Detection and confirmation of the ring-opened carboxylic acid metabolite of a new synthetic opioid furanylfentanyl

Tatsuyuki Kanamori¹ · Yuki Okada¹ · Hiroki Segawa¹ · Tadashi Yamamuro¹ · Kenji Kuwayama¹ · Kenji Tsujikawa¹ · Yuko Togawa-Iwata¹

Received: 7 April 2020 / Accepted: 3 August 2020 / Published online: 18 August 2020
© The Author(s) 2020

Abstract

Purpose Recently, the opioid epidemic has become a serious problem, particularly in North America and Europe. The aim of this study was to clarify the metabolic fate of a new synthetic opioid furanylfentanyl.

Methods The metabolism of furanylfentanyl was investigated by incubating fresh human hepatocytes with 10 μ M furanylfentanyl at 37 °C for 48 h in an atmosphere of 5% CO₂. After incubation, the culture medium was deproteinized and analyzed by liquid chromatography/mass spectrometry.

Results On the chromatogram, four metabolites of furanylfentanyl were presumably detected: 4'-hydroxy-furanylfentanyl, β -hydroxy-furanylfentanyl, 4'-hydroxy-3'-methoxy-furanylfentanyl, and a ring-opened carboxylic acid metabolite. These newly found metabolites of furanylfentanyl were then definitely identified using chemically synthesized authentic standards.

Conclusions Four metabolites of furanylfentanyl were newly identified. Although it has been proposed over recent years that a dihydrodiol metabolite, which has the same molecular weight as the ring-opened carboxylic acid metabolite, is formed from furanylfentanyl, this study demonstrated that the ring-opened carboxylic acid metabolite, rather than the dihydrodiol metabolite, is formed from furanylfentanyl.

Keywords Furanylfentanyl · Hepatocytes · Metabolism · Liquid chromatography · Mass spectrometry

Introduction

Recently, the opioid epidemic has become a serious problem, particularly in the United States, causing many opioid-related deaths. According to the annual report of the United Nations Office on Drugs and Crime (UNODC), 47,600 people died from opioid use in 2017 in the United States [1]. Among the opioids, fentanyl analogs have gained popularity in the drug market in recent years [2]. Furanylfentanyl is a new fentanyl analog that has a furan ring in its structure (Fig. 1), and is said to be pharmacologically more active than fentanyl [3]. Furanylfentanyl appeared in the United States in 2015 for the first time, and from December 2015 to September 2016, a total of 494 cases relevant to furanylfentanyl were reported [4]. In addition, acute intoxication cases associated with furanylfentanyl were reported by several

European countries [5]. According to the increasing public health concern caused by this drug, furanylfentanyl has been controlled under the United Nations Single Convention on Narcotic Drugs of 1961.

Several reports of the metabolism of furanylfentanyl have been published. Goggin et al. analyzed urine samples obtained from furanylfentanyl users and detected 4-anilino-*N*-phenethylpiperidine (4-ANPP), 4-ANPP-sulfate, and a unique dihydrodiol metabolite as the metabolites of furanylfentanyl (Fig. 2) [6]. In addition, Watanabe et al. reported that 4-ANPP and the dihydrodiol metabolite were formed from furanylfentanyl in human hepatocytes [7]. However, in the reports mentioned above, the metabolites were merely presumed based on their product ion spectra and/or their behavior in liquid chromatography/mass spectrometry (LC/MS), and were never confirmed using authentic standards.

In this study, furanylfentanyl was metabolized by fresh human hepatocytes isolated from human liver chimeric mice [8] and its metabolites were definitely identified using authentic standards of the presumed metabolites, which were chemically synthesized here.

