STUDIES ON THE FATE OF FENTANYL

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INTRODUCTION

Fentanyl citrate,1,2 N-(1-phenethyl-4-piperidinyl) propioanilide dihydrogen citrate, is an extremely potent narcotic analgesic derived from meperidine and synthesized by Janssen in 1959. As the onset of its action is rapid and the duration of activity is very short, fentanyl has been widely used in Europe as a component of “Neuroleptanalgesia” since 1959.3-9 The term implies a state or condition of surgical analgesia with marked mental indifference induced by analgesics and neuroleptics.

As to the metabolism and excretion of fentanyl, it has been found that only up to 10% of it is excreted unchanged in the urine and the metabolic pathway of most of the remaining fentanyl is assumed to be as shown in Fig. 1.10,11 In any case,
the main pathway of the metabolism of fentanyl is not yet clearly determined, because the usual dosage of fentanyl is very small and it is excreted into urine very rapidly.

In this paper, we examined the possible pathway of fentanyl in rats by thinlayer-chromatography (TLC), gaschromatography (GC) and mass-spectrography (MS).

METHODS

1. Extraction of pure fentanyl from the commercially available fentanyl vial.

It is explained in the circular that one vial (10 ml) contains 5 mg of methyl-p-oxybenzoate, 500 microgram of propyl-p-oxybenzoate and 500 microgram of fentanyl (Fig. 2). Separation of fentanyl from these phenolic compounds is as follows (Fig. 3):

Fig. 2. Ingredients of one vial. (10 ml)

One vial (10 ml)  
| Evaporation  
Residue  
| 10%–NaOH  
Alk. solution  
| Extraction  
(CHCl₃)  
Aqueous layer  
| Evaporation  
Residue  
| Methanol  

Fig. 3. Extraction procedure of fentanyl.  
Fentanyl and phenolic groups are separated under this procedure.
Evaporate the solution in one vial to 1 ml in vacuo at 60°C and adjust pH to 9.0 with 10% NaOH. Extraction is carried out with 0.5 ml of CHCl₃ three times from the alkaline solution after adding about 50 mg of NaCl as salting out. Evaporate up CHCl₃ solution to dryness and dissolve the residue with 0.5 ml of methanol (sample-1).

2) Extraction of fentanyl from urine (Fig. 4).

One hundred microgram of fentanyl is added to human urine (50 ml) of healthy young male. Pass the urine through a charcoal column (Activated charcoal: 1 g, column: 1 × 30 cm). Elute with 20 ml of methanol containing 30% acetic acid. After the evaporation of the eluant, dissolve the residue with 5 ml of water and adjust pH to 9.0 with 10% NaOH. And then, extract with 2.0 ml of CHCl₃ three times and evaporate to 0.1 ml (sample-2).

3) Extraction of fentanyl and its metabolites from rat urine.

The same extraction procedure as discribed above was carried out to the urine of female rat to which 3.5 mg/kg of fentanyl had been administrated subcutaneously (sample-3).

4) Hydrolysis of fentanyl and sample-3.

Hydrolysis of fentanyl and sample-3 were carried out with 0.5 ml of 6N-HCl for 1 hr at 120°C in sealed tube and hydrolisates were extracted with small CHCl₃ after adjusting pH to 9.0 with 10% NaOH (extract of fentanyl-hydrolisate: sample-4, hydrolisate of sample-3: sample-5).
5) Continuous extraction procedure on hydrolyzed solution of fentanyl. The procedure was carried out on hydrolyzed solution of fentanyl for 10 hrs after adjusting pH to 3.0 with 3N NaOH with ether.

6) Quantitative determination of fentanyl and its metabolites in rat urine by TLC.

Three mg/kg of fentanyl were administrated to rat subcutaneously and urine samples were collected every 4 hrs for 24 hrs. Each sample was treated with the procedure as described above and determined quantitatively.

7) Extraction procedure of fentanyl and its metabolites from blood sample of rats.

Eight rats were divided into four groups, and 50, 100, 200 and 400 micro-gram of fentanyl are administrated subcutaneously to each group respectively.

