Urine Concentrations of Fentanyl and Norfentanyl During Application of Duragesic® Transdermal Patches

Alphonse Poklis*
Department of Pathology, Virginia Commonwealth University School of Medicine, Richmond, Virginia 23298-0165

Ronald Backer
Ameritox Laboratory, 9930 West Highway 80, Midland, Texas 79706

Abstract

A study of the urinary concentration of fentanyl (F) and its major metabolite norfentanyl (NF) in chronic pain patients treated with the Duragesic continuous release transdermal patches is presented. These patches are available in 10, 20, 30, and 40 cm² sizes releasing 25, 50, 75, and 100 µg/h F, respectively. F is rapidly and extensively metabolized, with NF as the major metabolite. Five hundred-forty six random urine specimens were collected from chronic pain patients wearing 25, 50, 75, or 100 µg F transdermal patches. Urine specimens were collected from hours after application to several days later after continuous F release. Each specimen was analyzed for F, NF, creatinine, and pH. Additionally, each was screened by enzyme immunoassay for the following: amphetamines, barbiturates, benzodiazepines, cocaine metabolite, methadone, phenycyclidine, d-propoxyphene, opiates, and marijuana metabolites. All positive screening results were confirmed by gas chromatography–mass spectrometry (GC–MS). The LODs and LOQs for F and NF were 3 ng/mL, respectively. The results of F and NF analysis of urine form those wearing 25 µg patches (N = 142) was mean F, 47 ng/mL with a range of 0 to 983 ng/mL, and 97% of the specimens contained < 200 ng/mL and mean NF, 175 ng/mL with a range of 0-980 ng/mL, while 95% of the specimens contained < 400 ng/mL. The results of F and NF analysis of urine form those wearing 50 µg patches (N = 184) was: mean F, 74 ng/mL with a range of 0 to 589 ng/mL, and 92% of the specimens contained < 200 ng/mL and mean NF, 257 ng/mL with a range of 0-2200 ng/mL, and 98% of the specimens contained < 1000 ng/mL. The results of F and NF analysis of urine form those wearing 75 µg patches (N = 85) was mean F, 107 ng/mL with a range of 0 to 1280 ng/mL, and 98% of the specimens contained < 400 ng/mL and mean NF, 328 ng/mL with a range of 0-5630 ng/mL, and 99% of the specimens contained < 1000 ng/mL. The results of F and NF analysis of urine form those wearing 100 µg patches (N = 135) was mean F, 100 ng/mL with a range of 0 to 1080 ng/mL, while 96% of the specimens contained < 400 ng/mL and mean NF, 373 ng/mL with a range of 0–5730 ng/mL, and 95% of the specimens contained < 1000 ng/mL. The incidence of other drugs detected as a percentage the specimens was opiates, 48%, benzodiazepines, 43%; barbiturates, 3%; methadone, 4%; marijuana metabolite, 3%; and cocaine metabolite, 1%. With the exception of F and/or NF, no other drugs were detected in 25% of the specimens. These data demonstrate the wide variation in concentrations of F and NF in random urine specimens following application of Duragesic patches. However, these values obtained during therapeutic use far exceed concentrations previously reported in fatal poisoning. In general, one may expect to find urine NF concentrations 3–4 times higher than those of F.

