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Note

High-performance liquid chromatographic analysis of naloxone in human serum

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Naloxone is an opiate antagonist widely used for the reversal of narcotic overdose. Recently, case reports have shown naloxone to be possibly effective in reversing some of the detrimental hemodynamic effects of septic and hypovolemic shock [1—4]. Other investigational uses of naloxone include management of alcohol intoxication [5, 6], schizophrenia [7, 8], mania [9] and Alzheimer's disease [10, 11]. At the present time little is known concerning the pharmacokinetic disposition of naloxone [12, 13] and information concerning therapeutic and toxic serum concentrations and dosage adjustment in concomitant disease states is lacking. The limited clinical pharmacologic knowledge concerning this drug can be attributed, in part, to the absence of suitably sensitive, specific and rapid analytical methods capable of measuring naloxone in biological samples following therapeutic doses.

Two analytical methods for naloxone in biological media have been reported, namely, a gas—liquid chromatographic method [14] and radioimmunoassay [15]. However, while both report sensitivities consistent with concentrations observed following typical 0.4-mg bolus doses, concerns of complex derivatization and extraction in the case of the chromatographic procedure, and the lack of general availability of specific antibody in the case of the radioimmunoassay procedure, make these methods difficult to utilize in clinical situations. In the present paper we report a sensitive, rapid and reproducible high-performance liquid chromatographic (HPLC) analytical method applicable to the measurement of naloxone in patient samples following typical dosing schedules.

EXPERIMENTAL

Chemicals and reagents

Naloxone·HCl was obtained as a pure, unformulated standard from Endo

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Labs. (Lot No. 82-037, Garden City, NJ, U.S.A.) and was used directly. The internal standard, codeine sulfate USP, was obtained from Merck (Rahway, NJ, U.S.A.). All extraction and chromatography solvents were HPLC grade (Omnisolve, MCB, Cincinnati, OH, U.S.A.) and all other chemicals and reagents were analytical-reagent grade (J.T. Baker, Phillipsburg, NJ, U.S.A.) and were used as received. Distilled water was purified by passing through a reversed-osmosis four-filter system (Millipore, Bedford, MA, U.S.A.). Stock standard solutions of naloxone·HCl (0.01 mg/ml) and codeine sulfate (0.02 mg/ml) were prepared individually in methanol. These were protected from light and refrigerated at 4°C and were determined to be stable for at least one month.

Chromatographic conditions and instrumentation

A Waters Assoc. (Milford, MA, U.S.A.) Model 202 liquid chromatograph, equipped with a Model M6000A pump and a Model U6K injector, and interfaced with a Tracor Model 970A (Austin, TX, U.S.A.) variable-wavelength detector was used for the analysis. Chromatography was performed on a 30 cm × 4 mm I.D. stainless-steel μBondapak C_{18} reversed-phase column, 5 μm particle size (Analytical Systems, Santa Clara, CA, U.S.A.). The mobile phase consisted of 13% acetonitrile in 0.1 *M* sodium dihydrogen phosphate buffer, pH 4.8, at a flow-rate of 1.5 ml/min and a pressure of 1700 p.s.i. The separation was run at ambient temperature using a detector wavelength of 220 nm.

Extraction procedure

To 2.0 ml of a serum sample or standard in a 13 × 100 mm glass culture tube was added 1.0 ml of carbonate buffer, pH 8.95 (500 ml of 0.01 *M* sodium bicarbonate and 40 ml of 0.1 *M* sodium carbonate), 15 μl of internal standard solution (300 ng of codeine sulfate in methanol) and 2.0 ml of benzene. The tube was vortexed for 45 sec and centrifuged at 1500 *g* for 5 min. The upper organic layer was pipetted into a 10 × 75 mm glass culture tube and dried under a stream of filtered air at room temperature. The residue was then reconstituted with 50 μl of mobile phase and 40 μl were injected into the chromatograph.

Quantitation

Standard curves were generated over the range 10—80 ng/ml naloxone concentrations in serum and unknown concentrations were determined by calculating peak height ratios of drug:internal standard. Linear regression analysis, using a Hewlett-Packard Model 9825A computer (Palo Alto, CA, U.S.A.) was used to compute the standard curves on a daily basis.

Recovery

Recovery studies were performed by comparing peak height ratios from 10, 20 and 60 ng/ml naloxone serum standards, to which internal standard was added after extraction, to comparable unextracted standards.