Blood samples are taken at 3 and 60 minutes after fentanyl administration through carotid artery. The plasma was separated by centrifugation and protein was precipitated.

8) Mass-spectrography of hydrolysate of fentanyl, norfentanyl (C) and despropionyl-norfentanyl (D).

The mass-spectrometric analysis of these three compounds has been carried out in order to prove which of the three compounds would be identified as the hydrolysate of fentanyl. Mass-spectra were measured by a Hitachi RMU-6 Mass-spectrometer, with an 80 micro-amp. ionizing current and 70 ev, and the temperature of the ion source was 245-250°C.

RESULTS AND DISCUSSION

Results of TLC (sample-1 to 5) were as follows (Fig. 5, Table 1):

|---------|----------------------|-----------------------------|--------------------------|---------------------------|---------------------------|

Stationary phase: Kieselgel-G
Solvent: Methanol containing 1% NH₄OH
Reagent: Iodoplatinate

Fig. 5. Results of TLC.
FATE OF FENTANYL

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.64</td>
<td>0.61</td>
<td>0.82</td>
<td>0.77</td>
<td>0.81</td>
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<tr>
<td>2</td>
<td>0.46</td>
<td>0.48</td>
<td>0.68</td>
<td>0.62</td>
<td>0.67</td>
</tr>
<tr>
<td>3</td>
<td>0.64</td>
<td>0.60</td>
<td>0.80</td>
<td>0.77</td>
<td>0.81</td>
</tr>
<tr>
<td>4</td>
<td>0.46</td>
<td>0.48</td>
<td>0.67</td>
<td>0.61</td>
<td>0.68</td>
</tr>
<tr>
<td>5</td>
<td>0.45</td>
<td>0.47</td>
<td>0.68</td>
<td>0.60</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Solvent: (1) Butanol: acetone: $\text{NH}_2\text{OH}$
(90:10:1)
(2) Butanol: $\text{NH}_2\text{OH}$ (99:1)
(3) Ethanol only
(4) Methanol only
(5) Methanol containing 1%-$\text{NH}_2\text{OH}$

1. By the extraction procedure as shown in Fig. 3, pure fentanyl was detected as Rf. of 0.81 from sample-1 and this sample showed no reaction of phenolic hydroxyl group by ferric chloride test.

2. From human urine to which 100 microgram of fentanyl was added, same Rf. of 0.81 (sample-2) as in sample 1 was observed by the procedure as shown in Fig. 4.

3. From the urine of female rat to which 3.5 mg/kg of fentanyl was administrated subcutaneously, two spots (sample-3) were detected as Rf. of 0.81 and of 0.67 by the same extraction procedure discrived above.

4. After the hydrolysis of fentanyl and sample-3, only one spot of Rf. of 0.67 (sample-4 and 5) is detected and the spot of Rf. of 0.81 in sample-3 disap-
According to these results, it was found that one of the two spots (Rf.: 0.81) was fentanyl and the other (Rf.: 0.67) was a spot of fentanyl-metabolite hydrolysed.

5. By the continuous extraction procedure on hydrolyzed solution of fentanyl, propionic acid could be detected by TLC and GC (Fig. 6 and 7). This fact seemed to point that the moiety of propionylanilide of fentanyl was hydrolyzed into despropionate and propionic acid (Fig. 8).

6. Furthermore, it was proved that fentanyl-hydrolisate, showed Rf. of 0.67, was consisted of the secondary amine moiety by the following methods. a. Nitrosamine test: The first and the secondary amines form insoluble white precipitate of nitrosamine by reaction with NaNO in acidic solution by usual way.

\[
\text{Fentanyl} \quad \xrightarrow{\text{Hydrolysis}} \quad \text{Despropionyl derivative (DPD)} \quad \text{Propionic acid}
\]

Fig. 8. Hydrolysis of fentanyl.
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Fig. 9. Formation of dinitrophenyl derivative (DNP-Deriv.).