✉ Tatsuyuki Kanamori
kanamori@nrips.go.jp

¹ National Research Institute of Police Science, 6-3-1, Kashiwanoha, Kashiwa, Chiba 277-0882, Japan

Fig. 1 Chemical structure of furanylfentanyl

Materials and methods

Chemicals and reagents

Fresh human hepatocytes (PXB-cells™, seeded in a 24-well plate, 2.1×10^5 cells/cm²) and the culture medium for these cells were purchased from PhoenixBio (Higashihiroshima, Japan). *Helix pomatia* β -glucuronidase/aryl sulfatase (β -glucuronidase, 32 units/mL; aryl sulfatase, 102 units/mL) was purchased from Merck (Darmstadt, Germany). All other reagents used here were of analytical grade.

Synthesis of authentic standards of the furanylfentanyl metabolites

All synthesized standards were confirmed by high-resolution (HR) positive electrospray ionization (ESI) mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. HR-ESI mass spectra were obtained using a time-of-flight mass spectrometer (6530 Q-TOF, mass resolution in excess of 14,000 (FWHM) at m/z 322.0481, Agilent Technologies, Santa Clara, CA, USA). Mass accuracies were < 5 ppm for all compounds. NMR spectra were

recorded on an NMR spectrometer (JNM-ECA600; JEOL, Akishima, Japan). Tetramethylsilane was used as an internal standard.

N-Phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]furan-2-carboxamide (Furanylfentanyl)

Furanylfentanyl was synthesized in our laboratory as reported previously [9].

N-Phenyl-*N*-(piperidin-4-yl)furan-2-carboxamide (Nor-furanylfentanyl)

1-Benzyl-4-piperidone was condensed with aniline, and the resulting imine was reduced using sodium borohydride, as reported previously, to obtain *N*-phenyl-1-benzyl-4-piperidinamine [10]. A mixture of 300 mg of *N*-phenyl-1-benzyl-4-piperidinamine (1.1 mmol), 300 mg of 2-furoyl chloride (2.3 mmol), and 300 μ L of pyridine in 10 mL of tetrahydrofuran was heated at 90 °C for 20 h. Water was added to the reaction mixture and the solution was acidified with 3 M hydrochloric acid and extracted with chloroform. The solvent was evaporated to dryness under vacuum, and the residue was dissolved in a small amount of methanol, followed by the addition of water. The solution was made basic with ammonium hydroxide, then extracted with chloroform. The solvent was evaporated to dryness under vacuum, and the residue was purified by flash chromatography (column, silica gel; solvent, chloroform/ethyl acetate) to obtain 275 mg of *N*-phenyl-*N*-[1-benzylpiperidin-4-yl]furan-2-carboxamide (0.76 mmol, 69%).

To the solution of 170 mg of *N*-phenyl-*N*-[1-benzylpiperidin-4-yl]furan-2-carboxamide (0.47 mmol) in 6 mL of dichloromethane that was cooled in an ice bath, 135 mg of 1-chloroethyl-chloroformate (0.94 mmol) was added and the mixture was stirred for 60 min at room temperature. The solvent was evaporated to dryness under vacuum and the residue was dissolved in 5 mL of methanol and refluxed

Fig. 2 Metabolic pathway of furanylfentanyl proposed previously

for 30 min. The solvent was evaporated to dryness under vacuum and the residue was purified by flash chromatography (column, silica gel; solvent, chloroform/methanol). The residue was dissolved in chloroform and back-extracted using 3 M hydrochloric acid. The aqueous layer was basified with 10 M sodium hydroxide solution, then extracted with chloroform. The solvent was evaporated to dryness under vacuum to obtain 117 mg of *N*-phenyl-*N*-(piperidin-4-yl) furan-2-carboxamide (nor-furanylfentanyl) as a white solid (0.43 mmol, 91%).

Nor-furanylfentanyl (free base)—measured accurate mass of protonated molecule: 271.1430 (exact mass, 271.1441; mass accuracy, 4.1 ppm). ¹H-NMR (CD₃OD) δ: 1.43 (2H, qd, *J*=4.2 Hz, 12.4 Hz), 1.91 (2H, br d, *J*=12.0 Hz), 2.72 (2H, td, *J*=2.6 Hz, 12.6 Hz), 3.04–3.09 (2H, m), 4.78 (1H, tt, *J*=3.9 Hz, 12.1 Hz), 5.52 (1H, br s), 6.24 (1H, dd, *J*=1.2 Hz, 3.6 Hz), 7.23–7.28 (2H, m), 7.46 (1H, br s), 7.47–7.52 (3H, m).

