INVESTIGATION OF OESTROGEN AND PROGESTERONE INTERFERENCE WITH MORPHINE IDENTIFICATION IN 24 HOURS URINE OF RATS BY TLC TECHNIQUE

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ABSTRACT

Various screening techniques are employed by laboratories for rapid detection of morphine in urine including TLC, EIA (EMIT) and etc. There have been reports of hormonal drug-induced interference with morphine clearance from the body. The aim of this study was to evaluate the influence of oestrogen and progesterone on morphine detection in 24hr urine samples of rats by TLC. Male Wistar albino rats were housed in metabolic cages and were administered intraperitoneally oestradiol valerate or progesterone each at 10 & 20 mg/kg and morphine at 25 mg/kg once a day for 8 days. Urine samples were collected every 24 hr, rapidly checked by spot tests and assessed by TLC using Iodoplatinate and/or Dragendorff reagents. Results show that neither oestradiol valerate nor progesterone interfere with morphine detection (administered before or after) in 24hr urine samples. These findings could lead to the conclusion that these drugs do not interfere with morphine detection in urine by TLC but do not exclude the possibility of interference with enzyme immunoassay techniques (EMIT). Although EMIT is a sensitive technique but its specificity can be influenced by other drugs (i.e. steroid hormones). Therefore, the interference of oestradiol and progesterone with morphine detection by EMIT remains to be further investigated. However, other factors including higher doses of oestradiol valerate, progesterone or morphine, shortening of sampling time as well as application of an alternative sample preparation technique to increase the detection sensitivity, could also be important in this regard.

Key words: Morphine, Oestrogen, Progesterone, Urine, Interference, TLC, Rat

INTRODUCTION

Worldwide opium production has increased significantly since the 1980s and usage has been elevated due to higher production, increasing purity and lower prices. Opium is an extract of the juice of the poppy, *Papaver somniferum*, which has been used for social and medical purposes for thousands of years as an agent to produce euphoria, analgesia and sleep, and to prevent diarrhoea. It contains more than 20 distinct alkaloids, including morphine, codeine, thebaine and papaverine. The principal alkaloid in opium is morphine, in a concentration of about 10% (1, 2). In general, opioids become readily absorbed from the gastrointestinal tract or after subcutaneous and intramuscular injection. Most of opioids are metabolised to more polar metabolites which can be easily excreted by kidneys (1-4). Various screening techniques are employed by laboratories for rapid detection of morphine in urine including Thin Layer Chromatography (TLC), Enzyme Immunoassay (EIA) and etc. There have been reports of oral contraceptive drugs (OCPs) effects on morphine clearance from the body (6,10). The purpose of this study was to evaluate the influence of pre- and post-treatment of male rats with oestradiol valerate and progesterone on morphine detection in 24hr urine samples of rats by TLC.

MATERIALS AND METHODS

Chemicals: Morphine sulphate (20 mg/ml) ampoules were supplied by Darou-Pakhsh

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Pharmaceutical Company. oestradiol valerate (10 mg/ml) and progesterone (25 mg/ml) ampoules were purchased from Iran Hormone Pharmaceutical Company. All other chemicals were obtained from commercial sources and were of the highest purity available.

Animals and drug treatment: Male Wistar albino rats (6 weeks old, 200-250 g, Institute of Razi, Karaj, Iran) were housed in metabolic cages (2 rats in each cage) and allowed food and water *ad libitum*. The animals were dosed intraperitoneally, once a day for 8 days according to Tables 1 & 2, at the following dose levels: oestradiol valerate and progesterone each at 10 & 20 mg/kg and morphine at 25 mg/kg. These dose levels were chosen based on previous studies that indicated urinary excretion of oestradiol valerate or progesterone following intraperitoneal (i.p.) administration (5,6), and a pre-test for urinary excretion of morphine (Table 3). Urine samples were collected every 24 hr, filtered and stored at -20°C for subsequent detection of morphine.

