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LETTER TO THE EDITOR

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Postmortem distribution pattern of morphine and morphine glucuronides in heroin overdose

Skopp G et al.: Int J Legal Med (1996) 109 : 118–124

Sir, I have read with great interest an article of G. Skopp et al. [1] on post-mortem distribution of morphine and its glucuronides in heroin overdose cases. The authors have determined morphine, morphine-3-glucuronide (M3G), morphine-6-glucuronide (M6G) as well as water content and hematocrit values in blood samples, taken from different sites in four cases of acute, fatal heroin overdose. In three cases, cerebrospinal fluid was also investigated, in one case together with vitreous humor. The analytes were determined by means of HPLC with fluorescent detection, using external calibration curve.

In discussion the authors stated, that the concentrations of morphine, M3G and M6G exhibited a marked site dependent concentration range.

However, a close look on the results presented did not reveal any specific pattern, which the concentrations of all analytes or their molar ratios should follow. The results, and particularly the molar ratios seemed to be erratically scattered over the whole concentration range observed. Particular concern deserved the determination of M6G; in four samples this substance was not detected, despite pretty high concentrations of morphine and M3G, and the ratio M6G/morphine showed variations exceeding 800% in one case.

The available toxicological literature contains a good amount of observations evidencing, that the concentrations of drugs in post-mortem blood are site- and time dependent. In general, the concentrations were higher in central vessels and in heart and are more subjected to post-mortem increase than those in peripheral veins. This was observed for barbiturates [2, 3], antidepressants [3–6], benzodiazepines [6], cocaine [7] and non-opiate analgesics [8, 9]. The distribution data for opiates (morphine and codeine) are scarce but show similar trend [4, 6]. In our own study on the determination of morphine, M3G, M6M and 6-monoacetylmorphine in post-mortem femoral blood, cerebrospinal fluid and vitreous humor a strong correlation between the values in all fluids was observed [10].

This obvious disagreement of presented results with the published data prompted us to perform a short own study on the same topic, applying more specific method of identification and quantification by means of deuterated internal standards.

Material

The samples of body fluids were taken from two cases of acute heroin overdose.

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Case 1 (S-541/96)

31-year-old jobless man was found in the city park. Used syringe and heroin utensils were found nearby. The autopsy was performed 20 hr later.

Case 2 (S-555/96)

40-year-old man with known story of drug abuse was found dead in his apartment. Used syringe was found nearby. The autopsy was performed 3 days after discovery of the body.

Immunochemical screening of femoral blood samples from both cases, performed by means of EMIT-ETS assay [11], revealed positive reaction on opiates. The samples of blood (ca. 5 ml each) were taken from thoracic aorta, superior vena cava, inferior vena cava, left subclavian vein, right subclavian vein, left femoral vein and right femoral vein. The samples of cerebrospinal fluid and vitreous humor were also taken. The samples were centrifuged, the supernatants were collected and frozen until analyzed.

Sample preparation

1 ml of thawed sample was mixed with 2 ml of 0.01 M ammonium carbonate buffer (pH 9.3) and the mixture of internal standards, containing each 100 ng of morphine-d_3 (M-d_3), M3G-d_3, M6G-d_3, codeine-d_6 (C-d_6), codeine-6-glucuronide-d_3 (C6G-d_3) and 6-monoacetylmorphine-d_6 (MAM-d_6) was added. After vortexing and centrifugation 2 ml of supernatant was passed through the activated SPE-C_{18} column. After rinsing with ammonium carbonate buffer the drugs were eluted with 0.5 ml methanol-0.5 N acetic acid (9 : 1) and evaporated. The residue as reconstituted in 100 µl mobile phase and 20 µl were injected into chromatograph.

Analysis

The determination of all drugs in one analytical run was performed by means of atmospheric pressure chemical ionization mass spectrometry - liquid chromatography (APCI-LC-MS). The mobile phase consisted of 50 mM ammonium formate buffer (pH 3.0) and acetonitrile (95 : 5), the flow rate was programmed from 0.6 to 1.1 ml/min, the analysis time was 17 min. A SSQ 7000 instrument (Finnigan MAT, San Jose, USA) was used in positive ionization mode. Following ion were monitored: m/z 286 (for M, M3G and M6G aglycone), 289 (for M-d_3, M3G-d_3 and M6G-d_3 aglycone), 300 (for C and C6G aglycone), 303 (for C6G-d_3 aglycone), 306 (for C-d_6), 328 (for MAM), 334 (for MAM-d_6), 462 (for M3G and M6G) and 476 (for C6G). The quantitations were performed using internal standardization method and adequate area ratios for each

Table 1 Concentrations (μg/l) and molar ratios of analytes in Case 1

	1	2	3	4	5	6	7	8	9
M3G	132	155	100	173	171	150	124	172	100
M6G	66	39	34	35	28	29	28	57	36
M	136	121	132	132	118	93	118	94	63
C6G	49	60	49	54	66	55	59	94	63
C	9	11	12	9	6	+	+	10	+
MAM	12	8	6	9	9	8	9	17	24
M3G/M	0.60	0.80	0.47	0.81	0.90	1.0	0.65	0.92	1.13
M6G/M	0.30	0.20	0.16	0.16	0.15	0.19	0.15	0.30	0.41
C6G/C	3.42	3.43	2.57	3.77	6.92			5.91	

Table 2 Concentrations (μg/l) and molar ratios of analytes in Case 2

1: thoracic aorta, *2*: superior vena cava, *3*: inferior vena cava, *4*: left subclavian vein, *5*: right subclavian vein, *6*: left femoral vein, *7*: right femoral vein, *8*: cerebrospinal fluid, *9*: vitreous humor
+ = concentration below the limit of quantitation (for codeine 5 μg/l)

	1	2	3	4	5	6	7	8	9
M3G	162	306	149	193	199	156	127	432	358
M6G	276	298	300	165	206	48	32	165	87
M	380	359	449	513	406	201	136	245	179
C6G	97	129	124	119	87	87	58	133	114
C	21	26	23	19	21	+	9	19	15
MAM	10	11	11	6	11	11	11	33	17
M3G/M	0.26	0.53	0.21	0.23	0.30	0.48	0.58	1.10	1.24
M6G/M	0.45	0.52	0.42	0.20	0.32	0.15	0.15	0.42	0.30
C6G/C	2.90	3.12	3.39	3.94	2.61		4.05	4.4	4.78

analyte. The calibration curves consisted of five points, covering the concentration range of 5 to 500 μg/l for each analyte. Appropriate standards were obtained from Lipomed AG, Arlesheim, Switzerland and High Standard Products Corporation, Inglewood, CA, USA.

Results

Tables 1 and 2 show the results obtained. The concentrations profile is pretty consistent with findings of other authors, i.e. the values of M, M3G and M6G observed in peripheral veins were lower than in central vessels. The molar ratios of analytes were, however, pretty stable. This is particularly important, because high proportion of free morphine in blood, together with high concentration of MAM, indicates short survival time after heroin intake [10]. This seems to be valid not only for peripheral, but also for central vein blood samples.

The determination of opiates and its glucuronides in postmortem body fluids is very challenging task, particularly when the results are used as indicators concerning the survival time. Our own experience with the application of HPLC with electrochemical detection for this purpose have learnt us, that the matrix interferences may create enormous problems. In our view, only API-LC-MS (electrospray or APCI) assures an appropriate specificity for this kind of analyses at the moment.

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