The Duration Time of Urine Morphine Detection in Heroin Addicts by Radioimmunoassay

PAITOON NARONGCHAI, M.D.*,
SUPARAT THAMPITHUG, B.Sc.*,
CHAROON CHITIVUTHIKARN, M.D.**
PONGRUG SRIBANDITMONKOL, M.D.*,
SIRIPUN NARONGCHAI, M.Sc.*

Abstract

Heroin is one of the most seriously abused drugs and its consumption is illegal. Therefore, the detection of the drug in addicts has to be highly accurate and reliable. Morphine detection in urine is the most common method to confirm consumption. Several methods of morphine detection in urine are described such as Latex Agglutination Inhibition (LAI), Thin Layer Chromatography (TLC), Radioimmunoassay (RIA), High Performance Liquid Chromatography (HPLC) and Gas Chromatography and Mass Spectrophotometry (GC/MS). In this study, we performed Radioimmunoassay (RIA) because it is rapid and cheap. Sixty-three male heroin addicts, average age 32 years, with an average time of heroin administration of about 3 years were studied. They used on average 0.635 grams of heroin per day. The time of detection in the urine after the last heroin administration to the first urine testing was about 8.5 hours, over 3-4 days. The amount of morphine in the urine was 17,897.9 ng/ml. Nevertheless, we found that morphine could be detected in the urine every day for seven days. The decreasing rate of daily morphine detection in the urine was 88.4, 74.2, 64.2, 57.1, 63.3 and 44.8 per cent. But there was no significant difference in the route of administration.

Key word : Heroin, Radioimmunoassay, Morphine

NARONGCHAI P, SRIBANDITMONKOL P, THAMPITHUG S, NARONGCHAI S, CHITIVUTHIKARN C
J Med Assoc Thai 2002; 85: 82-86

* Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200,
** Northern Narcotic Center, Chiang Mai 50180, Thailand.

Heroin is one of the most common street drugs in Thailand and in other parts of the world. The Golden Triangle, is made up of three countries Myanmar, Thailand and Laos which are connected by borders and are well known as the largest area of heroin production. Many foreigners from Europe, America and Africa, come to Thailand to get heroin. There are even hilltribe tours called "Tour Pa". During recent years, the number of heroin addicts has increased. So heroin addicts must be controlled and punished according to the law. The method of detection after heroin administration must be highly accurate. RIA is one of the most useful methods for the determination of morphine, the metabolite of heroin, which can be detected in the urine at the level of more than 300 ng/ml. It is available at many laboratories in Thailand.

The diagnosis of heroin users or addicts is difficult and must be made rapidly because of the short half life, about 5 minutes[1,2], since heroin is metabolized by hydrolysis to morphine. The 6-monoacetyl morphine (6 - MAM), the intermediate metabolites of heroin in the body, can be detected after only a few minutes[3]. The end metabolite product, morphine, cab still be detected for about 4 days after the last heroin administration by gas chromatography[6]. The metabolism of heroin in the liver depends on the processes of enzymatic deacetylation and hydrolysis[4,5,7]. Morphine is conjugated with glucuronide, as morphine-3-glucuronide (M3G) and morphine -6-glucuronide (M6G) which is excreted in the urine as the total morphine. It can be detected by RIA but it can not analyze the free morphine or total morphine. It can not demonstrate how long since they used heroin and for how long the morphine can be detected in the urine. The cut off point of the morphine level in the urine by RIA is still significantly at 300 ng/ml. We planned to study the duration time of morphine detection in urine after the last heroin administration for 7 days. We also studied the decreasing rate of morphine in the urine. The correlation between the concentration of morphine in the urine and timing was demonstrated with a Log graph. The concentration of morphine in urine declined rapidly during the first 12 hours after heroin administration and approached the level of sensitivity limit of the immunoassay within 72-120 hours[8]. There was general agreement between immunoassay result and GC/MS analyses. Concordance analysis indicated that there were no false positive results as defined by > 300 ng/ml of total morphine equivalents by immunoassay and < 300 ng/ml of total morphine by GC/MS. Generally less than 5 per cent of the heroin and morphine specimens were tested as false negative by each of the immunoassays.