***N*-Phenyl-*N*-[1-[2-(4-hydroxyphenyl)ethyl]piperidin-4-yl]furan-2-carboxamide (4'-Hydroxy-furanylfentanyl)**

A mixture of 40 mg of nor-furanylfentanyl (0.15 mmol), 90 mg of 2-(4-benzyloxyphenyl)-ethyl methanesulfonate (0.29 mmol, prepared from 2-(4-benzyloxyphenyl)-ethanol [11], and 80 mg of potassium carbonate in 6 mL of ethanol was heated at 90 °C for 20 h. Water was added to the reaction mixture; subsequently, the solution was basified with ammonium hydroxide and extracted with chloroform. The solvent was evaporated to dryness under vacuum and the residue was purified by flash chromatography (column, silica gel; solvent, chloroform/methanol), to obtain 37 mg of 4-benzyloxy-furanylfentanyl (0.077 mmol, 51%). This 37 mg sample of 4-benzyloxy-furanylfentanyl (0.077 mmol) was dissolved in the mixture of 0.31 mL of thioanisole and 1.03 mL of trifluoro acetic acid, which was then stirred at room temperature for 3 h. Water was added to the reaction mixture, the pH of the solution was adjusted to 9 with ammonium hydroxide, and the mixture was extracted with chloroform/2-propanol (3:1). The solvent was evaporated to dryness under vacuum and the residue was purified by flash chromatography (column, silica gel; solvent, chloroform/methanol). The residue obtained was converted to hydrochloride salt by the addition of concentrated hydrochloric acid/methanol (1:9), and the hydrochloride salt was precipitated by adding diethylether, to obtain 17 mg of *N*-phenyl-*N*-[1-[2-(4-hydroxyphenyl)ethyl]piperidin-4-yl]furan-2-carboxamide (4'-hydroxy-furanylfentanyl) hydrochloride as a white powder (0.040 mmol, 52%).

4'-Hydroxy-furanylfentanyl hydrochloride—measured accurate mass of protonated molecule: 391.2009 (exact mass, 391.2016; mass accuracy, 1.8 ppm). ¹H-NMR

(CD₃OD) δ: 1.83 (2H, br q, *J*=11.4 Hz), 2.24 (2H, br d, *J*=13.2 Hz), 2.87–2.91 (2H, m), 3.16–3.26 (4H, m), 3.66 (2H, br d, *J*=10.8 Hz), 5.63 (1H, br s), 6.27 (1H, dd, *J*=1.5 Hz, 3.3 Hz), 6.74 (2H, d, *J*=8.4 Hz), 7.07 (2H, d, *J*=9.0 Hz), 7.31–7.34 (2H, m), 7.49 (1H, br s), 7.52–7.56 (3H, m). A proton signal (4.9 ppm) was overlapped with the signal derived from the solvent.

***N*-Phenyl-*N*-[1-(2-hydroxy-2-phenylethyl)piperidin-4-yl]furan-2-carboxamide (β-Hydroxy-furanylfentanyl)**

A mixture of 20 mg of nor-furanylfentanyl (0.074 mmol), 23 mg of phenacyl chloride (0.15 mmol), and 10 mg of sodium bicarbonate in 2 mL of ethanol was heated at 90 °C for 3 h. After cooling the reaction mixture to room temperature, 26 mg of sodium borohydride (0.69 mmol) was added and the solution was stirred for 30 min. Water was added to the reaction mixture, which was then basified with ammonium hydroxide and extracted with chloroform/2-propanol (3:1). The solvent was evaporated to dryness under vacuum and the residue was purified by preparative thin-layer chromatography (plate, silica gel; solvent, chloroform/ethyl acetate (1:1)). The purified product was converted to hydrochloride salt as described above, to obtain 17 mg of *N*-phenyl-*N*-[1-(2-hydroxy-2-phenylethyl)piperidin-4-yl]furan-2-carboxamide (β-hydroxy-furanylfentanyl) hydrochloride as a white powder (0.040 mmol, 54%).

