Determination of Morphine in the Hair of Heroin Addicts by High Performance Liquid Chromatography with Fluorimetric Detection*

M. Marigo, F. Tagliaro**, C. Poiesi, S. Lafisca, and C. Neri
Istituto di Medicina Legale e della Assicurazioni, Università di Verona, Policlinico B.go Roma, 37134 Verona, Italy

Abstract

A procedure has been presented for the quantitative determination of morphine contained in the hair of heroin addicts, by means of heat-acid hydrolysis, pre-column dansyl derivatization, straight phase liquid chromatography, and fluorescence detection. External standardization was used. Intra-assay and day-to-day variation coefficients were 5.6 and 7.8%, respectively (n = 10), when hair containing 1 ng/mg of morphine was assayed. Hair samples of 22 heroin addicts showed positive results in the range 0.08 to 15.7 ng/mg. No false positive results were found in 20 control subjects. A close correlation was shown between high performance liquid chromatography and radioimmunoassay results (y = 0.97x + 0.26) (r = 0.997, n = 15). Morphine hair content results significantly correlated with the grade of heroin use roughly estimated by means of serial determinations of morphine in urines during the last months before hair sampling.

Methods

Reagents

Commercially available ready-to-use tubes for the extraction of alkaline compounds (Toxi-Tubes A), containing Na₂CO₃ and NaHCO₃ as buffering salts and a mixture of organic solvents were supplied by Analytical Systems. HPLC grade solvents were provided by Merck and all other chemicals (analytical grade) by Carlo Erba.

Morphine RIA determinations were carried out using a commercially available kit from Diagnostic Product Corporation which has a sensitivity of 1 ng/L of free morphine. Toxico-logical assays in the urine of the studied addicts were carried out by EMIT-d.a.u.® (Syva) and by HPTLC using plates covered with Kieselgel 60 F 254 provided by Merck.

Equipment

HPLC was carried out using two model 302 pumps (Gilson), a model 802 manometric module, a model 811 dynamic mixer, a model 7125 injection valve (Rheodyne), a model 121 filter fluorimeter with a 9-µL flow cell, and a model 620 integrator (both from Gilson). The 250 × 4.6-mm column (Bischoff) used was packed with 5-µm sized spherical silica (Spherisorb).

Samples and Subjects

Human hair samples (4 cm in length, weighing 100 to 200 mg), which had no pre-treatment, were cut near the scalp of 22 heroin addicts, ages 18 to 30, who were admitted at a metha-nique reported until now (5), needs at least 1 g of hair for morphine identification; this fact clearly hampers its practical application. High performance liquid chromatography (HPLC), in spite of its widespread applications in the field of drug analysis, and particularly in the determination of morphine in biological fluids (6-8), has never been used for the assay of hair morphine content.

Recently, the authors have developed an original HPLC method, that allows morphine determination in biological fluids with a sensitivity comparable to RIA (9). A different extraction procedure and slightly modified chromatography conditions are proposed for assaying morphine content in the hair of heroin addicts with clinical and forensic aims.

Reprints

Reproduction (photocopying) of editorial content of this journal is prohibited without publisher’s permission.
done detoxication program at the Centro Medico e di Assistenza Sociale per le Tossicodipendenze in Verona. The samples were prepared as described below.

Blank control hair samples were collected from 20 well-known workers and students of the authors’ Institute, of matched age, without any anamnestic, clinical, or toxicological (EMIT assays of the urines) evidence of opiate use.

Initially, and at intervals during the detoxication program, urine samples were collected and assayed randomly by EMIT. The positive urines were re-assayed by HPTLC in order to exclude codeine interference. All the samples assayed showed codeine-free results.

### Results

Serial washings with ethyl ether and diluted HCl allowed the removal of loosely bound morphine. The complete removal of loosely bound drug was verified in four cases by measuring morphine levels in all washing solutions. Results are shown in Table I. On the basis of these results, a single-step acid washing with 12 mL 0.01 mol/L HCl was adopted. The percentage of removable morphone by washing, evaluated in 12 cases, was extremely variable, ranging from 0 to 66% of the whole drug extracted from the hair.

The recovery of morphine extracted from the hair matrix carried out in HCl was 87.5% (mean of 12 tests) of the one obtained with alkaline hydrolysis. In spite of a higher recovery, alkaline extraction gave many spurious peaks which, though eluting after morphine, required extensive and time-consuming clean-up for the column. Therefore, HCl extraction was preferred. No differences were observed between the results of the two acidic extraction procedures providing for different incubation temperatures and HCl concentrations. Absolute extraction recovery of morphine from the hair matrix was not investigated, because of the lack of hair with known morphine content. The Toxi-Tube extraction step gave a morphine mean recovery of 85.1% with a coefficient of variation (CV) of 3.7% (n=10) from a standard morphine solution. The overall recovery of the sample preparation procedures (HCl incubation, Toxi-Tube extraction, derivatization, and toluene extraction of derivatives), calculated by using standard morphine amounts, in the range of 5 to 1000 ng, resulted in 58.8% (CV=3.8%; n=10).

