

## تحديد وتقدير تركيز الفنتانيل في أنسجة الجرذان بعد الوفاة

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### عادة فؤاد

تم في هذا البحث تقدير تركيز الفنتانيل في أنسجة الجرذان المختلفة ، التي تم حقنها بالعضل بتركيزين مختلفين للفنتانيل ، كما تم تتبع تركيزه في أنسجة الكبد وعضلة القلب وعضلة الفخذ والطحال والمخ والدم والشعر بعد الوفاة مباشرة ، وكذا تتبع ثبات الفنتانيل في هذه الأنسجة بعد التعفن لتحديد أي الأنسجة يمكن أن تكون بديلا للدم في حالات التعفن .

وتوصل البحث إلى طريقة ميسرة وبسيطة للكشف عن الفنتانيل باستخدام جهاز GC/MS وتقدير تركيزه في الأنسجة المختلفة بعد الوفاة مباشرة ، ثم تتبع تركيزه في الأنسجة بعد التعفن . وانتهى البحث إلى أنه في حالة الجرعة العالية للفنتانيل كان أعلى تركيز له بعد الوفاة مباشرة موجود في : الطحال < الدم < عضلة القلب < المخ < الكبد < عضلة الفخذ < الكلى < الشعر . وكان أكثر ثبات للفنتانيل في أنسجة العضلات والشعر . وتم تحديده حتى الأسبوع السادس من الوفاة ، مما يجعل هذه الأنسجة هي الأنسجة المناسبة للكشف عن الفنتانيل في حالات الوفاة والتعفن ، وبديلا جيدا في حالة عدم وجود عينات دماء .

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Table (2)  
 degree of fentanyl abundance in different tissues of rats 5 hours after treatment

| Dose               | Descending order of Fentanyl concentration |       |              |              |       |                |        |      |
|--------------------|--|-------|--------------|--------------|-------|----------------|--------|------|
|                    | 1  | 2     | 3            | 4            | 5     | 6              | 7      | 8    |
| 0.1mg /Kg<br>b.wt. | Spleen                                     | Blood | Brain        | Heart muscle | Liver | Femoral muscle | Kidney | Hair |
| 0.2mg /Kg<br>b.wt. | Spleen                                     | Blood | Heart muscle | Brain        | Liver | Femoral muscle | Kidney | Hair |

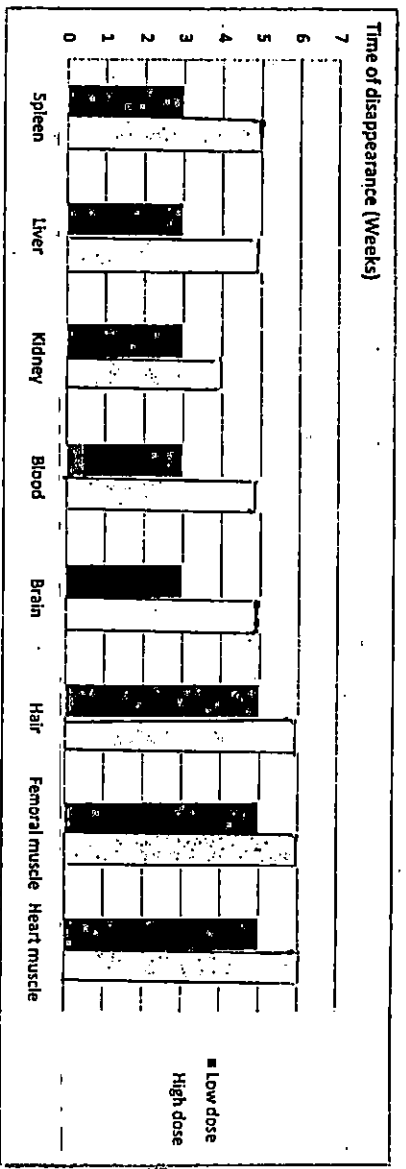


Fig. (3): Relationship between the administered dose and disappearance time of fentanyl from tissues of rats.