Introduction

Fentanyl [N-(1-phenethyl-4-piperidyl)propionanilide] is a synthetic narcotic analgesic of high potency and a short duration of action. The drug is 80 times more potent than morphine (1). Injections of 50–100 µg produce analgesia and rapid unconsciousness. The incidences of incomplete amnesia, hypotension, or hypertension are less than that associated with morphine and the duration of respiratory depression is shorter. For these reasons, it is the primary analgesic in surgical procedures performed in the United States; indicated as a preanesthetic medication, a primary anesthetic, and a postsurgical anesthetic (2). Fentanyl is also available in a continuous release transdermal patch (Duragesic) that is designed to release 25 µg/h per 10 cm² of surface area. The patches are available in 10, 20, 30, and 40 cm² sizes releasing 25, 50, 75, and 100 µg/h, respectively (3). The fentanyl patch is indicated in the management of chronic pain for patients requiring opioid analgesia. Serum, plasma, and blood concentrations 8 to 12 h after patch application are generally similar to those achieved with equal doses of fentanyl administered by continuous intravenous infusion (4). In controlled studies of non-opiate-tolerant patients, Duragesic (100 µg/h) provided pain relief equivalent to 60 mg/day of morphine.
Fentanyl is rapidly and extensively metabolized, with norfentanyl as its major urinary metabolite (5-7). At present, the literature contains little data concerning urine concentrations of fentanyl following therapeutic administration, particularly during use of the Duragesic. We present the urine concentrations of fentanyl and norfentanyl in chronic pain patients treated with Duragesic transdermal patches.

Material and Methods

Specimens

Random urine specimens were collected from 546 chronic pain patients wearing 25, 50, 75, or 100 µg/h Duragesic transdermal patches.

Urine specimens were collected from hours after application to several days after continuous fentanyl release. The specimens were submitted from pain clinics for compliance testing and the prescribed dose for each opiate drug including Duragesic transdermal patch was indicated on the request form.

Study protocol

Each specimen was initially screened by enzyme immunoassay for the following drugs or drug classes: amphetamines, barbiturates, benzodiazepines, cocaine metabolite, dextropropoxyphene, methadone, phencyclidine, opiates, and marijuana metabolites. All positive immunoassay results were confirmed by gas chromatography–mass spectrometry (GC–MS). Additionally, each urine specimen was analyzed for fentanyl and norfentanyl by GC–MS and creatinine and pH. The results of fentanyl testing were divided into four groups dependent on the dose of the fentanyl transdermal patch.

Initial immunoassay

The DRI® amphetamines (cut-off, 1000 ng/mL), barbiturates (cut-off, 200 ng/mL), benzodiazepines (cut-off, 200 ng/mL), cocaine metabolite (cut-off, 300 ng/mL), dextropropoxyphene (cut-off, 300 ng/mL) methadone (cut-off, 300 ng/mL), phencyclidine (cut-off, 25 ng/mL), opiates (cut-off, 300 ng/mL), and marijuana metabolites (cut-off, 25 ng/mL) enzyme immunoassays were obtained from Diagnostic Reagents, Inc. (Sunnyvale, CA). All DRI assays were performed on Hitachi 717 Automatic Analyzer as recommended by the manufacturer. Each assay was calibrated with a drug-free calibrator and its appropriate cut-off calibrator. Appropriate control urines for each assay containing a drug-free sample, 75% of the cutoff, and 125% of cutoff were obtained from Diagnostic Reagents, Inc.

Extraction of fentanyl and norfentanyl

To 2.0 mL of drug-free calibrator, 25 ng/mL calibrator, control urine of 30 ng/mL, or test samples was added 10 µL of internal standard solution containing fentanyl-d₅ and norfentanyl-d₅ (final concentration of 50 ng/mL). To each sample was added 5 mL of Detectabasic Buffer (pH 9.1, Biochemical Diagnostics). The samples were then added to solid-phase extraction columns (Biochemical Diagnostics GV-65) previously washed with methanol. The samples were allowed to flow through by gravity, then each column was washed with 5 mL of pH 9.1 buffer. The columns were dried by applying a vacuum (5-15 in. Hg) for 10 min. Fentanyl and norfentanyl were eluted with 1.5 mL of acetonitrile/n-butylchloride (55:45). The elution solution was then evaporated to dryness with gentle heat under nitrogen. The acetyl derivative of norfentanyl was prepared by adding 50 µL of acetic anhydride and 50 µL pyridine to each dried extract and heating at 80°C for 15 min in a bead bath. The mixtures were then evaporated to dryness with gentle heat under nitrogen. Resultant residues were reconstituted with 100 µL of ethyl acetate and transferred to GC–MS automatic sampler vials. Aliquots (2.5 µL) were injected into the GC–MS.