The standard curve was linear in the range of 5 to 100 ng of injected dansyl to morphine, according to the equation

\[
y = 1.01x - 1.40 \quad (r = 0.999; n=9)
\]

HPLC determination of morphine in hair samples of 22 heroin addicts showed measurable amounts of morphine from 0.08 to 15.7 ng/mg as shown in Figure 1. The assay of the blank samples (n=20) did not show any peaks interfering with morphine. Figure 2 shows typical chromatograms of a positive and a blank sample.

Intra-assay and day-to-day variation coefficients were 5.6 and 7.8%, respectively (n=10), for a morphine level of 1 ng/mg. Four subjects, who claimed to have abstained from heroin for three months before sampling, sustained by negative RIA of plasma and EMIT of urine, showed slightly positive results in the range 0.08 to 0.4 ng/mg. High levels were found in cases with a recent history of heavy heroin addiction, confirmed by toxicological assays of biological fluids. A significant positive correlation (p<0.01) was demonstrated between the hair morphine content of 13 addicts and the urine mean morphine levels, assayed randomly (from 8 to 16 times per subject) in the last four months before hair sampling (Figure 3). A good correlation, according to the equation of first degree, y = 0.97x + 0.26 (r = 0.997; n=15), was found between RIA and HPLC results (Figure 4).

### Discussion

The method presented gives a sensitive, accurate, and repro-

---

**Table I. Morphine Washed Away from 200 mg of Hair by Serial Washings with Ethyl Ether and HCl**

<table>
<thead>
<tr>
<th>Washings</th>
<th>Samples (ng of Eluted Morphine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Ethyl ether (10 mL)</td>
<td>2.4</td>
</tr>
<tr>
<td>0.01 mol/L HCl (2 mL)</td>
<td>0.0</td>
</tr>
<tr>
<td>0.01 mol/L HCl (2 mL)</td>
<td>0.0</td>
</tr>
<tr>
<td>0.01 mol/L HCl (2 mL)</td>
<td>0.0</td>
</tr>
<tr>
<td>0.01 mol/L HCl (2 mL)</td>
<td>0.0</td>
</tr>
</tbody>
</table>
It was found that morphine is present in hair mainly in two forms. The first, removable by washing with ethyl ether and diluted HCl, is present in variable amounts (0 to 66% of the whole morphine hair content) and presumably comes from external contamination; the second, likely bound to hair matrix, can be extracted by vigorous hydrolysis in HCl or NaOH. Other authors (1,2) found lower or negligible amounts of loosely bound morphine in human hair. This difference can be explained by the use of lighter washings (soap solutions). According to the authors' data, firmly bound morphine correlates with the "degree" of heroin intake, as evaluated on the basis of mean morphine levels measured in the urine during the last four months before hair sampling.

Derivatization and chromatographic features have been discussed elsewhere (9). Yet it must be pointed out that in the present work it was necessary to use external standardization because of interfering peaks co-eluting with the internal standard (nalorphine) that was previously used. Other compounds (dihydromorphine or normorphine), which are structurally related to morphine and more suitable for this purpose, are not available as yet in most Italian laboratories, owing to the enforcement of narcotics laws. The analytical precision is nonetheless quite comparable to that of other HPLC methods.
The high sensitivity achieved by fluorimetric detection allows the investigation of morphine content in small hair samples that can be collected without any aesthetic complications. This feature seems important for screening applications of this assay.

Finally, the specificity of this HPLC assay, which does not suffer from morphine congener interferences, makes it suitable for confirming RIA results. Different authors reported that RIA can be affected either by aspecific interferences (2) or by the cross-reactivity of anti-morphine antisera with other opiate drugs, such as codeine, that can also be found in the hair (4). For this reason, it has become a widely accepted rule that positive RIA results must be validated by a more specific technique such as gas or liquid chromatography (10).

Acknowledgements

The authors would like to thank Dr. A. Parolin and Dr. F. Capra of the Centro Medico e di Assistenza Sociale per le Tossicodipendenze of Verona for collaborating on this study and Mrs. Giovanna Carli for her technical assistance.

This work was partially supported by Grant #84.02409.56 from the Consiglio Nazionale delle Ricerche (CNR).

References


Manuscript received January 23, 1986; revision received April 28, 1986.

161