TECHNICAL NOTE

F. P. Smith,[1] Ph.D.; R. C. Shaler,[2] Ph.D.; C. E. Mortimer,[3] Ph.D.; and L. T. Errichetto,[4] M.S.

Detection of Drugs in Bloodstains. II: Morphine

REFERENCE: Smith, F. P., Shaler, R. C., Mortimer, C. E., and Errichetto, L. T., **"Detection of Drugs in Bloodstains. II: Morphine,"** *Journal of Forensic Sciences*, JFSCA, Vol. 25, No. 2, April 1980, pp. 369-373.

ABSTRACT: Picogram quantities of the opiate alkaloid, morphine, were detected in a 50-μl samples of dried bloodstain by using radioimmunoassay. The age of the stain versus detectability of morphine, the separation of endogenous interfering substances, and morphine extractibility by various agents were investigated.

KEY WORDS: toxicology, morphine, blood

In an earlier account [*1*] we reported the enhanced persistence of the antiepileptic drug phenytoin over proteinaceous genetic markers and the other factors in dried bloodstains through the use of the radioimmunoassay (RIA) technique.

The advantages of RIA, including increased sensitivity over existing methods, have recently been pointed out with increased emphasis on forensic science utility [*2,3*]. After the application of RIA to opiate alkaloids was presented by Spector and Parker [*4*] in 1970 it was possible to detect picogram (10^{-12} g) quantities of morphine in human plasma and urine. Morphine, a biological metabolite of heroin, was detected by Möller et al [*3*] in bloodstains created from blood that had been spiked with high concentrations of morphine. Through these in-vitro studies Möller et al drew the conclusion that the presence of morphine could be reliably excluded under the conditions of his experiments [*3*].

In our study, picogram quantities of morphine were detected in 50-μl samples of bloodstains from an individual on morphine therapy. Furthermore, the age of the stain versus detectability of morphine, the effect of endogenous cross-reacting substances, and morphine extractibility by various agents were investigated. This study points out the expanding use of RIA in forensic science.

Received for publication 1 May 1979; revised manuscript received 21 Sept. 1979; accepted for publication 19 Oct. 1979.

[1] Assistant professor, Department of Criminal Justice, University of Alabama in Birmingham.
[2] Director of serology, Office of the Chief Medical Examiner, New York, N.Y.
[3] Research assistant professor, Chemistry Department, University of Pittsburgh, Pittsburgh, Pa.
[4] Criminalist, Metropolitan Police Department, Criminalistics Bureau, Las Vegas, Nev.

Experimental Procedure

Standard Curve

A standard concentration curve for morphine was prepared by assaying, in duplicate, 0.1-ml samples of morphine in 0, 2.5, 5.0, 10.0, 20.0, and 40.0 ng/ml standard solutions. These solutions were prepared by dilution of the 40.0 ng/ml standard solution provided by the RIA kit manufacturer. The actual amounts of morphine assayed were 250, 500, 1000, 2000, and 4000 pg. Next, 0.2 ml of ^{125}I-labeled morphine and 0.2 ml of morphine antiserum were added. The assay was completed as described by the manufacturer [5]. All duplicates agreed within the manufacturer's ±12% experimental error. Data obtained are plotted in Fig. 1.

Sample Preparation

Dried Bloodstains—Morphine-positive bloodstains were created by drying 50 μl of ethylenediaminetetraacetic acid-anticoagulated blood onto a cloth sheet, as was done with phenytoin [*1*]. The blood was obtained from an individual on morphine therapy in a clinical environment (Central Medical Pavilion, Pittsburgh, Pa.).

Plasma—Whole blood was centrifuged for 3 min at 1000 *g* to separate the plasma from the red blood cells (particulates). Morphine concentration was determined directly from 0.1 ml plasma by the RIA method described.

Whole Blood—Ethylenediaminetetraacetic acid-anticoagulated whole blood (100 μl) was analyzed for the presence of morphine by the RIA method described without sample dilution. Necessary column chromatographic purification was performed before the RIA

