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Morphine intake from poppy seed food

KJELL BJERVER†, JOHN JONSSON, ANNA NILSSON, JAN SCHUBERTH* AND JUTTA SCHUBERTH

† *Swedish Administration of Prison and Probation, Stockholm, and National Laboratory of Forensic Chemistry, Toxicology Department, University Hospital, S-581 85 Linköping, Sweden*

Morphine was determined in commercially available poppy seed and seed cake as well as in urine from healthy normal human adults 3 and 15 h after ingestion of poppy seed cake. The following morphine concentrations were determined: between 374 ± 51 and 9·4 ± 1·0 μmol kg^{-1} poppy seed of different brands, 290 ± 11 μmol kg^{-1} poppy seed paste, 1·43 ± 0·07 and 0·53 ± 0·15 μmol $litre^{-1}$ urine sampled 3 and 15 h, respectively, after ingestion of two cakes and 0·67 ± 0·17 and 0·30 ± 0·06 μmol $litre^{-1}$ urine sampled 3 and 15 h, respectively, after ingestion of one cake. It is concluded that a positive finding of morphine in the urine from a person suspected of heroin abuse calls on some attention due to possible accidental morphine intake from poppy seed food.

Morphine constitutes one of the main metabolites of heroin and its identification in urine may indicate possible heroin abuse. The present study was initiated by a case of suspected heroin abuse submitted to our laboratory for chemical investigation. The preliminary analysis with the 'enzyme multiplied immuno-assay technique' (EMIT) revealed the presence of opiates in the urine, a finding which was extended to the identification of morphine by thin-layer chromatography (t.l.c.) and by gas chromatography-mass spectrometry (g.c.-m.s.). The suspect, however, denied any heroin or morphine abuse but a few hours before urine sampling he had eaten a poppy seed cake. Since the question whether the seed of the opium poppy (*Papaver somniferum*) contains morphine is a matter of controversy (Mika 1955; Preininger et al 1965; Fairbairn & El-Masry 1968; Sarkany et al 1970; Adler et al 1972; Grove et al 1976), we investigated the possibility of this being the source of morphine intake.

To this end, one or two helpings of poppy seed cake of the same type as that eaten by the suspect were given to healthy subjects and the morphine content in the urine, in the cake filling ingested, and also in poppy seed of different brands assayed.

MATERIALS

Unspecified blue poppy seed (500 g) and cakes containing this material were purchased at a shopping centre in downtown Stockholm. White seed with the trade names of 'Durkee' or of 'Santa Maria' and blue seed with the trade name of 'Kockens, Licensee of McCormick' were obtained at shopping centres in Linköping. Morphine labelled with three deuterium atoms in the *N*-methyl group ([^{2}H]morphine) was purchased from Massanalys, Stockholm, Sweden. Its purity controlled by g.c.-m.s. was found to be 99·9%. Seven healthy normal human adults with no previous history of abuse or medicinal record of taking opiates were used in the experiment.

* Correspondence.

METHODS

Detection of morphine by g.c.-m.s. Blue poppy seed (5·0 g) was shaken in 10 ml 0·1 M citric acid (pH 4·0) for 1 h and then removed by filtration. Five ml urine was heated to 100 °C for 1 h after addition of 5 ml 2 M HCl. The following procedure was then used to extract morphine from water extracts of seed or from urine into organic solvents: The pH of 10 ml water phase was adjusted to pH 9·0 and the final volume to 20 ml by addition of water. After absorption of the solution on a dry kieselguhr column (15 g Extrelut, 800 × 28 mm, Merck), elution was carried out with 60 ml dichloromethane–2-propanol (9:1). The eluate was evaporated to dryness and the residue dissolved in 5·0 ml chloroform. One to 2 ml chloroform solution was taken to dryness and the residue heated at 65 °C with 100 μl pentafluoropropionic anhydride (PFPA) (Pierce Chemical Co.) for 30 min to yield morphine-PFPA. Excess PFPA was removed by evaporation under a stream of N_2 and 10 μl ethyl acetate was added to solve the residue. One to 2 μl of the ethyl acetate solution was injected into the g.c.-m.s. (Finnigan 4000 on line with the Incos data system). The separation was in a glass column (1·2 m; 2 mm i.d.) filled with 3% SP 2250, operated at 215 °C and eluted with 20 ml min^{-1} He. Silanization of glass wares and chromatography column was carried out with Silyl-8 (Pierce Chemical

Co.). The m.s. was operated in the electron impact mode and set at one scan per 2 s over the mass range of *m/z* 100 to *m/z* 600.