β-Hydroxy-furanylfentanyl hydrochloride—measured accurate mass of protonated molecule: 391.2007 (exact mass, 391.2016; mass accuracy, 2.3 ppm). ¹H-NMR (CD₃OD) δ: 1.89 (1H, br q, *J*=13.6 Hz), 1.96 (1H, br q, *J*=12.8 Hz), 2.20 (1H, br d, *J*=12.6 Hz), 2.28 (1H, br d, *J*=12.6 Hz), 3.17–3.30 (4H, m), 3.69 (1H, br d, *J*=11.4 Hz), 3.86 (1H, br d, *J*=9.6 Hz), 4.90–4.98 (1H, m), 5.06 (1H, br d, *J*=9.0 Hz), 5.62 (1H, br s), 6.27 (1H, dd, *J*=2.1 Hz, 3.3 Hz), 7.30–7.35 (3H, m), 7.36–7.44 (4H, m), 7.49 (1H, br s), 7.52–7.57 (3H, m).

***N*-Phenyl-*N*-[1-[2-(4-hydroxy-3-methoxyphenyl)ethyl]piperidin-4-yl]furan-2-carboxamide (4'-Hydroxy-3'-methoxy-furanylfentanyl)**

A 10 mg sample of 4'-hydroxy-3'-methoxy-furanylfentanyl hydrochloride (0.022 mmol, 15%) was prepared from 40 mg of nor-furanylfentanyl (0.15 mmol) via the method used for the synthesis of 4'-hydroxy-furanylfentanyl.

4'-Hydroxy-3'-methoxy-furanylfentanyl hydrochloride—measured accurate mass of protonated molecule: 421.2122 (exact mass, 421.2122; mass accuracy, 0 ppm). ¹H-NMR (CD₃OD) δ: 1.84 (2H, br q, *J*=13.2 Hz), 2.26 (2H, br d, *J*=12.6 Hz), 2.88–2.93 (2H, m), 3.32 (2H, br t, *J*=13.2 Hz), 3.25–3.30 (2H, m), 3.68 (2H, br d, *J*=11.4 Hz), 3.84 (3H, s),

4.90–4.97 (1H, m), 5.63 (1H, br s), 6.27 (1H, dd, $J=2.1$ Hz, 3.3 Hz), 6.68 (1H, dd, $J=1.8$ Hz, 7.8 Hz), 6.74 (1H, d, $J=8.4$ Hz), 6.83 (1H, d, $J=1.8$ Hz), 7.30–7.34 (2H, m), 7.49 (1H, br s), 7.51–7.56 (3H, m).

***N*-Phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]-2-oxo-4-carboxybutanamide (Ring-opened carboxylic acid metabolite, ring-opened-CBA)**

A 95 mg sample of *N*-phenyl-*N*-[1-(2-phenylethyl)piperidine-4-yl]-2-hydroxy-4-carboxybutanamide (0.23 mmol, 68%) was prepared from a 94 mg sample of 1-phenethyl-*N*-phenylpiperidin-4-amine (4-ANPP, 0.34 mmol) using a method reported previously [9]. To the solution of 40 mg of *N*-phenyl-*N*-[1-(2-phenylethyl)piperidine-4-yl]-2-hydroxy-4-carboxybutanamide (0.097 mmol) in 4 mL of acetone that was cooled in an ice bath, 0.1 mL of the Jones reagent was added. The solution was then sonicated at room temperature for 30 min. The Jones reagent was prepared as follows: to 1 mL of water that was cooled in an ice bath, 0.5 mL of concentrated sulfuric acid was added, followed by the incorporation of 0.5 g of chromium (VI) oxide and 1.2 mL of water. The reaction mixture was extracted with chloroform/2-propanol (3:1). The solvent was evaporated to dryness under vacuum and the residue was purified by flash chromatography (column, silica gel; solvent, chloroform/methanol), to obtain 15 mg of *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]-2-oxo-4-carboxybutanamide (ring-opened carboxylic acid metabolite (ring-opened CBA)) as a clear oil (0.037 mmol, 38%).