Table (1)  
Fentanyl concentrations (ug/g rat tissue) in samples of two different doses

| Tissue         | Dose | Time of detection |             |             |             |              |             |             |
|----------------|------|-------------------|-------------|-------------|-------------|--------------|-------------|-------------|
|                |      | Zero Time         | 1st Week    | 2nd Week    | 3rd Week    | 4th Week     | 5th Week    | 6th Week    |
| Spleen         | A    | 1.46 ± 0.07       | 0.78 ± 0.11 | 0.35 ± 0.07 | 0.17 ± 0.05 |              |             |             |
|                | B    | 3.21 ± 0.08       | 2.53 ± 0.11 | 2.13 ± 0.05 | 1.63 ± 0.07 | 0.94 ± 0.05  | 0.62 ± 0.06 |             |
| Liver          | A    | 0.97 ± 0.12       | 0.47 ± 0.10 | 0.25 ± 0.05 | 0.11 ± 0.04 |              |             |             |
|                | B    | 2.12 ± 0.07       | 1.36 ± 0.07 | 0.89 ± 0.06 | 0.55 ± 0.05 | 0.27 ± 0.05  | 0.12 ± 0.03 |             |
| Kidney         | A    | 0.86 ± 0.12       | 0.65 ± 0.11 | 0.37 ± 0.07 | 0.18 ± 0.04 |              |             |             |
|                | B    | 1.75 ± 0.11       | 1.19 ± 0.08 | 0.81 ± 0.09 | 0.66 ± 0.07 | 0.49 ± 0.07  |             |             |
| Blood          | A    | 1.42 ± 0.12       | 0.88 ± 0.10 | 0.43 ± 0.05 | 0.25 ± 0.05 |              |             |             |
|                | B    | 3.19 ± 0.08       | 2.24 ± 0.1  | 1.62 ± 0.06 | 0.81 ± 0.07 | 0.51 ± 0.11  | 0.37 ± 0.06 |             |
| Brain          | A    | 1.21 ± 0.1        | 0.67 ± 0.11 | 0.19 ± 0.03 |             |              |             |             |
|                | B    | 2.51 ± 0.06       | 1.66 ± 0.09 | 0.84 ± 0.07 | 0.37 ± 0.05 | 0.15 ± 0.06  |             |             |
| Hair           | A    | 0.35 ± 0.13       | 0.26 ± 0.07 | 0.22 ± 0.04 | 0.17 ± 0.02 | 0.12 ± 0.02  | 0.10 ± 0.03 |             |
|                | B    | 1.27 ± 0.1        | 0.97 ± 0.09 | 0.77 ± 0.05 | 0.57 ± 0.04 | 0.49 ± 0.08  | 0.33 ± 0.05 | 0.28 ± 0.24 |
| Femoral muscle | A    | 0.92 ± 0.14       | 0.79 ± 0.13 | 0.47 ± 0.05 | 0.25 ± 0.04 | 0.19 ± 0.02  | 0.11 ± 0.02 |             |
|                | B    | 1.84 ± 0.12       | 1.16 ± 0.07 | 0.87 ± 0.06 | 0.65 ± 0.05 | 0.742 ± 0.06 | 0.27 ± 0.07 | 0.13 ± 0.03 |
| Heart muscle   | A    | 1.19 ± 0.13       | 0.89 ± 0.12 | 0.65 ± 0.04 | 0.37 ± 0.05 | 0.22 ± 0.03  | 0.14 ± 0.03 |             |
|                | B    | 2.86 ± 0.01       | 2.31 ± 0.09 | 1.85 ± 0.07 | 0.87 ± 0.06 | 0.65 ± 0.05  | 0.42 ± 0.06 | 0.27 ± 0.06 |

\*All data are present as means of 5 rats ± SD, DEV. \*0 time= Immediately after dissection.

\*A=0.1 mg/kg b.wt.

B=0.2 mg/kg b.wt.

Postmortem kidney tissue showed a marked degree of abundance with the different doses of the studied drug. This finding may be related to the excretory route of drugs <sup>(22)</sup> and/or to the specific structure of the kidney. <sup>(23)</sup>

The present finding showed that postmortem brain tissue had moderate concentration of fentanyl. This may be explained by the assumption mentioned by Kuan-Hang *et al.*, 2008 that the low molecular weight, high potency and lipid solubility of fentanyl which helps it to cross the blood brain barrier. <sup>(24)</sup>

