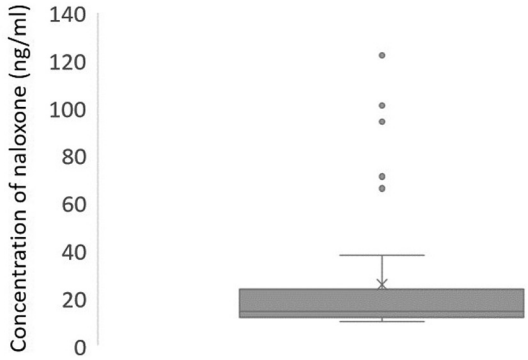

Fig. 1. Distribution of naloxone concentration among individuals that consumed naloxegol. Naloxone was detected in the urine of 45 patients that consumed movantik™ using a dilute and shoot LCMS method.

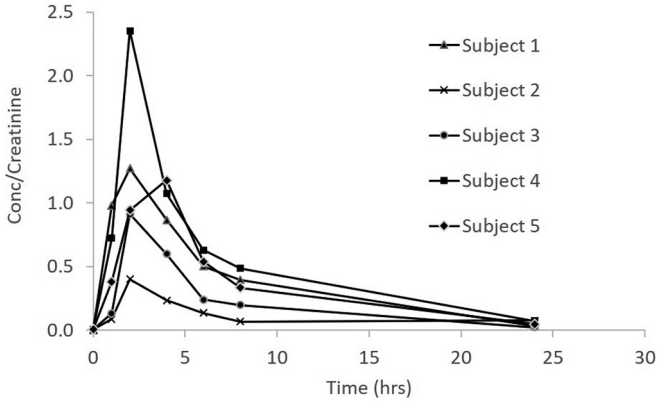

Fig. 2. Urinary concentration of naloxone in 5 volunteers that consumed 25 mg of naloxegol. Naloxone was measured using a dilute and shoot LCMS method. Naloxone concentration is normalized for urinary creatinine (ng/mg).

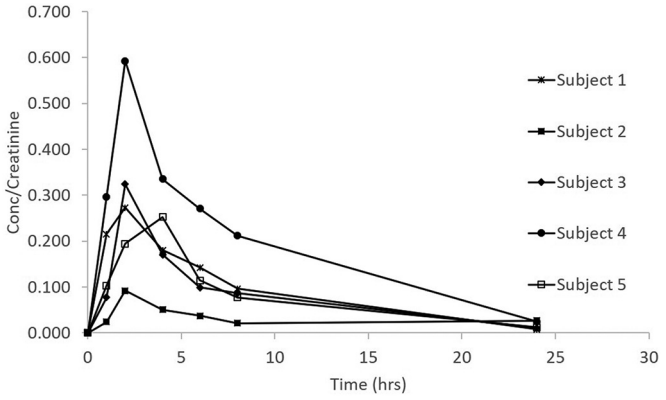

Fig. 3. Urinary concentration of naloxol in 5 volunteers that consumed 25 mg of naloxegol. Naloxol was measured using a dilute and shoot LCMS method. Naloxol concentration is normalized for urinary creatinine (ng/mg).

3, 5, 7, 9, 15, 22, 24, and 28 h after consumption of the pill. Urinary concentration of naloxone fluctuated around 20–30% of naloxol in all time points.

3.3. Urinary naloxol and naloxone were glucuronidized

The assays that were primarily used in the study were able to detect naloxone and naloxol and not the glucuronidized forms of analytes. Therefore, when we used a sample preparation protocol that did not utilize glucuronidase enzyme, detection of naloxone and naloxol in the urine of patient that consumed naloxegol was not possible. Suggesting that in the urine of individuals that took naloxegol, only naloxone glucuronide and naloxol glucuronide are present and not naloxone and naloxol (Fig. 4). We examined other factors in the sample preparation procedures such as exposure of sample to 55 °C for 30 min or to the hydrolysis buffer. None of the factors except glucuronidase enzyme was necessary for detection of naloxone and naloxol. This was confirmed when we developed a method to detect naloxone glucuronide. After utilizing the method without pretreatment of urine with glucuronidase, only naloxone glucuronide was detected in the urine of volunteers that consumed naloxegol (Fig. 4). After consumption of naloxegol glucuronidation of naloxol and naloxone occurs very fast. Half an hour after consumption of the pill we could not detect any naloxol or naloxone without exposure of urine to glucuronidase enzyme. Using morphine glucuronide as a control, our hydrolysis efficiency was > 85% (data not shown). When high concentration solutions of naloxone and naloxol stored at room temperature for 24 h or were exposed to 55 °C for 2 h no spontaneous conversion of naloxone to naloxol or reverse occurred (data not shown). Suggesting that interconversion of naloxone and naloxol is most likely an enzymatic reaction.