MATERIAL AND METHOD

There were 63 healthy male heroin addicts in the study. Twenty-three heroin addicts injected heroin by the intravenouse route and 40 addicts inhaled it. Over a one month period all of the addicts used heroin within 24 hours from the last time they had used it. Anyone with a disease especially AIDS, liver disease or renal disease etc was excluded from the study. The first urine specimens were collected at their first visit, and we collected urine at 5-6 am every day thereafter. Urine specimens were kept at 2-8°C in a refrigerator until analysis. The specimens were tested by RIA with the Coat - A - Count Morphine kit from Diagnostic Products Corporation company. All quantitative results are shown as morphine over 7 days respectively by Gamma Counter. We used the calibrators provided with the kits. Appropriate morphine dilutions of the specimens were set as the standard curve.

RESULT

The primary goal of these studies was immunoassay testing of urine that was collected under controlled conditions after heroin administration, by the intravenous and inhalation routes. The study was conducted during treatment at the center of drug abuse and each volunteer gave informed consent. All subjects were drug free, and none of them had chronic diseases of the liver, kidney, infection, prior to study participation. The study was performed with volunteer subjects who had been addicted for more than one month. All of them were males aged 15-57 years, on average 32 years old. On average they become addicted at 26 years old and the average duration of addiction was 3.7 years. They used about 0.635 grams of heroin daily. The volume of urine morphine detected in the volunteers was 638-77870 ng/ml, average 17897.9 ng/ml. RIA testing was performed for all specimens according to the manufacturer's specifications. The result of analysis is presented in Table 1. The detection times (time to addiction of last positive at specified cut off < 300 ng/ml by RIA are demonstrated for each

Table 1. General information of heroin addicts.

Heroin addicts by inhalation						
Code	Age (year)	Started addiction (year)	Duration addiction (year)	Amount of heroin (gram)	The last usage until detection (h)	Morphine concentration (ng/ml)
Range	16-57	14-54	0.25-5	0.15-1.2	1-25	638-43,020
N	23	23	23	23	23	19
Mean	33.5	28.8	4.0	0.53	11.3	10,181.78
SD	11	9.7	5.8	0.27	2.06	10,458.59
Heroin addicts by intravenouse route						
Code	Age (year)	Started addiction (year)	Duration addiction (year)	Amount of heroin (gram)	The last usage until detection (h)	Morphine concentration (ng/ml)
Range	15-56	12-48	0.08-12	0.15-1.2	0.3-26	1,022-77,870
N	40	40	40	40	40	35
Mean	30.5	24.7	2.8	0.697	6.92	19,952.69
SD	10	8	3.3	0.69	6.33	19,754.59

Table 2. Morphine concentration in urine of heroin addicts by inhalation over 7 days (ng/ml).

Code	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Range	638-4,0320	39-23,340	19-2,400	15-4,340	0-2,140	0-256	0-33
N	18	23	23	23	23	23	23
Mean	10,181.78	2,441.52	325.22	291.96	129.57	25.04	13.52
SD	11,032.24	4,948.60	576.27	907.82	441.73	51.97	11.68

Table 3. Morphine concentration in urine of heroin addicts by intravenous route over 7 days (ng/ml).

Code	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Range	1,432-7,780	127-6,840	17-5,820	9-800	11-600	0-440	0-58
N	34	40	40	40	40	40	40
Mean	19,952.69	1,855.65	660.6	140.15	58.15	34.32	20.32
SD	19,754.59	2,030.31	1,273.7	218.93	99.17	67.36	15.60

day as an average value as presented in Table 2 and 3. Table 4 demonstrates the decreasing rate of urine morphine each day and the two ways of heroin administration are compared.

DISCUSSION

The validity of the method depends its ability to detect the parent drugs and their metabolites in urine after administration. It depends on pharmacological variables such as dose, route of administration and variability of subject or chemical factors such as sensitivity, specificity and accuracy. Consequently, RIA should not only detect drugs by a sensitive and selective methods, but also quantitative analysis in a concentration range. At least four kinds of commercial opiate screening immunoassays have been evaluated for their ability to accurately detect drug exposure after heroin, and morphine administration[8]. The false negative rate was low, < 5 per cent and there were no false positives identified after heroin and morphine administration[8]. RIA is based on detection of free mor-

Table 4. Decreasing rate of urine morphine concentration over 7 days.

