

Urinary morphine and codeine concentrations after ingestion of bean-jam buns decorated with poppy seeds

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Dear Editor:

Poppy seeds are a common food ingredient. Cakes and bread decorated with or containing poppy seeds are quite popular in many countries. In Japan, one of the most popular foods with poppy seeds is bean-jam buns decorated with poppy seeds. However, the so-called poppy seeds defense has been often used in forensic urinalysis for opiates. It is sometimes difficult to determine the source of morphine and codeine in urine, because morphine-positive results can be caused by ingestion of foods containing poppy seeds [1–3]. Following a number of studies on opiates in poppy seeds, it has become clear that the opiate levels inside the seeds are very low; the alkaloid content of the seeds is affected by external contamination during harvest and changes in harvesting technology [2]. The seeds are contaminated with the chyle of unripe poppy capsules during harvesting. Other factors affecting morphine contents of poppy seeds are the poppy variety, geographical origin, time of harvest, and the processing method for foods containing poppy seeds [2]. In some countries, governmental food agencies have identified products with high morphine contents and removed them from the market. This has led to reduced morphine levels in poppy seeds over the past 4 years, especially in the German market [2]. It is actually easy for producers to reduce morphine contents in poppy seeds by washing or soaking them in water. However, with the reported variation of

morphine content in poppy seeds, more data on the urinary levels of morphine and its metabolites after intake of foods containing poppy seeds are required. To date, some investigators have reported urinary concentrations of morphine and codeine after ingestion of poppy seeds or foods containing poppy seeds [4–14]. Furthermore, as a recent trend, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry equipped with databases [15, 16] are now being utilized for drug screening in place of conventional immunochemical methods. The instrumental screening methods give much more sensitive and reliable results than those of the immunochemical tests, and show positive results even in very low concentration ranges of drugs in blood and urine, including opiates after ingestion of foods containing poppy seeds. This is the second reason why we need such data at low concentrations of opiates of food origin in human urine for discrimination from opiate abuse.

In this brief communication, we report the concentrations of morphine and codeine in urine after ingesting the most commonly eaten amounts of poppy seeds (i.e., two bean-jam buns decorated with poppy seeds). We determined the urinary concentrations of free (i.e., unconjugated form) opiates as well as those of total (i.e., sum of free and conjugated forms) opiates.

Bean-jam buns decorated with poppy seeds (Kimuraya Bakery, Tokyo, Japan) were purchased from a local market. Healthy volunteers (5 males and 9 females, aged 14–60 years old) who had no history of drug abuse or medical record of taking opiates, each ingested two bean-jam buns. Urine specimens were collected between 6 and 11 h after consumption. Morphine hydrochloride and codeine phosphate were purchased from Takeda Pharmaceutical (Osaka, Japan); *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane from

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Pierce Chemical (Rockford, IL, USA); solid-phase extraction (SPE) cartridges, Bond Elut Certify (3 ml), from Varian (Harbor City, CA, USA); enzyme immunoassay EMIT d.a.u. kits for opiates from Syva (San Jose, CA, USA). Morphine- d_3 used as internal standard (IS) was synthesized at the National Institute of Health Sciences (Tokyo, Japan).

GC-MS analysis for opiates in urine was performed according to our previous report [17] with a minor modification. Two milliliters of each specimen was mixed with 5 M $\text{NH}_4\text{Cl}/\text{NH}_4\text{OH}$ buffer (pH 8.9) and 200 ng of morphine- d_3 (IS). The mixture was loaded to a Bond Elut Certify cartridge that was preactivated by elution with methanol and water. The cartridge was washed with 2 ml each of water, 0.1 M acetic acid in water, and water, and then it was dried under vacuum for 10 min. The analytes were eluted with 3 ml of a mixture of dichloromethane/isopropanol/ NH_4OH (80:20:2, v/v/v), and the extract was evaporated to dryness. To the residue, 50 μl of BSTFA/pyridine (1:1, v/v) was added, and the tube was sealed and heated at 90°C for 30 min. The reaction mixture was evaporated to dryness, and the residue was redissolved in 50 μl of ethyl acetate. A 1- μl aliquot of the extract solution was subjected to GC-MS analysis. To measure total morphine and codeine concentrations, each specimen was hydrolyzed by adding concentrated HCl and heating at 100°C for 15 min. After cooling to room temperature, the pH was adjusted to 6 and the opiates were extracted by the same solid-phase extraction method as described above for free opiates.