GC–MS of fentanyl and norfentanyl

GC–MS analysis was performed on a Hewlett-Packard (Avondale, CA) 5890 GC equipped with a cross-linked 5% phenylmethylsilicone capillary column with a 12-m guard column (HP-5, 30 m × 0.25-mm i.d., 0.25-µm film thickness, Hewlett-Packard, Avondale, CA) connected to a Hewlett-Packard 5973-A mass selective detector. The GC–MS was operated in the splitless mode with a helium carrier gas linear velocity of 20 mL/min. Initial oven temperature was 100°C for 1 min. with an injection port temperature of 250°C. The temperature was ramped at 25°C/min to a final temperature of 280°C that was held for 6 min, resulting in a total run time of 14.2 min. Data were collected in the SIM mode with a dwell time of 50 ms for each ion, monitoring m/z 245, 146, 246 (fentanyl); 250, 151, 194 (fentanyl-d₃); 231, 158, 132 (acetyl-norfentanyl derivative); and 236 (acetyl-norfentanyl-d₅). Quantification of fentanyl and norfentanyl was achieved by comparing ions 250/245 and 236/231, respectively. The lower limit of detection and lower limit of quantification for both fentanyl and norfentanyl was 3 ng/mL. The upper limits of linearity for fentanyl and norfentanyl were 250 and 400 ng/mL, respectively.

Results

The results of urine analysis for fentanyl and norfentanyl are presented in Table I. Results are divided into four groups dependent upon the dose of the fentanyl transdermal patch: 25 µg/h, 50 µg/h, 75 µg/h, and 100 µg/h. Considering all specimens, the mean urine fentanyl concentration for each group show an apparent differentiation between expected fentanyl urine values following 25 µg/h (mean, 47 ng/mL), 50 µg/h (mean, 74 ng/mL) and those observed following 75 µg/h (mean, 107 ng/mL) and 100 µg/h (mean, 100 ng/mL) doses. A similar relationship appears apparent by considering the mean urine norfentanyl concentration observed in each fentanyl dose group. Norfentanyl urine values seem to differentiate between fentanyl doses of 25 µg/h (mean, 175 ng/mL) and 50 µg/h (mean, 257 ng/mL) and those observed following 75 µg/h (mean, 328 ng/mL) and 100 µg/h (mean, 373 ng/mL). Mean fentanyl and norfentanyl urine concentrations and the range of fentanyl and norfentanyl urine concentrations, following
75 μg/h and 100 μg/h fentanyl dose, were indistinguishable. However, in reviewing our cumulative data there was considerable overlap of the fentanyl and norfentanyl concentrations in individual specimens following the various doses. For example, following 25-μg fentanyl doses, many subjects had fentanyl and norfentanyl urine concentrations greater than values observed in urines collected following a 50-μg dose. No significant correlation was found between fentanyl and norfentanyl concentrations within each dose group. When fentanyl and norfentanyl urine concentrations were normalized against creatinine, no significant relationship was found within each dose group. A lack of such correlation is not surprising given the nature of the specimens. Urine was collected at random and at anytime within a dosage window of a few hours after application of the patch to 24 h after the patch was removed. Subjects may have applied the patch for the first time or had been wearing a patch for several days.

Within each dose group, several specimens contained fentanyl and/or norfentanyl concentrations an order of magnitude greater than the mean value (Table I). Although fentanyl concentrations ranged up to 983 ng/mL and norfentanyl values were as high as 980 ng/mL after use of the 25 μg/h patch, 98% of these specimens contained < 200 ng/mL of fentanyl and 95% of the specimens contained < 400 ng/mL of norfentanyl. Similarly, after application of the 50 μg/h patch, 92% contained < 200 ng/mL of fentanyl and 98% contained < 1000 ng/mL of norfentanyl. To evaluate a more representative sample of our data, 10% of specimens were removed from the data set; 5% of the lowest and 5% of the highest fentanyl concentrations, and the was data re-evaluated (Table I). Fentanyl concentrations were used for re-evaluations as most toxicology laboratories analyze urine for fentanyl and not norfentanyl (1). The resultant re-evaluated mean and range of fentanyl and norfentanyl concentrations were much lower than within the entire data set and provide a clearer presentation of typical concentrations observed in the specimens. Of particular note, fentanyl concentrations averaged less than 100 ng/mL following application of Duragesic at all four different doses, 25 μg/h to 100 μg/h. In general, fentanyl and norfentanyl urine concentrations following 25 μg/h dose average less than half the values observed during 75 μg/h and 100 μg/h doses.

