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Determination of Cross-Reactant Drugs with a New Morphine Radioimmunoassay Procedure

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ABSTRACT: A study to determine the specificity of a new morphine radioimmunoassay (RIA) procedure was performed on 23 drugs of forensic science interest. Spiked whole blood and urine specimens were analyzed and the apparent morphine concentrations determined. All compounds analyzed showed low cross-reactivity, indicating a high specificity for morphine.

KEYWORDS: toxicology, morphine, radioimmunoassay

The determination and quantitation of morphine in blood specimens poses special problems for the forensic toxicologist. Not only are the levels in the microgram/litre range, but the extraction procedures are usually quite involved, with the resultant recovery being lower than desired.

Radioimmunoassay (RIA) has proved to be an effective analytical tool for both the qualitative and quantitative analysis of morphine [*1,2*]. Results can be obtained using a small sample, as low as 25 μL, and the analysis can be performed directly on biological fluids or tissue homogenates, thus eliminating the extraction procedure and possible loss of morphine. A major disadvantage, for quantitative purposes, of morphine kits available to date has been the lack of specificity [*3*], resulting in cross-reactivity with other opiate condensed ring structures [*1*]. Abuscreen® Radioimmunoassay for Morphine (Roche Diagnostics, Nutley, NJ 07110), although sensitive to morphine (detection limit 10.0 μg/L) cross-reacts with codeine on the order of 100%. Other cross-reactants with this procedure include, but are not limited to, dihydromorphine, morphine-3-glucuronide, and meperidine [*3*]. This lack of specificity for morphine may lead to spurious results if one of these crossreactants is present in a specimen along with morphine.

This paper describes a cross-reactivity study conducted with the Coat-A-Count® Morphine RIA kit (supplied by Diagnostic Products Corp. 5700 W. 96 St., Los Angeles, CA 90045). Whole blood and urine were spiked with various drugs of forensic science interest. The samples were analyzed to determine the percent of cross-reactivity, if any, when compared to morphine.

Materials and Methods

Coat-A-Count Morphine RIA kits were used in the study. The kit consists of solid phase morphine antibody-coated polypropylene tubes (12 by 75 mm), lyophilized iodinated (I-125)

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morphine, and morphine calibrators (0, 1, 5, 10, 25, and 100 μg/L prepared in urine which are used to construct a standard curve. Whole blood, obtained from the South Florida Blood Bank, and urine, obtained from healthy adult male volunteers, were screened for the presence of morphine and common drugs of abuse by RIA and gas chromatography. Both blood and urine were found to be drug free. Aliquots of the blood and urine were spiked with methanolic or aqueous drug standards to the desired working concentrations (2 000 and 10 000 μg/L). The standards used were available to the laboratory as pure drug reference standards. The working concentrations of 2 000 and 10 000 μg/L were chosen since these were the same concentrations used by the suppliers of morphine RIA kits to determine cross-reactivity [*3,4*]. Blood and urine morphine quality control samples were prepared from a 1000 mg/L methanolic standard to the concentrations of 5, 10, 25, and 100 μg/L.

A Micromedic Isoflex (Micromedic Systems, Horsham, PA 19044) dual channel gamma radiation counter coupled to a Micromedic's Compucenter data system was used for instrumentation.

The basic procedure involves a 25-μL sample and 1.0 mL of iodinated morphine added to an antibody-coated tube, and incubated at room temperature for 1 h. Following incubation, the tubes are decanted to remove all visible moisture and counted in a gamma counter for 1 min. The total count and nonspecific binding tubes were prepared in noncoated tubes. Standards and samples were analyzed in triplicate.

Results and Discussion

Blood and urine morphine quality control data are presented in Tables 1 and 2. Cross-reactivity data are presented in Table 3.

Cross-reactivity for the drugs analyzed range from 0 to 11.39%. Codeine, with an actual concentration of 2000 μg/L, gave an apparent morphine concentration of 2.8 μg/L, in the blood and a value of zero in the urine. This blood level represents a cross-reactivity of 0.14% which is lower than the value of 0.24% reported by the manufacturer [*4*]. This low cross-reactivity with codeine should eliminate the possibility of false positive results with codeine levels expected in blood.

As seen in Table 3, nalorphine had a cross-reactivity in blood and urine of 11.39 and 7.02%, respectively. Although no longer produced for human use, nalorphine was included in this study because it is still available for veterinary use and could possibly filter into illicit drug trade.

Linearity of the morphine calibration curve (Fig. 1) decreases in the range of 25 to 100 μg/L. This decreased linearity is reflected in the results of the data reduction of the 100-μg/L blood and urine quality controls (Tables 1 and 2). Averaging the results from the 100-μg/L blood and urine quality controls yields a mean value of 74.75 and 70.53 μg/L, respectively. This large difference between the mean value and the target value of 100 μg/L is due to the dynamics of the calibration curve. The curve approaches a flat line above 25 μg/L (Fig. 1). A change in concen-

TABLE 1—*Blood morphine quality controls.*[a]

	Target Value			
	5 μg/L	10 μg/L	25 μg/L	100 μg/L
	8.0	10.9	24.2	78.6
	10.4	15.5	27.3	63.2
	5.9	9.9	30.7	82.5
$\bar{X}$	8.10	12.12	27.41	74.75
S.D.	2.28	2.99	3.23	10.22

[a]Between day comparison of quality control samples.