Route of administration	Urine morphine concentration each day (ng/ml) Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Number	Heroin per day (gram)
Intravenous route	19,952.69	1,855.65	660.6	140.15	58.15	34.32	20.32	40	0.697
	80.6%	87.1%	10.2%	55.6%	80.7%	46%			
Inhalation route	10,181.78	2,441.52	325.72	291.96	129.56	25.04	13.52	23	0.528
	90.7%	86.5%	78.8%	58.6%	41%	40.3%			
Total	17,897.9	2,082.61	537.52	192.37	83.71	30.77	16.98	63	0.635
	88.4%	74.2%	64.2%	57.1%	63.3%	44.8%			

phine and shows a very low cross reactivity to morphine metabolites. The detection times of RIA in this study were demonstrated at 3-4 days following the last time of heroin administration. There was no difference in the time of detection between the two routes of administration. The decreasing rate of both routes of administration were rapid in the first 24 hours after the last administration, 90, 80 and 88 per cent in the group of injection, inhalation and both. Compared to the study of Edward JC et al, the decreasing rate of urine morphine in the first 24 to 48 hours after the last administration was about 90 and 87 per cent. The concentration of morphine declined rapidly during the first 12 hours after heroin administration and approached the level of sensitivity limit of the immunoassay within 72-120 hours (7). There was general agreement between the immunoassay results and GC/MS analyses. Concordance analysis indicated that there were no false positive results as defined by > 300 ng/ml of morphine equivalents by immunoassay and < 300 ng/ml of total morphine and < 25 ng/ml for free morphine by GC/MS. Generally less than 5 per cent of the heroin and morphine specimens tested as false negatives by each of the immunoassays.

SUMMARY

The detection time of RIA in this study was 3-4 days after heroin administration in chronic abuse of more than one month at the cut off level 300 ng/ml. There was no difference in the values of detection time between the two routes of administration. However, RIA is still considered to be the method which is reliable, sensitive and rapid for detection of morphine in urine. RIA is suitable for developing countries because of its low price and high accuracy.

(Received for publication on April 26, 2001)

REFERENCES

1. Goldberger BA, Cone El, Grant TM, et al. Disposition of heroin and its metabolites in heroin related deaths. J Analyt Toxicol 1994; 18: 22-8.
2. Wright CI. The deacetylation of heroin and related compounds by mammalian tissues. J Pharmacol Exp Ther 1942; 75: 328-37.
3. Lockridge O, Mottershaw-Jackson N, Eckerson HW, Ladu BN. Hydrolysis of diacetyl morphine (heroin) by human serum cholinesterase. J Pharmacol Exp Ther 1980; 215: 1-8.
4. Smith DA, Cole WJ. Identification of an arylesterase as the enzyme hydrolysing diacetyl morphine (heroin) in human plasma. Biochem Pharmacol 1976; 25: 367-70.
5. Nakamaro GR, Thornton JL, Noguchi TT. Kinetics of heroin deacetylation in aqeous alkaline solution and in human serum and whole blood. J Chromatog 1975; 110: 81-8.
6. Yeh SY, Gorodetzky CW, Mc Quinn RL. Urinary excretion of heroin and its metabolites in man. J Pharmacol Exp Ther 1976; 196: 249-56.
7. Edward JC, Sandra D, Buddha DP, John MM. Forensic drug testing for opiates. V. Urine testing

for heroin, morphine and codeine with comercial opiate immunoassays. J Analyt Toxicol 1993; 17: 156-64.
8. Cone EJ, Dickerson S, Paul BD, Mitchell JM. Forensic drug testing for opiates IV. Analytical sensitivity specificity and accuracy of commercial urine opiate immunoassays. J Analyt Toxicol 1992; 16: 72-8.