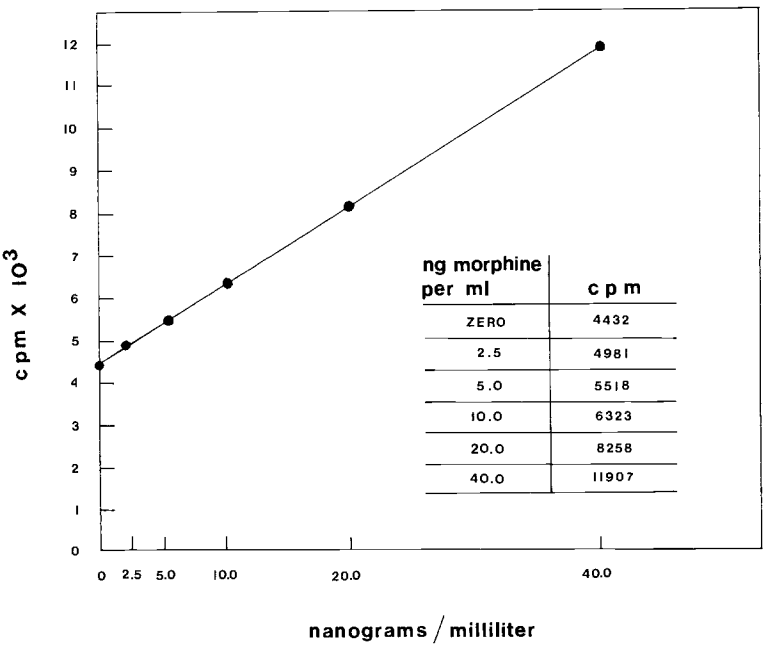

FIG. 1—*This standard curve illustrates the sensitivity of the RIA procedure for morphine determinations. Since only 0.1 ml of the standard solutions were tested, the actual amounts of morphine assayed correspond to 250, 500, 1000, 2000, and 4000 pg.*

analysis to remove interfering blood chromophores which caused quenching when using the beta scintillation counter in this laboratory.

Column Chromatography—Bloodstain eluates and whole blood were purified by quantitative transfer to #1001 Jet® tube columns. The pH of the eluates was adjusted so that the drugs were preferentially soluble in the organic phase. This was accomplished by the pretreatment of the Jet tube with 1.0 ml saturated ammonium chloride buffer, pH 9.3, as recommended by the manufacturer.[5] The eluting solvent was 90:10, methylene chloride/isopropanol. Three successive 4-ml washes were spaced by 2- to 5-min equilibration periods. The drug was recovered from Jet tube columns at 84% efficiency as described by the manufacturer and confirmed by radioactive tracer recovery in this laboratory.

Elution by Detergents

For studies on the extractability of morphine, bloodstains were prepared by pipetting 50 μl of morphine-spiked donor blood onto white unbleached cotton cloth. The concentration of morphine in the blood was 1.0 ng/μl (50 ng/50 μl dried bloodstain). The purity of the drug used to spike blood was verified first by silica gel thin-layer chromatography (TLC) with 100:1.5 methanol/ammonium hydroxide as the developing solvent and acidified iodoplatinate spray as the visualizing agent [*6*]. Only one spot was detected and its R_f value was equivalent to that described in the literature [*7*, p. 807]. Second, an infrared spectrum using 1% drug in a KBr disk was taken as a positive check against the known spectrum of morphine [*7*, p. 747].

An ionic detergent, sodium dodecyl sulfate, $CH_3(CH_2)_{11}OSO_3^-Na^+$(SDS), and a nonionic detergent, Triton X-100, $CH_3C(CH_3)_2CH_2C(CH_3)_2C_6H_4O[CH_2CH_2O]_nH$ (n = 10), were examined for their efficiency at extracting morphine from dried bloodstains. Detergent solutions (0.1 and 1.0%) of each were prepared in physiological saline.

Materials

Authentic drug standards were donated by Mr. Dennis Hahn, Allegheny County Crime Laboratory, Pittsburgh, Pa. Jet tubes may be obtained from Har-Len Associates, Pittsburgh, Pa. Uniplate silica gel TLC plates were purchased from Analtech Inc., Newark, Del. Spray reagents (iodoplatinate) were purchased from Quantum Industries, Fairfield, N.J. Morphine-positive physiological fluids were obtained from the Central Medical Pavilion, Pittsburgh, Pa. Negative control blood was obtained from the Central Blood Bank of Pittsburgh, Pa. The RIA kit for morphine was purchased from Hoffmann-LaRoche, Inc., Roche Diagnostics, Inc., Nutley, N.J.

Results and Discussion

The standard curve (Fig. 1) illustrates the sensitivity of the RIA procedure for morphine determinations. The detectable morphine amount in the 50-μl bloodstain of a 64-kg individual receiving a single oral 5-mg morphine dose is comfortably within the standard curve. Blood, blood plasma, and bloodstains aged for nine months contained 2000, 1220, and 700 pg/0.05 ml, respectively. The whole blood samples were tested to give amounts that might be expected from bloodstains of the same volume. These data, as expected, indicate that blood plasma has larger concentrations of morphine than whole blood; the difference in morphine concentration between whole blood and blood plasma represents the dilution factor of the red blood cells in the specific blood sample. The

[5] P. Harris, Tel Instrument Co., Lawndale, Calif., personal communication.

recovery rate of morphine in the nine-month-old stain was 57%. Negative control bloodstains, blood plasma, and whole blood contained no cross-reacting substances.