Detection of morphine by thin-layer chromatography. Urine was hydrolysed and extracted as described above up to the step of dissolving the residue from the Extrelut column. After application of extracts on a silica gel plate (100 × 100 mm; 60F-254; Merck, Darmstadt) it was exposed to ammonia vapour for 3 min and then developed with ethyl acetate–methanol–ammonium hydroxide (85:10:5) in a Camag Micro Chamber (28510). The spots were localized after spraying the plate with iodoplatinate in 0·5 M H_2SO_4.

Quantitative assay of morphine. To 1·0 ml of urine was added 0·2 nmol [^{2}H]morphine as an internal standard. After acid hydrolysis of some of the samples, all of them were next extracted into organic solvents and finally treated with PFPA to yield morphine-PFPA as earlier described (Dahlström et al 1977). One to 2 µl of the extracts were analysed by g.c.-m.s. under g.c. conditions described above under 'Detection of morphine by g.c.-m.s.'. The m.s., operated in the electron impact and multiple ion detection modes, was focused at the *m/z* numbers of 361, 414, 430 and 577, which correspond to the major fragments of morphine derivative, and at the *m/z* number of 417, which corresponds to one of those of [^{2}H]morphine derivative. The concentration of morphine in the sample was calculated from the estimated peak area ratio of *m/z* 414 and *m/z* 417 at a scan number between 104 and 125, which corresponds to the retention time for morphine-PFPA.

Water extracts of poppy seed were prepared by homogenization of 5 g seeds in 100 ml or of 50 mg in 2 ml 0·1 M citric acid (pH 4·0) containing [^{2}H]morphine as an internal standard. Water extracts were also obtained by washing 5 g seeds for 1 h in 10 ml citric acid containing [^{2}H]morphine. After filtration the clear solutions were extracted into organic solvents, treated with PFPA as described by Dahlström et al (1977), and analysed by g.c.-m.s. as explained above.

RESULTS

Three h and 15 h after intake of one poppy seed cake by four healthy volunteers and of two cakes by three volunteers, urine samples were collected. In all samples taken after 3 h, we identified an iodoplatinate positive spot with a R_F-value of 0·3, which corresponds to that of authentic morphine. To establish more firmly the presence of morphine, the urine extracts were analysed by g.c.-m.s. after derivatization with PFPA to yield morphine-PFPA. As shown in Fig. 1a, the extracts generated a mass spectrum almost identical to that of standard morphine derived with PFPA (Fig. 1c).

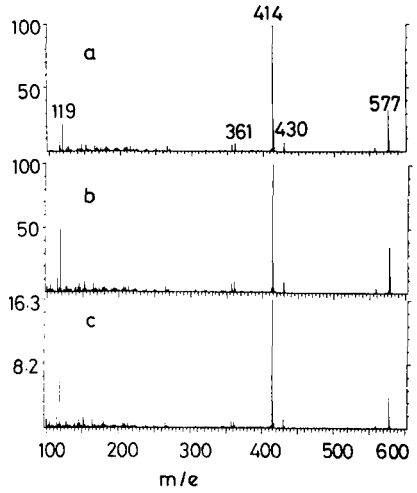

FIG. 1. Mass spectrum generated by extracts of urine, poppy seed or morphine standard. Morphine in washings of blue poppy seed or in urine sample taken from a healthy subject 3 h after intake of two poppy seed cakes was enriched by a liquid/solid phase extraction technique on Extrelut columns. After derivatization with PFPA to yield morphine-PFPA the extracts were analysed by g.c.-m.s. Mass spectrum (a) is generated from urine extracts and represents a summation of 12 scans. Mass spectrum (b) is generated from poppy seed extracts at scan number 119. Mass spectrum (c) recorded at scan number 119 is generated from a standard solution of morphine derivative with PFPA.

The concentrations of morphine excreted in the urine after intake of one or two poppy seed cakes were measured by g.c.-m.s. The results demonstrated in Table 1 show clearly measurable amounts of both 'free' and 'free plus conjugated' morphine 3 h and even 15 h after intake of poppy seed cake. Higher morphine concentrations were found following ingestion of two cakes compared with ingestion of one cake.

To estimate the dose of morphine taken, we quantitatively assayed the cakes of the same type and origin as those tested. The determinations made on the paste-like filling, which was taken through the same procedures for extraction, derivatization, and analysis as used for the urine samples, yielded morphine at 290 ± 11 µmol kg^{-1} paste (mean ± s.e.m. of four determinations). Since the amount of filling per cake was estimated at 50–70 g, this means that each cake held about 5 mg morphine.