The GC-MS analysis was carried out on an HP 5890 series II GC instrument interfaced to an HP 5971 MS

detector (Agilent, Palo Alto, CA, USA). The separation column was an Ultra-2 fused silica capillary (12 m \times 0.22 mm i.d., 0.33 μm film thickness; Agilent). GC-MS conditions were: carrier gas, helium; injection temperature, 250°C; transfer line temperature, 280°C; oven temperatures, 100°C (1-min hold) raised to 300°C at 20°C/min for 10 min; ions monitored, m/z 414 and 429 for morphine, m/z 417 and 432 for morphine- d_3 , and m/z 343 and 371 for codeine.

Morphine and codeine contents in the bean-jam buns were measured by the method of Struempfer [8] and found to be 2.20 and 0.77 μg per one piece of bun, respectively. The contents of morphine and codeine in poppy seeds were reported to be in the range of 1.5–294 $\mu\text{g/g}$ and 0.1–14 $\mu\text{g/g}$, respectively [1–3,18]. The urinary concentrations of free and total morphine and codeine at 6–11 h after ingestion of the two bean-jam buns are shown in Table 1. Through screening for opiates by EMIT, all specimens showed negative results. However, free and total morphine in urine could be measured by GC-MS. The free morphine concentrations were not higher than 5.3 ng/ml in all subjects. Total morphine concentrations were varied and the highest was 62.8 ng/ml in Subject 12, whereas it was below the detection limit (<1.0 ng/ml) in Subject 7. The concentrations of free codeine were below the detection limit for all subjects; the total codeine concentration was below the detection limit for 11 subjects, and very low levels were found for 3 subjects. In 1998, the US government raised the cutoff concentrations of morphine and codeine for urine drug testing from 300 to 2000 ng/ml [19]. The results in Table 1 show that the urinary morphine and codeine concentrations after ingestion of the two bean-jam buns were much lower than the cutoff values.

Table 1 Morphine and codeine concentrations in urine after ingestion of two bean-jam buns

Subject	Hours after ingestion	EMIT Opiate assay	GC-MS results			
			Morphine (ng/ml)		Codeine (ng/ml)	
			Free	Total	Free	Total
1	9.5	–	3.8	9.1	ND	1.5
2	10.5	–	2.5	3.2	ND	ND
3	10.0	–	2.5	3.1	ND	ND
4	10.0	–	1.6	4.3	ND	ND
5	8.0	–	1.4	6.7	ND	1.0
6	7.5	–	1.4	4.8	ND	1.0
7	6.5	–	1.5	ND	ND	ND
8	8.0	–	1.0	1.5	ND	ND
9	7.0	–	2.2	8.2	ND	ND
10	6.0	–	1.7	5.4	ND	ND
11	11.0	–	ND	6.7	ND	ND
12	8.0	–	5.3	62.8	ND	ND
13	8.0	–	1.8	9.3	ND	ND
14	8.0	–	2.4	24.5	ND	ND

EMIT enzyme multiplied immunoassay technique, ND not detected

Immunoassay kits, such as EMIT and Triage, commonly used in urinary drug screening, have the same cutoff value for opiates (300 ng/ml); thus, it is not surprising that neither opiate showed positive results by the EMIT test. The total morphine and codeine concentrations in urine after ingestion of the two bean-jam buns are comparable with those reported by Hasegawa et al. [5]. In their report, the levels of total morphine and codeine in urine 6–12 h after ingestion of two bean-jam buns were 2.5–48.2 ng/ml and less than 2.5 ng/ml, respectively.

In most of the previous reports on urinary concentrations of morphine and codeine, only total concentrations (combined concentrations of free and conjugated forms) of morphine and codeine were measured [1, 4–10, 12, 13]. ElSohly et al. [11] analyzed urine specimens without hydrolysis by GC-MS and reported that neither free morphine nor codeine was detected. Recently, Trafkowski et al. [14] reported LC-MS-MS analysis of urinary concentrations of free morphine, free codeine, and their conjugates after consumption of poppy seed cakes, which contained as much as 10–60 g of the seeds. Their study was unique in reporting concentrations of both free and conjugated opiates until our present study. We analyzed both free and total concentrations after consumption of quite common amounts of food with poppy seeds and revealed that free morphine concentrations were below 6 ng/ml, while free codeine was not detectable in all specimens. It seems that urinary concentrations of free morphine and codeine after consumption of poppy seed bean-jam buns are lower than those in opiate abuse cases. So far as we experienced, the free concentrations of morphine and codeine in urine obtained from an opiates abuser were more than 1500 and 160 ng/ml, respectively. On the other hand, even after ingestion of 3 g of poppy seeds, the free morphine and codeine concentrations were lower than 80 and 10 ng/ml, respectively (unpublished results). Although it requires further investigation, urinary concentrations of free morphine and codeine may become useful indicators for determining whether detected opiates are due to foods with poppy seeds or due to opiate abuse.

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