Sample analysis: The 24hr urine samples were thawed, acidified to pH 3 by 1N HCl and hydrolysed for 30 minutes at 100°C in a water bath (7). Following a further pH adjustment to 8.5 by sodium bicarbonate, samples were extracted twice by chloroform. The tubes were spun at 400g for 10 minutes to clarify phases, after which the organic layers were transferred to fresh test tubes and evaporated to dryness. The samples were re-suspended in 0.5ml of chloroform and rapidly checked by spot tests including Bertrand, Bouchardat, Dragendorff and Mayer reagents (8) and assessed by thin layer chromatography (TLC, stationary phase: silicagel G60, mobile phase; benzene, acetone, methanol, ammonium hydroxide 50:40:5:5 v/v). The bands on TLC plates were visualised by spraying Iodoplatinate and/or Dragendorff reagent (9).

RESULTS

Morphine was found in rat urine samples collected 24hr following the last i.p. administration of 10 & 15 mg/kg of the drug (Table 3). However, it could not be detected in urine samples collected 48hr after the last dose (Table 3). Administration of higher doses of morphine (25 & 30 mg/kg) increased the period of its urinary excretion up to 48hr (Table 3). Treatment by oestrogen valerate, either at 10 or 20 mg/kg doses, in animals receiving 25 mg/kg of morphine did not interfere with morphine detection in 24hr urine samples (Table 4). In addition, administration of oestrogen valerate (for 3 days at 10 or 20 mg/kg) to rats before receiving 25 mg/kg of morphine, did not affect TLC detection of morphine in urine (Table 5). In the same manner for the oestrogen valerate data, administration of either 10 or 20 mg/kg of progesterone, to the animals receiving 25 mg/kg of morphine, did not interfere with morphine detection in 24hr urine samples (Table 4). Moreover, animal treatment for 3 days by 10 or 20 mg/kg of progesterone prior to the morphine administration (25 mg/kg) did not influence morphine detection by TLC (Table 5).

DISCUSSION

OCPs inhibit metabolic oxidation and reduce clearance of drugs, thus can potentiate pharmacological activity or toxicity of drugs by increasing their blood concentrations (6,10). On the other hand, OCPs induce hepatic glucuronidation that facilitate excretion of some drugs including morphine from the body (6,10). This effect has been shown to be mainly due to oestradiol (10) and women using OCPs have to take twice as normal dose for the same analgesic effect (11). However, glucuronidation varies by sex, although no information is available about the effect of OCPs on glucuronidation in males and females. Additionally, OCPs increase clearance of morphine taken orally and/or systemic by 120% and 75%, respectively (10). Watson *et al.* showed that induction of glucuronosyltransferase is responsible for OCPs increase of morphine clearance and is not due to change in flow rate (10). It has also been shown that

Table 1. Pattern of animal dosing by morphine prior to administration of oestradiol valerate or progesterone

Day	Control group	Test group
1	No drug	No drug
2	No drug	No drug
3	Morphine	Morphine
4	Morphine	Morphine
5	Morphine	Morphine
6	Morphine + Sesame oil	Morphine + Oestradiol or Progesterone
7	Morphine + Sesame oil	Morphine + Oestradiol or Progesterone
8	Morphine + Sesame oil	Morphine + Oestradiol or Progesterone
9	Sesame oil	Oestradiol or Progesterone
10	Sesame oil	Oestradiol or Progesterone

Male Wistar albino rats (6 weeks old, 200-250 g, Institute of Razi, Karaj, Iran) were housed in metabolic cages (2 rats in each cage) and treated i.p., once a day at the following dose levels: oestradiol valerate and progesterone each at 10 & 20 mg/kg and morphine at 25 mg/kg; urine samples were collected every 24 hr.

Table 2. Pattern of animal dosing by oestradiol valerate or progesterone prior to morphine administration

Day	Control group	Test group
1	No drug	No drug
2	No drug	No drug
3	Sesame oil	Oestradiol or Progesterone
4	Sesame oil	Oestradiol or Progesterone
5	Sesame oil	Oestradiol or Progesterone
6	Morphine + Sesame oil	Morphine + Oestradiol or Progesterone
7	Morphine + Sesame oil	Morphine + Oestradiol or Progesterone
8	Morphine + Sesame oil	Morphine + Oestradiol or Progesterone
9	Sesame oil	Oestradiol or Progesterone
10	Sesame oil	Oestradiol or Progesterone

Male rats were housed in metabolic cages (2 rats in each cage) and dosed i.p., once daily at the following dose levels: oestradiol valerate and progesterone each at 10 & 20 mg/kg and morphine at 25 mg/kg; urine samples were collected every 24 hr.