The present study reveals that the spleen and blood have the highest concentration of fentanyl. This finding may be related to the assumption of Drummer, 2008, who stated that deep-tissue sites are usually the 'fast to reach equilibrium. These are also relatively poorly perfused by blood further retarding the rate at which equilibrium is reached. He added, in single dose situations equilibrium will probably never be reached, since the drug is being continually removed by the body through metabolism and excretion. <sup>(25)</sup> In addition, spleen tissue is highly rich in blood, this may explain the near similarity of fentanyl concentration. <sup>(26)</sup>

Nowadays, besides blood and urine, hair is being recognized as an alternative and fundamental biological specimen for drug testing<sup>(11)</sup> because it is resistant to autolysis, easy to obtain and store<sup>(12)</sup> and its collection is almost non invasive.<sup>(13)</sup> Palmeri *et al.*, 2000 mentioned that drug analysis in keratinized matrices, such as hair and nails can provide a reliable tool for the detection of drug abuse.<sup>(14)</sup> The aforementioned information may explain the present finding that fentanyl has been detected in postmortem hair samples for longer periods after death as compared to other studied tissue specimens. Similarly, Gruszecki *et al.*, 2000 reported that drug can be detected in hair samples for longer periods than in blood and urine.<sup>(15)</sup> Musshoff and Madea, 2007 reported that it is important to take into account the fact that there is only a limited correlation between the frequency of drug use or the ingested dose and the drug concentration in hair which is based on inter individual difference in the rate of metabolism and thus serum peak concentrations, as well as on differing drug incorporation rates.<sup>(16)</sup> Also, Cirimele *et al.*, 2000 suggested that drug administration can be traced by hair analysis for months or even for years.

<sup>(17)</sup> The incorporation of drugs from plasma into hair distinctly depends upon the physiochemical properties of such drug.<sup>(18)</sup>

It is interesting enough to notice that inspite of the presence of several factors which may affect the accumulation of chemicals in the hair matrix, there is consensus on the usefulness of hair analysis in the study of drug abuse.<sup>(19)</sup>

Skeletal muscles are proposed as a useful alternative specimen to postmortem blood as the ratios between the concentration of the drugs in muscle and blood were often near unity.<sup>(20)</sup>

Ito *et al.*, 1997 mentioned that the skeletal muscle is considered to be a suitable sample for toxicological examinations of paraquat drug.<sup>(21)</sup> Therefore, the present finding that the studied drug was detected for a long period in postmortem muscle samples (femoral, heart) may confirm the importance of such tissues in evaluation of fatal cases of drug overdose.



At the low dose (1/10 LD50), fentanyl was not detected in the brain tissue on 4th week of death. While fentanyl was not detected on the 6th week postmortem in three tissues (femoral muscle, heart muscle and hair), it was detected until the 3rd week after death in blood, liver and kidney tissues.

At the high dose (1/5 LD50), fentanyl was detected until the 6th week postmortem in femoral muscle, heart muscle and hair. Meanwhile, fentanyl was detected in brain and kidney until the 4th week postmortem and it was detected in blood, spleen and liver tissues until the 5th week.

It was interesting to notice that the postmortem detection time of fentanyl in most studied tissues was found directly correlated with the administrated dose (Fig. 1).

#### Discussion:

Some changes in drug concentration occur for all drugs, particularly for lipid soluble drugs. Moreover, in cases of putrefaction further changes in the composition of the blood, instability of the substance and diffusion of other fluids from neighboring sites.<sup>(7)</sup>

Flanagan and Connally, 2005 mentioned that, in order to properly interpret blood concentration in postmortem specimen, it is preferable to quantify the substances in another tissues specimen.<sup>(8)</sup>

Liver has been one of the most common second specimens, this solid specimen is the major metabolic organ and is reasonably homogenous and importantly to be readily homogenized to provide a useful medium for extraction techniques.<sup>(9)</sup> Since fentanyl major metabolism occur in liver, this may explain the present finding that postmortem liver tissues showed a higher abundance of fentanyl as compared to the kidney tissues. Also, there are no essential postmortem changes in the liver tissues when kept under different conditions for 48 hours or for 15 days postmortem.<sup>(10)</sup>

LD50 /24h Injected intramuscularly which was 1mg/kg body weight of rat <sup>(6)</sup>.

◇ 1/10 LD50, 0.1mg/kg b.wt. (low dose).

◇ 1/5 LD50, 0.2mg/kg b.wt. (high dose).