3.4. Consumption of products that contain naloxone leads to excretion of naloxol

Analysis of urine from 25 patients (21 males and 4 females) that consumed combination of buprenorphine and naloxone (suboxone™) led to the detection of naloxone (180 ± 187, range 19–631 ng/ml). Naloxol was also detected in the patient's urine (6.3 ± 7.2 ng/ml range 1.2–14 ng/ml). On average the concentration of naloxol was 3.5% of naloxone (3.5 ± 6.7%, range, 0.5–18% ng/ml).

4. Discussion

Our studies demonstrated that consumption of naloxegol leads to detection of naloxone and naloxol in the urine of patients. To the best of our knowledge this is the first study to report the detection of naloxol in the urine of patients that consumed naloxegol or suboxone™.

In a letter to editor Wotring et al. [18] recently reported the detection of naloxone in urine of 14 patients that took naloxegol for opioid-induced constipation. The authors also reported the detection of naloxone in the urine of an individual that consumed naloxegol for a day [18]. Our findings are in line with the study for the detection of the low levels of naloxone in the patients` urine. However, in contrast to our report the study did not investigate the role of naloxol as a source of naloxone in the patients that consumed naloxegol. Presence of naloxol in the urine of patients that took suboxone™ was not evaluated in Wotring's study [18].

There is a general assumption that mass spectrometry-based methods using for confirmatory urine drug testing are very specific and less prone to false positive results compared to immunoassay screening methods. This is true considering this fact that in contrast to immunoassay screening methods mass spectrometry-based methods are not susceptible to cross reaction by other chemicals. Alternative sources can generate an unexpected positive result for a targeted analyte in mass spectrometry-based confirmatory methods. It is well known that consumption of poppy seed leads to detection of morphine and codeine [19] and consumption of over the counter nasal decongestants (some brands of vapor inhalers) leads to detection of methamphetamine [20]. Our study added monantik™ as an alternative source for a positive result for naloxone in confirmatory urine drug testing.

Glucuronidation of morphine occurs in two carbon sites and leads to production of morphine-3- and − 6-glucuronides (M3G, M6G). It has been reported that beta glucuronidase efficiently hydrolyzed M3G, while the hydrolysis efficiency for M6G is lower [21,22]. If naloxone and naloxol are also glucuronidized at two carbon sites of 3 and 6, our assays may underestimate the concentrations of these analytes due to