When it became clear that ingestion of food containing poppy seed could bring about a positive finding of urinary morphine, we investigated this source for its possible content of morphine. Extracts from washings of poppy seed were therefore injected into the g.c.-m.s. and mass spectrum at scan number 119 corresponding to the retention time for morphine-PFPA was recorded. As shown in Fig. 1b, the spectrum contained all fragments typical of authentic morphine-PFPA: the main fragment at *m/z* 414, the molecular ion at *m/z* 577, as well as minor fragments at *m/z* 361, *m/z* 430, and *m/z* 558. The relative intensities of the fragments generated from the poppy seed extract did not differ from those obtained following injection into the g.c.-m.s. of authentic morphine-PFPA (Fig. 1c).

Table 1. Morphine in urine after ingestion of poppy seed cake. To 1·0 ml of urine, sampled 3 and 15 h after intake of poppy seed cakes was added 0·2 nmol [^{2}H]morphine as internal standard. After acid hydrolysis of those of the samples, in which 'total' (free plus conjugated) morphine was determined, all of samples were next extracted into organic solvents; the extracts were treated with PFPA to yield morphine-PFPA and finally analysed by g.c.-m.s. Data are given as the mean ± s.e.m. of three or four determinations.

Number of cakes ingested	Morphine (μ M) in urine sampled after 3 h		15 h	
	'Free'	'Total'	'Free'	'Total'
1	0·44 ± 0·06	0·67 ± 0·17	0·21 ± 0·05	0·30 ± 0·06
2	1·07 ± 0·02	1·43 ± 0·07	0·39 ± 0·12	0·53 ± 0·15

The concentrations of morphine in different types of seed were estimated. As shown in Table 2, blue seed of unspecified trade-mark contained substantial amounts of morphine as did both types of white seed investigated. However, the data also showed that the two brands of blue poppy seed investigated differed markedly in content of morphine. Furthermore, the values on the individual estimates were spread more widely when the assay was on a small amount than when made on a large amount of sample. Yet even the assays on a large sample amount yielded a 'standard error of the mean' higher than that accounted for by the analytical procedure.

DISCUSSION

The results show that commercially available poppy seed contains morphine and the highest mean value found, 107 mg kg^{-1}, is 6–35 times greater than that reported by Grove et al (1976) for blue or white seed. However, it is still well below the morphine concentration of poppy straw which was found to hold 4500 mg kg^{-1} by Wu et al (1979). The wide variation between batches of blue seed suggests the presence of morphine may be due to inadequate cleaning of the seed with most of the opium alkaloid coming from debris or latex on the seed surface. Moreover, the results presented in Table 2 show that about 40% of the total morphine content in the blue poppy seed could be isolated from the seeds by a single simple washing procedure with slightly acidic water.

Table 2. Morphine concentrations in commercially available poppy seed. Water extracts of poppy seed were prepared by homogenization or by washing of seeds in citric acid containing [^{3}H]morphine as an internal standard. After filtration the clear solutions were extracted into organic solvents, the extracts treated with PFPA, and analysed by g.c.-m.s. The data are given as the mean ± s.e.m. with the number of experiments within parentheses.

Specimen and amount taken for assay (g)		Morphine μmol kg^{-1} seed	Morphine μmol litre^{-1} washings of 1 kg seed
Blue seed	0·05	300 ± 129 (7)	
Blue seed (not specified)	5·0	374 ± 51 (5)	142 ± 6 (5)
Blue seed (Kockens)	5·0	9 ± 1 (5)	
White seed (Durkee)	5·0	181 ± 10 (3)	
White seed (Santa Maria)	5·0	212 ± 13 (5)	

The seed *Papaver somniferum,* is a common ingredient in many dishes served in Europe, e.g. poppy seed and maple syrup bread, veal with poppy seeds, pork chops with apples and poppy seeds, fillets of pompano with toasted poppy seeds, poppy seed sauce, poppy seed breakfast buns, Hungarian poppy seed torte or Ukranian poppy seed cake (Polvay 1981). We have shown that poppy seed cake, commercially available in Sweden, may contain morphine. The amount of the alkaloid per helping is estimated at about 5 mg, which corresponds to half a therapeutic dose of morphine when given as an injection. However, due to an effective 'first-pass' extraction of morphine from the blood into the bile where the alkaloid is accumulated, a single oral dose of 5 mg is conceivably not sufficient to precipitate any pharmacological effects. Supporting this is the observation that apart from some constipation none of the persons taking the cakes was able to recognize any signs of morphine effects after intake of 5–10 mg of the alkaloid. Nonetheless, a single dose of 5 mg morphine ingested from poppy seed food is sufficient to create a positive finding of morphine in the urine, even if detected by t.l.c. This simple analytical procedure contrasts with the more sophisticated

techniques that Hazum et al (1981) had to use to analyse trace amounts of morphine of enigmatic origin in cow and human milk. From the data here reported we thus conclude that caution should be exercised when attributing the presence of morphine in a body fluid or tissue to opiate abuse or to accidental morphine intake from poppy seed food.

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