Physiological saline was used for drug dilution, fentanyl was injected intramuscular into rats which were scarified five hours after drug administration.

Rats were dissected as soon as possible and samples of liver, kidney, femoral muscle, heart muscle, brain, spleen, hair and blood were collected for analysis. All samples for studying the effect of putrefaction on stability of fentanyl were kept separately in polyethylene bags and were left at room temperature during the experimental period, while samples taken for zero time determination analysis was carried out immediately.

Detection and concentration of fentanyl in tissues were achieved at zero time (time of sacrifice) and then every week until disappearance. Fentanyl was extracted from postmortem tissues by using ammonium sulphate for precipitation of protein and then fentanyl was extracted by liquid-liquid extraction using dichloromethane in alkaline medium, tissue extract were examined for drug detection and concentration estimation by Agilent technologies (USA) 6890 GC equipped with 5793 mass detector. ADB-17, 0.25 mm.i.d, 0.25 m film thickness 30m length column was used. Injector temperature was 250 °C, injection mode splitless. The oven was programmed from 70 °C for 1min, ramped at 35 °C/min to 310 °C, where it was held for 5min. the transfer line was held at 280 °C, the ion source was held at 230 °C and the quadropole at 150 °C.

### Result:

Table (1) shows the concentration of fentanyl in tissues obtained from rats administrated different doses of fentanyl and sacrificed five hours after the treatment.

Fentanyl is one of the medicinal used synthetic opioid and is abused, it has similar properties to morphine yet up to 200 times more potent <sup>(2)</sup>.

Fentanyl or 1-(2-phenylethyl)-4-(n-propionylphenyl amino) piperidine, <sup>(3)</sup> caused intoxication due to ingestion of transdermal patches, intravenous injection and transnasal sprays <sup>(4)</sup>. In addition, fentanyl theft has been reported from nursing homes and other long term care facilities. Recently, clandestine production and distribution of fentanyl powder and tablets have also surfaced <sup>(5)</sup>.

Reports on analysis of fentanyl have been published, but most focused on the determination of the drug in biological fluids such as plasma, cerebrospinal fluids and urine. In forensic toxicology, examination of fresh body fluids is not always available because of putrefaction, degradation and contamination have been occurred.

The need for applying sensitive, selective and reliable methods applicable to both detection and determination of fentanyl in solid tissues such as liver, brain, muscles, kidney, hair and spleen and in biological fluids such as blood. Also, studying the effect of putrefaction on the drugs stability in tissue and determine which organ and/or organs will be of choice in having the highest concentration and long duration of drug stability in case of putrefied tissues.

## **Materials and Methods**

### **Experimental animals:**

Male albino rats, weighing 120-180g were provided by Laboratory Animal House in The National Research Center, standard pellet diet and water were given *ad libitum* throughout the study.

Fentanyl which is a synthetic opioid was purchased from Janssen- Cilag Pty Ltd.

### **Dosage and experimental design:**

Two doses of fentanyl were chosen as follow according to fentanyl

# DETECTION AND DETERMINATION OF FENTANYL CONCENTRATIONS IN POSTMORTEM RAT TISSUES

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The aim of this study was to investigate the postmortem tissue concentrations of fentanyl in a rat model. Fentanyl was administered intramuscularly, with different doses, into rats 5 hours prior to sacrifice. Different tissues (liver, kidney, heart muscle, femoral muscle, spleen, brain, blood and hair) were removed immediately after decapitation and left at room temperature during the experimental periods. Detection of fentanyl and determination of its concentration in the tissues were performed at the time of decapitation and every week until their complete disappearance. Fentanyl was extracted from postmortem specimens and drug identification and evaluation was performed using GC/MS. Immediately after decapitation, fentanyl concentration in postmortem tissues of rats treated with higher dose was as follows: spleen > blood > heart > muscle > brain > liver > femoral muscle > kidney > hair. Fentanyl was detected in postmortem specimens of heart muscle, femoral muscle and hair for a longer period than in other tissues. Consequently, these tissues can provide a good forensic matrix in case of absence of blood or blood putrefaction.

## Introduction

The dangers of opioid overdose have been recognized for as long as the use of opium itself. In many countries, the use of heroin or their opioids in medicinal and non-medicinal context is associated with an increasing rate of overdose<sup>(1)</sup>.

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