Ring-opened CBA (free base)—measured accurate mass of protonated molecule: 409.2120 (exact mass, 409.2122; mass accuracy, 0.5 ppm). $^1\text{H-NMR}$ (DMSO- d_6) δ : 1.33 (2H, qd, $J=3.4$ Hz, 12.2 Hz), 1.78 (2H, br d, $J=12.0$ Hz), 2.05 (2H, t, $J=11.7$ Hz), 2.16 (2H, t, $J=6.6$ Hz), 2.45 (2H, t, $J=7.5$ Hz), 2.65 (2H, t, $J=7.8$ Hz), 2.81 (2H, t, $J=6.6$ Hz), 2.95 (2H, br d, $J=12.0$ Hz), 4.32 (1H, tt, $J=3.7$ Hz, 11.9 Hz), 7.11–7.18 (3H, m), 7.21–7.29 (4H, m), 7.37–7.42 (3H, m).

Incubation of PXB-cells with furanylfentanyl

PXB-cells seeded in a 24-well plate were incubated for 11 days at 37 °C and 5% CO₂ before the addition of the drug. The culture medium was replaced every 2 days. Furanylfentanyl hydrochloride (1 mM, dissolved in dimethylsulfoxide) was added to the cells at a final concentration of 10 μM , followed by continuous incubation of the cells. The medium was sampled 48 h after the addition of the drug and stored at –20 °C until analysis.

LC/MS analysis for the identification of the metabolites of furanylfentanyl

To identify the metabolites of furanylfentanyl, the culture medium was hydrolyzed, deproteinized, and analyzed by LC/MS. The culture medium (25 μL) was incubated with 15 μL of 0.25 M acetate buffer (pH 5.0) containing β -glucuronidase/aryl sulfatase (β -glucuronidase, 0.01 unit) at 60 °C for 1.5 h. Acetonitrile (250 μL) was added to the hydrolyzed sample and the mixture was vortexed and centrifuged at 10,000 $\times g$ for 5 min. The supernatant was evaporated to dryness under a gentle stream of nitrogen and the residue was dissolved in the initial mobile phase (100 μL) for LC/MS analysis. LC/MS analyses were carried out using an Exion LC system connected to a QTRAP 4500 mass spectrometer (SCIEX, Framingham, MA, USA). The conditions were as follows: column, XBridge BEH C18 (2.1 $\times$ 150 mm; particle diameter, 3.5 μm ; Waters, Milford, MA, USA) maintained at 40 °C; mobile phase composition, 10 mM ammonium acetate (A) and methanol (B); linear gradient mode, 20% to 90% B over 15 min, 90% B for 5 min, and 90% to 20% B over 0.1 min; flow rate, 0.2 mL/min; MS interface, ESI (positive and negative); analysis mode, scan (m/z 100–650), and product ion analysis (ESI-positive mode; collision energy 40 eV; precursor ions, protonated molecule of each compound).

Results

Synthesis of the metabolites of furanylfentanyl

Five putative metabolites of furanylfentanyl were chemically synthesized in this study. Nor-furanylfentanyl was synthesized by *N*-debenzylation of the precursor compound. Catalytic hydrogenation is preferably used for *N*-debenzylation, because the procedure is simple and easy and the product is usually pure. However, in the present case, catalytic hydrogenation was not applicable, because the hydrogenation of the furan ring occurs at the same time. 1-Chloroethyl-chloroformate is a useful reagent for *N*-dealkylation [12]; therefore, it was used successfully for *N*-debenzylation in this study. On the other hand, 4'-hydroxy-furanylfentanyl and 4'-hydroxy-3'-methoxy-furanylfentanyl were synthesized by *O*-debenzylation of the precursor compounds. For the reason detailed above, catalytic hydrogenation was not applicable for these reactions; however, 4'-hydroxy-furanylfentanyl and 4'-hydroxy-3'-methoxy-furanylfentanyl were successfully synthesized by *O*-debenzylation using thioanisole/trifluoroacetic acid [13]. Ring-opened CBA was synthesized by oxidation of the precursor compound, which contains a 2-hydroxy-4-carboxy-butanamide moiety. The oxidation