The results of the initial screening and confirmation of other drugs in the specimens is presented in Table II. As expected in a population of patients treated for chronic pain, the most commonly encountered drugs were semi-synthetic opiates and benzodiazepines. Several patients were also using illicit drugs. The use of drugs that have not been prescribed is a common practice of self-medication by pain patients to deal with their chronic pain (8).

### Discussion

Fentanyl is rapidly and extensively metabolized, with oxidative N-dealkylation to norfentanyl as the major metabolite (5–7). Amide hydrolysis to the despropionyl metabolite also occurs. Minor pathways include hydroxylation of the piperidine ring, the phenyl rings, or the propionyl side chain (6). None of these metabolites has analgesic activity. Following an intravenous dose, fentanyl is eliminated within 72 h, with nearly one-half of this amount excreted in the first 8 h (4,5). Less than 6% of the administered dose is excreted as unchanged drug. Urine concentrations in overdose cases, primarily following intravenous injection, have only ranged from 5 to 93 ng/mL (9). However, during cardiac bypass surgery with administration of up to a total of 5 mg of fentanyl over 5 h, peak urinary concentrations ranged as high as 500 ng/mL (10). Urine concentrations of fentanyl in overdose cases associated with Duragesic transdermal patches have been reported to 3–895 ng/mL (11). In our study, fentanyl urine concentrations in many specimens during therapeutic Duragesic far exceed these concentrations previously reported in fatal poisoning. Urine norfentanyl concentrations have not been reported in these other studies.

The terminal plasma half-life of fentanyl varies widely, from 1.5 to 6.0 h. Such a wide variation is likely related to differences in individual rates of biotransformation, intrahepatic recirculation, age, and the physiological status of patients (1,12). In ad-

---

**Table I. Urine Concentrations of Fentanyl and Norfentanyl Following the Application of Duragesic Transdermal Patch**

<table>
<thead>
<tr>
<th>Duragesic Patch, Dose of Fentanyl</th>
<th>25 μg/h</th>
<th>50 μg/h</th>
<th>75 μg/h</th>
<th>100 μg/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>All urine specimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of specimens</td>
<td>142</td>
<td>184</td>
<td>85</td>
<td>135</td>
</tr>
<tr>
<td>Fentanyl mean (ng/mL)</td>
<td>47</td>
<td>74</td>
<td>107</td>
<td>100</td>
</tr>
<tr>
<td>Range (ng/mL)</td>
<td>0–983</td>
<td>0–589</td>
<td>0–1280</td>
<td>0–1080</td>
</tr>
<tr>
<td>Norfentanyl mean (ng/mL)</td>
<td>175</td>
<td>257</td>
<td>328</td>
<td>373</td>
</tr>
<tr>
<td>Range (ng/mL)</td>
<td>0–980</td>
<td>0–2200</td>
<td>0–5630</td>
<td>0–5730</td>
</tr>
<tr>
<td>90% of urine specimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of specimens</td>
<td>128</td>
<td>166</td>
<td>77</td>
<td>121</td>
</tr>
<tr>
<td>Fentanyl mean (ng/mL)</td>
<td>32</td>
<td>58</td>
<td>95</td>
<td>79</td>
</tr>
<tr>
<td>Range (ng/mL)</td>
<td>0–167</td>
<td>0–250</td>
<td>4–444</td>
<td>0–150</td>
</tr>
<tr>
<td>Norfentanyl mean (ng/mL)</td>
<td>173</td>
<td>251</td>
<td>285</td>
<td>327</td>
</tr>
<tr>
<td>Range (ng/mL)</td>
<td>0–980</td>
<td>0–860</td>
<td>4–1330</td>
<td>0–1670</td>
</tr>
</tbody>
</table>