Precision

Between-run variability of the method was assessed by comparing standard curves from five consecutive daily runs. Within-run variability was evaluated from five replicate analyses of 15 and 50 ng/ml control samples.

RESULTS AND DISCUSSION

Typical chromatograms from a human serum blank, a spiked human serum sample and a patient specimen are shown in Fig. 1. Internal standard was added to all three samples. Under the analytical conditions described, retention times for naloxone and codeine were 6.3 and 7.7 min, respectively. No significant interferences near the drug and internal standard peaks were observed from extracted human serum blanks. Peak shape was generally symmetrical and allowed a calculation of naloxone concentrations from peak height ratio measurements. Standard curves prepared from spiked human serum were linear over the range 10—80 ng/ml and statistics for a typical daily curve demonstrated a slope of 0.0044, an intercept of —0.001 and r^2 of 0.993. The mean r^2 observed for nine standard curves was 0.987 with a standard deviation of 0.014.

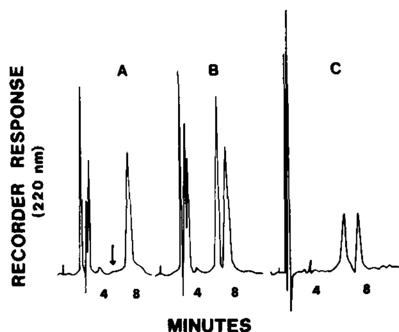

Fig. 1. High-performance liquid chromatograms of (A) extracted blank human serum, to which codeine, internal standard, had been added; (B) spiked human serum sample of 20 ng/ml naloxone; and (C) patient sample containing 12 ng/ml. All were run at 0.01 a.u.f.s. and the arrow indicates the expected elution of naloxone.

The percent recovery from 10, 20 and 60 ng/ml serum standards was 67%, 70%, and 65%, respectively, and the mean recovery was 67% with a standard deviation of 2%. The between-run coefficients of variation for control concentrations (n=5) of 20 and 40 ng/ml were 13.9% and 10.4%, respectively. The within-run coefficients of variation for 15 and 50 ng/ml controls (n=5) were 9.0% and 8.3%, respectively. The detector response demonstrated linearity up to 2000 ng/ml and the practical lower limit of sensitivity, which demonstrated a 3:1 signal-to-baseline noise ratio, was 5 ng/ml from a 2.0-ml serum sample.

Fig. 2 illustrates the application of the method to the analysis of serum samples over the typical time course of naloxone therapy in a septic shock patient. Naloxone was initiated as a 0.4-mg bolus followed by a continuous infusion of 2 mg/h. This infusion rate was increased in stepwise increments of 2 mg/h over 80 min until the rate reached 10 mg/h. Prior to each increase in the in-

fusion rate an additional 0.4-mg bolus was administered. Serial blood samples were drawn over a 6-h time period and naloxone was detected for 1 h after stopping the infusion.

The procedure presented here provides a satisfactory method for the analysis of clinical naloxone samples. It avoids the lengthy organic extraction and derivatization procedures needed in the gas chromatographic assay [14] and circumvents the difficulty in obtaining specific antibodies for the radioimmunoassay procedure [15]. There is also a question of cross-reactivity by metabolites in the immunoassay method. Since the primary metabolite of naloxone in humans is the 3-glucuronide [16], it is not extracted in the present system and, as such, does not interfere in the HPLC analysis. Additionally, other narcotic agents with similar chemical structures were evaluated and all, including morphine, meperidine, and hydromorphone, eluted with the solvent front in this system.

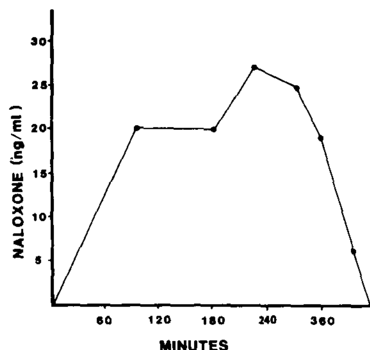

Fig. 2. Time course of serum naloxone concentrations in a patient in septic shock receiving typical intravenous administration (see text for details of dosing protocol).

In summary, the present procedure appears to be selective, sensitive, reproducible and rapid enough to support typical clinical pharmacology studies with naloxone and continuing clinical studies are presently being conducted in our laboratory.

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