of the hydroxyl group of 2-hydroxy-4-carboxy-butanamide moiety to 2-oxo-4-carboxy-butanamide moiety was successfully achieved using the Jones reagent.

Fig. 3 Total ion chromatogram (TIC) and extracted ion chromatograms (EICs) of the culture medium, as acquired using liquid chromatography/mass spectrometry (LC/MS)

Fig. 4 EICs of the authentic standards of the furanylfentanyl metabolites, as acquired using LC/MS

Detection and identification of the metabolites of furanylfentanyl

The total ion chromatogram (TIC) and extracted ion chromatograms (EICs) under positive ESI mode obtained from the culture medium of human hepatocytes incubated with furanylfentanyl are shown in Fig. 3. Peaks corresponding to the unchanged furanylfentanyl (peak 1) and its four metabolites (peaks 3–6) were detected on the EICs. Peaks 3–6 were presumed to be 4'-hydroxy-furanylfentanyl, β -hydroxy-furanylfentanyl, 4'-hydroxy-3'-methoxy-furanylfentanyl, and ring-opened CBA, respectively. The EICs obtained from the authentic standards are depicted in Fig. 4. The retention times of peaks 1 and 3–6 presented in Fig. 3 were coincident with those presented in Fig. 4. Figures 5 and 6 depict the product ion spectra of the peaks illustrated in Figs. 3 and 4, respectively. The product ion spectra of the metabolites in the culture medium (peaks 3–6, Fig. 5) were coincident well with those of authentic standards (Fig. 6). In Fig. 3, the intensity of peak 6 was extremely high, whereas peaks 3–5 were detected at trace levels, indicating that ring-opened CBA was the main metabolite of furanylfentanyl. Nor-furanylfentanyl (peak 2), which is a desphenethylated metabolite of furanylfentanyl, was not detected in the culture medium (Fig. 3).

Fig. 5 Product ion spectra of furanylfentanyl metabolites detected in the culture medium of fresh human hepatocytes

Discussion

The proposed metabolic pathway of furanylfentanyl is shown in Fig. 7. The present study identified 4'-hydroxy-furanylfentanyl, β -hydroxy-furanylfentanyl, 4'-hydroxy-3'-methoxy-furanylfentanyl, and ring-opened CBA as the metabolites of furanylfentanyl. Ring-opened CBA was considered as the main metabolite of furanylfentanyl, based on the peak intensities of the metabolites. In case of fentanyl, the desphenethylated metabolite (nor-fentanyl) is a main metabolite *in vivo* [14] and *in vitro* [15]; however, here, this type of metabolite was not formed from furanylfentanyl. In addition, the deamide metabolite 4-ANPP was not detected, although it had been detected in previous studies [6, 7]. It is unclear why 4-ANPP was not detected in our study; 4-ANPP is thought to be formed by amide-hydrolysis of furanylfentanyl, catalyzed by a certain kind of amidase. PXB-cells, fresh human hepatocytes used in this study, may lack the activity of amidase involved in the formation of 4-ANPP.