Sample: 1. Fentanyl, 2. DPD, 3. DPD, 2,4-D, 4. 2,4-D, 5. 2,4-D, DNP-Deriv.

Solvent: A. Methanol containing 1% NH\textsubscript{2}OH
B. Butanol : Ethanol : NH\textsubscript{4}OH
( 50 : 50 : 1 )

Stationary phase: Kieselgel-G
Reagent: Iodoplatinate

Fig. 10. Detection of DNP-Deriv. by TIC.

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td></td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
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<tr>
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<td>0.78</td>
<td>0.90</td>
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Rf. value of Fig. 10
b. Formation of dinitrophenyl derivative (DNP-deriv.): As shown in Fig. 9, by usual method of formation of DNP-deriv., fentanyl hydrolisate with Rf. of 0.67, formed DNP-deriv. by the reaction with 2,4-dinitrofluorobenzen (2,4-D) and it was proved by TLC and GC (Fig. 10 and 11, Table 2).
7. Moreover, it was found that Rf. value of hydrolisate (Rf.: 0.67) was different apparently from the authentic norfentanyl (C) (Rf.: 0.30) and from despropionyl-norfentanyl (D) (Rf.: 0.15) by TLC (solvent: methanol containing 1% NH₄OH, Reagent: iodoplatinate, Stationary phase: Kieselgel-G).

8. Finally, chemical structure of hydrolisate of fentanyl was identified with (B), i.e. despropionyl derivative (DPD), by mass-spectrometric analysis (Fig. 12 and 13).16,17

9. It was found further that when 3.00 mg/kg of fentanyl was administered to rat subcutaneously, fentanyl were excreted unchanged (22.5%) as well as DPD (7.5%) in urine until 8 hrs by quantitative determination by TLC and that no spots were detected on TLC from blood samples of sacrificed rats.

Fig. 13. Fragmentation of despropionyl derivative (DPD) (B) and other related compounds.
SUMMARY AND CONCLUSION

1) Fentanyl is extracted from urine and detected by TLC (detection limit: 1.0 to 2.0 microgram) and GC (1.0 to 2.0 microgram).

2) When 2.00 mg/kg or more of fentanyl is injected to rat subcutaneously, two spots (Rf. of 0.81 and of 0.67) are detected from its urine on TLC and it is proved that the spot at Rf. of 0.81 is unchanged fentanyl and the other (Rf.: 0.67) is its despropionyl derivative (DPD) by mass-spectrometric analysis.

3) According to these results, it is suggested that fentanyl is metabolized mainly by hydrolytic pathway rather than by oxidation as shown in Fig. 1 and when 3.00 mg/kg or more of fentanyl is injected subcutaneously to rat, about 30% of the dose is excreted in urine unchanged as well as its despropionyl derivative.

4) To detect fentanyl and its DPD from rat's urine by TLC and GC, at least 2.00 mg/kg or more of fentanyl must be administered subcutaneously and accordingly, 3 to 5 microgram of fentanyl must exist in 50 ml of human urine to be detected by the same method.
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However, a single intravenous dose of 0.5 to 1 mg is enough to produce a pronounced state of surgical analgesia in man, so it is very difficult to detect fentanyl used by the normal dosage from human urine unless the fate of the remaining 70% substance is clarified.

ADDEDUM

During the course of this study, we received a report from Dr. W. Soudijn on the metabolism and the excretion of $^{3}$H-fentanyl in Wistar rats.

He reports that when 0.32 mg/kg (100 mC/mmole) of $^{3}$H-fentanyl was administrated intravenously to adult male rats, most of the radioactivity was excreted into urine during the first 24 hrs after administration as unchanged (about 10% of given dose) and norfentanyl (about 50%).

Due to the differences of dose-level, route of administration, the sex of animals and probably the strain of rats, our results on the metabolic pathway of fentanyl are not in accord with theirs.

We agree that 10% or more of fentanyl is excreted unchanged in urine during the first 24 hrs, but we would like to postulate that the hydrolysis is the main pathway in rats rather than oxidation because of the apparent fact that no trace of norfentanyl in urine through our experiments is detected.

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