**Table II. Incidence of Other Drugs Detected as a Percentage of the 546 Duragesic Urine Specimens**

<table>
<thead>
<tr>
<th>Opiates</th>
<th>48%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine</td>
<td></td>
</tr>
<tr>
<td>Hydromorphone</td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td></td>
</tr>
<tr>
<td>Oxycodone</td>
<td></td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>43%</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>3%</td>
</tr>
<tr>
<td>Methadone</td>
<td>4%</td>
</tr>
<tr>
<td>Marijuana metabolite</td>
<td>3%</td>
</tr>
<tr>
<td>Cocaine metabolite</td>
<td>1%</td>
</tr>
<tr>
<td>No drugs detected</td>
<td>25%</td>
</tr>
</tbody>
</table>
dition to the random collection of the specimens in the pre-
sented study, these physiological variations are also a factor in
the wide range of fentanyl and norfentanyl urine concentrations
observed in this study.

Our data could be manipulated by pharmacokinetic calcula-
tions to establish a relationship between fentanyl dose and
the mean urinary fentanyl and/or norfentanyl concentrations
observed. However, in terms of urine drug testing of a single
random specimen, we conclude that no precise estimate
of dose is possible given a specific fentanyl or norfentanyl
concentration. For interpretation of urine testing results, our
study may support the statement that < 200 ng/mL fentanyl
and/or < 1000 ng/mL norfentanyl is "consistent" with wearing
a 25 µg or 50 µg/h Duragesic transdermal patch. A urine
concentration of > 200 ng/mL fentanyl and/or > 1000 ng/mL
norfentanyl is "consistent" with wearing a 75 µg or 100 µg/h
Duragesic transdermal patch. However, it should be noted
that lower concentrations are also consistent with values ob-
served shortly after application or after removal of high-dose
patches, particularly in urine collected at random. As a general
statement, one may expect during Duragesic application urine
norfentanyl concentrations to be three to four times greater
than those of fentanyl during Duragesic therapy. In addition to
Duragesic therapy, chronic pain patients routinely are pre-
scribed other opiates and benzodiazepines.

Conclusions

This study demonstrated the wide variation in urine concen-
trations of fentanyl and norfentanyl in random specimens col-
lected during therapeutic use of the Duragesic transdermal
patch. Fentanyl and norfentanyl values may exceed 1000 ng/mL
in some specimens, values that exceed concentrations previ-
sely reported in fatal poisoning. In general, one may expect to
find urine norfentanyl concentrations three to four times higher
than those of fentanyl during Duragesic therapy.

References

1. A. Poklis. Fentanyl: a review for clinical and analytical toxicolo-
and Gilman's Pharmacological Basis of Therapeutics, 7th ed.,
4. K.A. Calis, D.R. Kohler, and D.M. Corso. Transdermally admin-
(1980).
6. P. Hess, G. Steibler, and A. Herz. Pharmacokinetics of fentanyl in
drug use among chronic pain clinic patients. Abstract. American
9. R.C. Baselt. Disposition of Toxic Drug and Chemicals in Man, 6th
ed. Chemical Toxicology Institute, Foster City, CA. 2003, pp
319–322.
10. J.G. Schwartz, J.C. Garnott, J.S. Somerset, E.J. Igler, R. Rodriguez,
and M.D. Orr. Measurements of fentanyl and sufentanil in blood
and urine after surgical application. Implications in detection of
11. D.T. Anderson and J.J. Muto. Duragesic® transdermal patch: post-
mortem tissue distribution of fentanyl in 25 cases. J. Anal. Toxicol.
of fentanyl as a possible explanation for recurrence of respiratory