In previous studies, a dihydrodiol metabolite was detected in *in vivo* and *in vitro* samples [6, 7]. Goggin et al. detected a metabolite of m/z 409 (protonated molecular) in a urine

sample, and suggested three compounds, i.e. dihydrodiol metabolite, dihydrodiol metabolite isomer, and ring-opened CBA, as candidates for this peak [6]. Those authors ruled out ring-opened CBA based on the behavior of the peak in LC/MS, and concluded that the peak corresponded to the dihydrodiol metabolite. However, definite identification of this metabolite using an authentic standard was not performed, and this meant that the exact structure of this metabolite remained obscure. In this study, ring-opened-CBA was definitely identified in the culture medium of hepatocytes using a synthesized authentic standard. The retention time and the product ion spectrum of the peak detected on the EIC of m/z 409 of the culture medium were identical to those of the authentic standard of ring-opened CBA. In addition, the above peak was detectable in a negative ESI mode (data not shown), corroborating the presence of a carboxyl group in this compound. Dihydrodiol metabolite is not thought to be detectable in a negative mode, because it does not have a functional group which can be an anion in the mobile phase. Other peaks were not detected on the EIC of m/z 409; therefore, we concluded that ring-opened CBA, rather than the dihydrodiol metabolite, was formed from furanylfentanyl in hepatocytes. It might be

Fig. 6 Product ion spectra of the authentic standards of furanyl/fentanyl metabolites

possible that the authors of the previous study [6, 7] misidentified the ring-opened CBA as the dihydrodiol metabolite, because they did not use an authentic standard to identify this metabolite. Needless to say, our above assertion would be distinctly verified if the authentic standard of dihydrodiol metabolite was available; however, the standard could not be obtained due to the difficulty in synthesizing this compound.

On the other hand, several studies have reported the formation of ring-opened CBAs from furan-ring-containing drugs. Erve et al. reported that a ring-opened-CBA metabolite (M7) of prazosin, which is a α_1 -blocker, was formed by human and animal liver microsomes, as well as human and rat hepatocytes (Fig. 8) [16]. However, those authors did not confirm the identity of the metabolite using an authentic standard.

Fig. 7 Newly proposed metabolic pathway of furanylfentanyl

Fig. 8 Formation of ring-opened carboxylic acid metabolites from furan-ring-containing drugs

Williams et al. detected a ring-opened CBA (γ -ketocarboxylic acid) of furosemide, which is a loop diuretic, in the bile sample of a rat that was administered furosemide (Fig. 8) [17]. It should be noted that those authors confirmed the structure of the ring-opened CBA by NMR spectra. These reports corroborate our finding that ring-opened-CBA is a metabolite of the furan-ring-containing drug furanylfentanyl.

Conclusions

The metabolism of a new synthetic opioid, furanylfentanyl, was investigated using fresh human hepatocytes; four new metabolites of furanylfentanyl were identified using authentic standards. The findings of this study revealed that the ring-opened carboxylic acid metabolite, rather than the dihydrodiol metabolite, was formed from furanylfentanyl. This is consistent with that observed for the other furan-ring-containing drugs.

Acknowledgement This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 19K10702.