TABLE 2—*Urine morphine quality controls.*[a]

	Target Values			
	5 μg/L	10 μg/L	25 μg/L	100 μg/L
	5.5	11.5	24.6	76.8
	5.5	11.4	21.9	78.1
	9.3	11.5	19.7	56.7
$\bar{X}$	6.77	11.45	22.08	70.53
S.D.	2.17	0.01	2.45	11.98

[a]Between day comparison of quality control samples.

TABLE 3—*Percent of cross-reactivity of spiked blood and urine samples.*[a]

Drug	Blood,[b] 2000 μg/L	Urine,[b] 2000 μg/L	Blood,[b] 10 000 μg/L	Urine,[b] 10 000 μg/L
Codeine	0.14	0	0.01	0
Nalorphine	11.39	7.02	0.65	2.46
Methadone	0.08	0.05	0	0.04
Meperidine	0	0.07	0	0.05
Oxycodone	0	0.06	0	0.05
Hydrocodone	0.12	0	0	0.04
Hydromorphone	0.55	0.24	0.12	0.25
Fentanyl	0.09	0.04	0	0.03
Butorphanol	0.05	0	0	0.04
Pentazocine	0	0.09	0.01	0
Propoxyphene	0.01	0.04	0	0
Naloxone	0.08	0	0	0.03
Levallorphan	0	0.04	0.01	0.07
Diphenoxylate	0.06	0.48	0	0.04
Diacetylmorphine	2.16	0.36	0.94	0.24
Dextromethorphan	0	0.04	0	0.05
Cocaine	0.05	0	0.01	0.04
Quinine	0.10	0.12	0	0
Apomorphine	0.09	0	0.21	0.04
Alphaprodine	0.10	0	0	0.03
L-alpha-acetylmethadol	0.4	0.07	0	0.03
Papaverine	0	0.06	0	0.05
Levorphanol	0.09	0.10	0.01	0.08

[a]Percent of cross-reactivity $= \frac{\text{Apparent morphine concentration of sample}}{\text{Actual concentration of sample}} = \text{X } 100.$

[b]Percent of cross-reactivity.

tration above this level results in only a minimal change in the ratio plotted on the abscissa. Linearity of the calibration curve may be improved by including standards in the range of 25 to 100 μg/L or by diluting specimens to a concentration approaching 25 μg/L.[2]

Table 3 shows the cross-reactivity of many drugs to be higher at the lower concentration (2000 μg/L) than at a higher concentration (10 000 μg/L). Because of the high specificity for morphine of this procedure, an increase in concentration of drug other than morphine does not cause an increase in binding of drug to morphine antibodies. The larger value of the denomi-

[2]Personal communication, Diagnostic Products Corp., Technical Services Department, Los Angeles, CA, Aug. 1984.

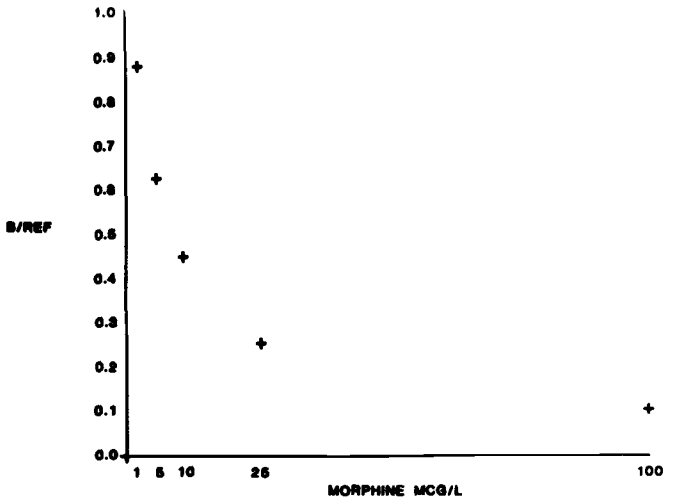

FIG. 1—*Morphine standard curve.*

nator (equation, Table 3) for the higher concentration causes the percent of cross-reactivity to appear to be less than that of the lower concentration.

Conclusion

A group of 23 drugs were analyzed for cross-reactivity by radioimmunoassay using the Coat-A-Count Morphine assay. This procedure was determined to be very specific for morphine. Because of its specificity, this procedure is better suited for the quantitative analysis of morphine rather than a general screening for opiates.

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References

[*1*] Spiehler, V.R., Reed, D., Cravey, R. H., Wilcox, W. P., Shaw, R. F., and Holland, S., "Comparison of Results for Quantitative Determination of Morphine by Radioimmunoassay, Enzyme Immunoassay, and Spectrofluorometry," *Journal of Forensic Sciences*, Vol. 20, No. 4, Oct. 1975, pp. 647-655.

[*2*] Catlin, D., Cleeland, R., and Grumberg, E., "A Sensitive, Rapid Radioimmunoassay for Morphine and Immunologically Related Substances, in Urine and Serum," *Clinical Chemistry*, Vol. 19, No. 2, 1973, pp. 216-220.

[*3*] Package insert for Abuscreen®, Radioimmunoassay Roche Diagnostic, Nutley, NJ, April 1983.

[*4*] Package insert for Coat-A-Count® Morphine in Urine, Diagnostic Products Corp., Los Angeles, CA, Nov. 1982.

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