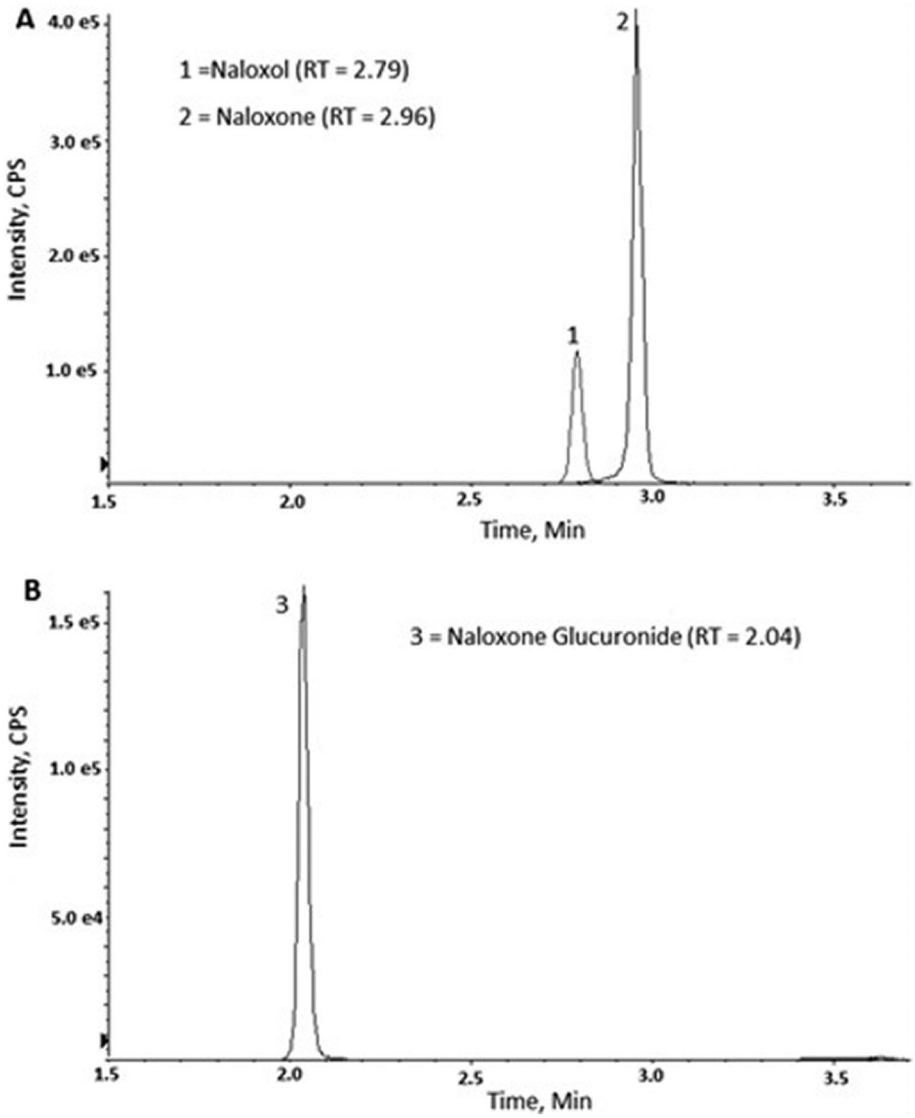

Fig. 4. Detection of naloxone after hydrolysis of glucuronide from naloxone glucuronide.
The method that was primarily developed could detect only naloxone and naloxol. Panels A, shows the sample preparation procedure that used glucuronidase enzyme and panels B, shows the same condition but without glucuronidase enzyme in sample preparation step and a method that was developed to detect naloxone glucuronide in addition to naloxone and naloxol.

possible lower hydrolysis recovery for naloxone-6 glucuronide and naloxol-6 glucuronide.

Naloxegol metabolism has been studied previously with fecal and urinary excretion of.

67.7% and 16.0%, respectively [13]. The structure that would result from loss of the entire PEG chain of naloxegol would be naloxol. However, metabolites with > 3 repeats of polyethylene glycol (PEG) were reported as the main metabolites of naloxegol and naloxol was not reported as a metabolite [13]. The radiolabel used for the metabolic studies was located on the PEG chain and not on the core opioid structure, naloxol. Therefore, in the radioprofiling detection of naloxol as a result of cleavage of full chain of PEG was not possible. In addition, the mass spectrometry method that was used by the researchers only targeted naloxegol and a method for detection of naloxol or naloxone was not developed [13]. These may explain why the cumulative radioactivity recovery of naloxegol in urine and feces was only 84% of the consumed dose by volunteers. The authors mentioned a “discrepancy in the recovery of naloxegol in urine when naloxegol was measured by LCMS/MS vs. radioprofiling” as a potential limitation for the study [13]. Identification of naloxol and naloxone as metabolites of naloxegol may question this claim that naloxegol has minimal penetration in central nervous system due to pegylation.

Limited information is available about the metabolic pathways that connect naloxone to naloxol. However, previous studies cited that naloxol is a derivative of naloxone [14,15]. If that statement is true, then the detection of naloxone after consumption of movantik™ is not expected unless movantik™ pill is contaminated by naloxone during production or naloxol is converted to naloxone inside human body. Some drugs that are produced by pharmaceutical companies contain impurity. For instance, oxycodone may have some impurity with hydrocodone or morphine may have some contamination with codeine. However, the levels of impurities in these drugs are very low and usually does not exceed 0.04% of the original drug [23,24].