ระยะเวลาการตรวจพบมอร์ฟีนในปัสสาวะของผู้ที่เสพเฮโรอินโดยวิธีการตรวจด้วยเรดิโออิมมูโนแอสเสย์

ไพฑูรย์ ณรงค์ชัย, พ.บ., พงษ์รักษ์ ศรีบัณฑิตมงคล, พ.บ.*,*
ศุภรัตน์ ธรรมพิทักษ์, วท.บ., สิริพันธ์ ณรงค์ชัย, วท.ม.*, จรูญ จิตติวุฒิการ, พ.บ.***

เฮโรอินเป็นสารเสพติดที่น่าสพึงกลัวชนิดหนึ่งและเป็นสิ่งที่ผิดกฎหมาย เพราะฉะนั้นการตรวจว่าผู้ใดเสพจริงหรือไม่จำเป็นต้องการวิธีตรวจที่แม่นยำและน่าเชื่อถือมากที่สุด ซึ่งพบว่าการตรวจพบมอร์ฟีนในปัสสาวะเป็นสารที่สามารถใช้เป็นสิ่งที่บ่งชี้ว่าผู้นั้นเสพเฮโรอินมาจริงหรือไม่ มีการตรวจด้วยกรรมวิธีต่าง ๆ หลายอย่าง เช่น Latex Agglutination Inhibition (LAI), Thin Layer Chromatography (TLC), Radioimmunoassay (RIA), High Performance Liquid Chromatography (HPLC) และ Gas Chromatography and Mass Spectrophotometry (GC/MS) ซึ่งในการศึกษาครั้งนี้ได้เลือกใช้วิธี RIA ทั้งนี้เพราะวิธีดังกล่าวเป็นวิธีที่รวดเร็ว เชื่อถือได้ดีและราคาถูก อีกทั้งยังเป็นการพัฒนาการตรวจให้สามารถนำไปใช้ประโยชน์ได้อย่างมีประสิทธิภาพ ในการศึกษาครั้งนี้มีผู้ที่เสพยาเสพติดให้โทษชนิดเฮโรอิน เป็นเพศชายทั้งหมด จำนวน 63 คน อายุเฉลี่ย 32 ปี ระยะเวลาของการเสพยาเฉลี่ย 3 ปี ปริมาณของเฮโรอินที่ใช้ในการเสพแต่ละวันเฉลี่ย 0.635 กรัม และมอร์ฟีนที่มีปริมาณที่น่าเชื่อถือได้นั้นสามารถถูกตรวจพบในปัสสาวะได้นาน 3-4 วัน ภายหลังการเสพครั้งสุดท้าย และพบว่าปริมาณของสารมอร์ฟีนในปัสสาวะของผู้ที่เสพเฮโรอินโดยเฉลี่ย 17,897.9 นาโนกรัมต่อมิลลิลิตร อย่างไรก็ตามเราสามารถตรวจพบมอร์ฟีนในปัสสาวะได้นานถึง 7 วัน แต่ตรวจพบในปริมาณที่ต่ำกว่าค่ามาตรฐาน นอกจากนี้ยังพบว่าอัตราการลดลงของมอร์ฟีนในปัสสาวะจะลดลงอย่างรวดเร็วในระยะเวลา 2 วัน หลังจากหยุดเสพ โดยมีอัตราการลดลงตลอดระยะเวลา 7 วัน เป็นร้อยละดังนี้ 88.7, 74.2, 64.2, 57.1, 63.3 และ 44.8 ตามลำดับ โดยที่อัตราการลดลงของมอร์ฟีน ไม่มีความแตกต่างกันระหว่างวิธีการเสพ คือ เสพโดยการฉีดเข้าเส้นเลือด และการเสพโดยการสูบ

คำสำคัญ : เฮโรอิน, มอร์ฟีน, เรดิโออิมมูโนแอสเสย์

ไพฑูรย์ ณรงค์ชัย, พงษ์รักษ์ ศรีบัณฑิตมงคล,
ศุภรัตน์ ธรรมพิทักษ์, สิริพันธ์ ณรงค์ชัย, จรูญ จิตติวุฒิการ
จดหมายเหตุทางแพทย์ ฯ 2545; 85: 82-86

* ภาควิชานิติเวชศาสตร์, คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่, เชียงใหม่ 50200
** ศูนย์บำบัดยาเสพติดภาคเหนือ, เชียงใหม่ 50180