Enhancement of extraction with different detergents was investigated. The stains were extracted with nonionic Triton X-100 and anionic SDS detergents for 1 h, as previously described [*1*]. The Triton X-100 extracted 86.8 and 63.0 ng of morphine at concentrations of 0.1 and 1.0%. This corresponds to 152 and 110% of the amount extracted by saline alone. The anionic detergent SDS extracted 84.8 and 26.4 ng of morphine (or 149 and 46% of the saline value) for 0.1 and 1.0% detergent solutions. Thus both detergents when used in the lower concentrations enhance extraction of the drug approximately 50% over the values obtained for physiological saline alone. The possibility that higher detergent concentrations may inhibit the RIA reaction was not investigated. The critical micelle concentration values (CMC values) for these detergents are 8.08×10^{-3} molar and 3.35×10^{-4} molar for SDS and Triton X-100 at room temperature [*8*]. Therefore, the concentrations of Triton X-100 used were greater than the CMC value (0.1% Triton X-100 equals 1.57×10^{-3} molar), although the SDS concentrations straddled the CMC value (0.1% SDS equals 3.47×10^{-3} molar). The precise role of these aqueous surfactants in eluting drugs from bloodstains was not explored but would provide an interesting topic for future research.

Since codeine, heroin, morphine, and normorphine cross-react with the antiserum commercially available [*9*], this RIA is an opiate screen. Two possible solutions to this specificity problem are either to use a more specific antiserum from the procedure of Gross et al [*10*] or to perform a preliminary chromatographic step to separate the opiate alkaloids into individual fractions. Although these alternatives may be necessary to distinguish the presence of a Schedule I substance (heroin) from a Schedule V substance (codeine), a negative result to this sensitive assay would go a long way toward eliminating the possibility of the presence of any of the opiate compounds in bloodstains.

Acknowledgments

This work is supported by the Law Enforcement Assistance Administration Grant No. 25-0965591.

References

[*1*] Shaler, R. C., Smith, F. P., and Mortimer, C. E., "Detection of Drugs in Bloodstains. I: Diphenylhydantoin," *Journal of Forensic Sciences*, Vol. 23, No. 4, Oct. 1978, pp. 701-706.

[*2*] Kido, Y., "Radioimmunoassay in Forensic Chemistry," *Eisei Kagaku*, Vol. 21, No. 5, 1975, pp. 239-255.

[*3*] Möller, M. R., Tausch, O., and Biro, G., "Radioimmunological Detection of Morphine in Stains of Blood and Urine," *Zeitschrift für Rechtsmedizin*, Vol. 79, No. 2, Feb. 1977, pp. 103-107.

[*4*] Spector, S. and Parker, C. W., "Morphine: Radioimmunoassay," *Science*, Vol. 168, No. 3937, June 1970, pp. 1347-1348.

[*5*] "Abuscreen Morphine," product brochure, Roche Diagnostics, Nutley, N.J.

[*6*] "Iodoplatinate Spray Reagent," product brochure, Quantum Industries, Fairfield, N.J.

[*7*] Clarke, E. G. C., *Isolation and Identification of Drugs*, Vol. 1, The Pharmaceutical Press, London, 1974.

[*8*] Muckerjee, P. and Mysels, K. J., "Critical Micelle Concentrations of Aqueous Surfactant Systems," National Bureau of Standards Report, Washington, D.C., Feb. 1971.

[*9*] Spector, S., "Quantitative Determination of Morphine in Serum by Radioimmunoassay," *Journal of Pharmacology and Experimental Therapeutics*, Vol. 178, No. 2, Aug. 1971, pp. 253-258.

[*10*] Gross, S. J., Grant, G. D., Wong, S. R., Schuster, R., Lomax, P., and Campbell, D. H., "Critical Antigenic Determinants for Production of Antibody to Distinguish Morphine from

Heroin, Codeine, and Dextromethorphan," *Immunochemistry,* Vol. 11, No. 8, Aug. 1974, pp. 453-456.

Address requests for reprints or additional information to
F. P. Smith, Ph.D.
Department of Criminal Justice
University of Alabama in Birmingham
Birmingham, Ala. 35294