Our studies demonstrated that following consumption of naloxegol the concentration of urinary naloxone was around 20–30% of naloxol concentration. This amount of naloxone is very unlikely to be due to contamination of movantik™ with naloxone in the manufacturing process.

We hypothesize that the detected naloxone is produced through the conversion of naloxol in the patient body. Furthermore, we found out

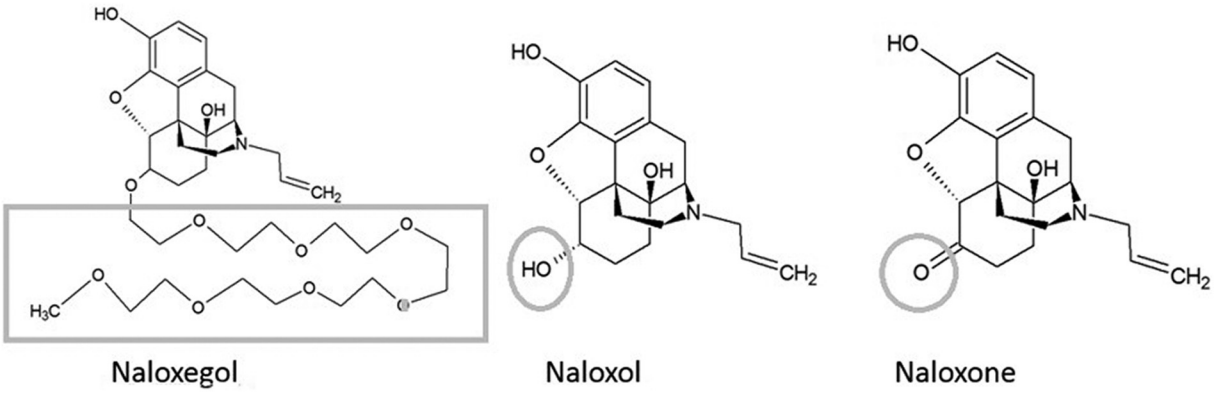

Fig. 5. Formulas of Naloxegol, Naloxol, and Naloxone. Circles indicate the difference between Naloxol and Naloxone formula.

that after consumption of suboxone™, naloxol is detected in the patients` urine with an average of 3.5% of naloxone concentration. *N*-dealkylation and the reduction of the 6-keto group of naloxone as well as conjugation with glucuronide was mentioned as the main metabolic pathway for naloxone in human [25,26]. The structural difference between naloxone and naloxol is in the presence of hydroxyl functional group in naloxol and a carbonyl (keto) functional group in naloxone on carbon at position 6 (Fig. 5). This slight structural difference led us to hypothesize that naloxol and naloxone are interconvertible while inside the human body through oxido-reductive reactions. Mammalian morphine 6-dehydrogenase converts morphine hydroxyl group at carbon position 6 into a carbonyl group and makes morphinone. Morphine 6-dehydrogenase belongs to the aldo-keto reductase (AKR) superfamily and has been detected in liver of hamster, guinea pig, rabbit and human [27–31]. Morphine 6-dehydrogenase exists in several isoforms and oxidizes various xenobiotic alicyclic alcohols [30-33]. Considering the resemblance between morphine and naloxone structure, it is likely that morphine 6-dehydrogenase converts naloxol to naloxone by oxidation. It has been demonstrated that naloxone can be reduced to naloxol by another enzyme, naloxone reductase [34,35]. The same investigator that discovered morphine 6-dehydrogenase and naloxone reductase, Yamano et al. [33] demonstrated that both enzymes are identical [30,35]. We hypothesize that in human morphine 6-dehydrogenase converts naloxone to naloxol, as seen in suboxone consumption and naloxol to naloxone, as seen in movantik™ consumption. Further studies are needed to investigate in vivo metabolism of naloxone and naloxol and particularly the role of morphine 6 dehydrogenase.

Clinical application

Clinicians who prescribe naloxegol for treatment of opioid-induced constipation should be aware of possibility of naloxone presence in urine specimen when they order confirmatory urine drug testing for their patients.

Conclusions

This study demonstrated that consumption of naloxegol leads to appearance of naloxone in the urine of patients receiving the pill.

Declarations of interest

None.

Acknowledgement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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