Compliance with ethical standards

Conflict of interest There are no conflicts to declare.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- United Nations Office on Drugs and Crime. World drug report 2019; 3 depressants. https://wdr.unodc.org/wdr2019/prelaunch/WDR19_Booklet_3_DEPRESSANTS.pdf (Accessed Mar 16, 2020)
- United Nations Office on Drugs and Crime. Fentanyl and its analogues - 50 years on. Global Smart Update. https://www.unodc.org/documents/scientific/Global_SMART_Update_17_web.pdf (Accessed Mar 16, 2020)
- Wilde M, Pichini S, Pacifici R, Tagliabracchi A, Busardò FP, Auwärter V, Solimini R (2019) Metabolic pathways and potencies of new fentanyl analogs. *Front Pharmacol*. <https://doi.org/10.3389/fphar.2019.00238>
- Drug Enforcement Administration, Department of Justice (2016) Schedules of controlled substances: temporary placement of furanylfentanyl into schedule I. *Fed Regist* 81:85873–85877
- European Monitoring Centre for Drugs and Drug Addiction (2017) Report on the risk assessment of *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]furan-2-carboxamide (furanylfentanyl) in the framework of the Council Decision on new psychoactive substances. Publications Office of the European Union, Luxembourg. https://www.emcdda.europa.eu/system/files/publications/6712/20176480_TDAK17002ENN_PDF.pdf (Accessed Mar 16, 2020)
- Goggin MM, Nguyen A, Janis GC (2017) Identification of unique metabolites of the designer opioid furanyl fentanyl. *J Anal Toxicol* 41:367–375. <https://doi.org/10.1093/jat/bkx022>
- Watanabe S, Vikingsson S, Roman M, Green H, Kronstrand R, Wohlfarth A (2017) In vitro and in vivo metabolite identification studies for the new synthetic opioids acetylfentanyl, acrylfentanyl, furanylfentanyl, and 4-fluoro-Isobutyrylfentanyl. *AAPS J* 19:1102–1122. <https://doi.org/10.1208/s12248-017-0070-z>
- Yamasaki C, Kataoka M, Kato Y, Kakuni M, Usuda S, Ohzone Y, Matsuda S, Adachi Y, Ninomiya S, Itamoto T, Asahara T, Yoshizato K, Tatenos C (2010) In vitro evaluation of cytochrome P450 and glucuronidation activities in hepatocytes isolated from liver-humanized mice. *Drug Metab Pharmacokinet* 25:539–550. <https://doi.org/10.2133/dmpk.dmpk-10-rg-047>
- Kanamori T, Segawa H, Yamamuro T, Kuwayama K, Tsujikawa K, Iwata YT (2020) Metabolism of a new synthetic opioid tetrahydrofuranylfentanyl in fresh isolated human hepatocytes: Detection and confirmation of ring-opened metabolites. *Drug Test Anal* 12:439–448. <https://doi.org/10.1002/dta.2743>
- Siegfried. Synthesis of fentanyl. <https://erowid.org/archive/rhodium/chemistry/fentanyl.html> (Accessed Mar 16, 2020)
- Crossland RK, Servis KL (1970) Synthesis of methanesulfonate esters (mesylates) from alcohols. *J Org Chem* 35:3195–3196
- Olofson RA (1988) New, useful reactions of novel haloformates and related reagents. *Pure Appl Chem* 60:1715–1724. <https://doi.org/10.1351/pac198860111715>
- Kiso Y, Ukawa K, Nakamura S, Ito K, Akita T (1980) Efficient removal of protecting groups by a 'push-pull' mechanism. II. Deprotection of *O*-benzyltyrosine with a thioanisole-trifluoroacetic acid system without *O*-to-*C* rearrangements. *Chem Pharm Bull* 28:673–676. <https://doi.org/10.1248/cpb.28.673>
- Goromaru T, Kawauchi T, Katashima M, Matsuura H, Yoshimura N, Sameshima T, Miyawaki T (1986) Urinary excretion of fentanyl and its metabolites at high-dose infusion (in Japanese with English abstract). *Masui* 35:35–39
- Kanamori T, Iwata YT, Segawa H, Yamamuro T, Kuwayama K, Tsujikawa K, Inoue H (2018) Use of hepatocytes isolated from a liver-humanized mouse for studies on the metabolism of drugs: application to the metabolism of fentanyl and acetylfentanyl. *Forensic Toxicol* 36:467–475. <https://doi.org/10.1007/s11419-018-0425-x>
- Erve JC, Vashishtha SC, DeMaio W, Talaat RE (2007) Metabolism of prazosin in rat, dog, and human liver microsomes and cryopreserved rat and human hepatocytes and characterization of metabolites by liquid chromatography/tandem mass spectrometry. *Drug Metab Dispos* 35:908–916. <https://doi.org/10.1124/dmd.106.013219>
- Williams DP, Antoine DJ, Butler PJ, Jones R, Randle L, Payne A, Howard M, Gardner I, Blagg J, Park BK (2007) The metabolism and toxicity of furosemide in the Wistar rat and CD-1 mouse: a chemical and biochemical definition of the toxicophore. *J Pharmacol Exp Ther* 322:1208–1220. <https://doi.org/10.1124/jpet.107.125302>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.