Table 3. TLC analysis of morphine in male rat urine

Hours	24	48	72
Control	-	-	-
Morphine (10 mg/kg)	+	-	-
Morphine (15 mg/kg)	+	-	-
Morphine (25 mg/kg)	+	+	-
Morphine (30 mg/kg)	+	+	-

Male rats were housed in metabolic cages (2 rats in each cage) and were treated for 3 days by i.p. injection of morphine; control animals were received 0.25-0.65 ml of saline. Urine samples were collected once a day and analysed by TLC as described in materials and methods (section 3). (-) morphine not detected, (+) morphine detected.

Table 4. Effects of *oestradiol valerate* or *progesterone* administration on morphine detection by TLC

Days	Control group	MOR detection	Test group	MOR detection
1	MOR	+	MOR	+
2	MOR	+	MOR	+
3	MOR	+	MOR	+
4	MOR+SEO	+	MOR+OEV/PRG	+
5	MOR+SEO	+	MOR+OEV/PRG	+
6	MOR+SEO	+	MOR+OEV/PRG	+
7	SEO	+	OEV/PRG	+
8	SEO	-	OEV/PRG	-

Male rats were housed in metabolic cages (2 rats in each cage) and were treated every 24 hr by i.p. injection of sesame oil (SEO), 25 mg/kg of morphine (MOR) and 10 or 20 mg/kg of oestradiol valerate (OEV) or progesterone (PRG); urine samples were collected once a day and analysed by TLC as described in materials and methods (section 3).
(-) morphine not detected. (+) morphine detected.

Table 5. Effects of early administration of *oestradiol valerate* or *progesterone* on morphine detection by TLC

Days	Control group	MOR detection	Test group	MOR detection
1	SEO	-	OEV/PRG	-
2	SEO	-	OEV/PRG	-
3	SEO	-	OEV/PRG	-
4	MOR+SEO	+	MOR+OEV/PRG	+
5	MOR+SEO	+	MOR+OEV/PRG	+
6	MOR+SEO	+	MOR+OEV/PRG	+
7	SEO	+	OEV/PRG	+
8	SEO	-	OEV/PRG	-

Male rats were housed in metabolic cages (2 rats in each cage) and were treated once daily by i.p. injection of sesame oil (SEO), 25 mg/kg of morphine (MOR) and 10 or 20 mg/kg of oestradiol valerate (OEV) or progesterone (PRG); urine samples were collected every 24 hr and analysed by TLC as described in materials and methods (section 3). (-) morphine not detected. (+) morphine detected.

morphine inhibits its own N-demethylation. after 2 weeks of repeated use (5). Administration of desipramine before morphine increases efficacy and duration of analgesic effect of morphine probably due to inhibition of hepatic N-demethylation (12). Based on TLC analysis, our results indicate that neither oestradiol valerate nor progesterone interfere with morphine detection (at dose level of 25 mg/kg) in 24hr urine samples. This finding could lead to the conclusion that these drugs do not interfere with morphine detection in urine of rats by TLC at the indicated dose levels. However, this finding does not exclude the possibility of interference with enzyme immunoassay techniques (i.e. EMIT) by these drugs. EIA is a homogeneous enzyme technique based on a competition between morphine in urine, and a labelled morphine added to the urine for binding to antibody (13). The EIA system most often described is the EMIT system (Syva Corporation, Palo Alto, California, USA). This test is sensitive but not specific (13,14). Therefore, the interference of oestradiol and progesterone with morphine detection by EMIT in rat and human urine samples remain to be further investigated. However, various other factors including higher doses of oestradiol valerate, progesterone or morphine, shortening of sampling time as well as application of an alternative sample preparation technique (e.g. column chromatography), to increase the detection sensitivity, could also be important in this regard.

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