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Note

High-performance liquid chromatographic analysis of plasma levels of nalbuphine in cardiac surgical patients

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Nalbuphine, chemically derived from the opiate analgesic oxymorphone, is one of a new class of analgesics described as an agonist-antagonist. The administration of nalbuphine to patients with cardiac disease for anesthesia or analgesia is characterized by hemodynamic stability and lack of respiratory depression, even after large doses (> 1 mg/kg). However, studies of drug disposition and correlation of plasma levels with its effects have not been carried out for nalbuphine because a rapid, sensitive method for analysis has not been available.

Previously described methods for determination of plasma levels of nalbuphine are cumbersome and time-consuming [1], and thus have greatly limited understanding of the uptake, distribution and elimination of this drug. Knowledge of these factors will help to develop rational patterns of administration of the drug to surgical patients.

High-performance liquid chromatography (HPLC) using electrochemical detection has been used for the rapid analysis of nanogram quantities of morphine, oxymorphone, and other opioid analgesics.

EXPERIMENTAL

Materials

The nalbuphine and naloxone were obtained from Endo Laboratories Division of E.I. Dupont de Nemours, Wilmington, DE, U.S.A. The nalbuphine is a 10 mg/ml concentration with 0.1% sodium chloride, 0.94% sodium citrate, 1.26% citric acid anhydrous, 0.1% sodium metabisulfite and 0.2% of a 9:1 mixture of methyl- and propylparaben. Naloxone is available as a 0.4 mg/ml pre-

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paration in 8.6 ml of sodium chloride and 2.0 mg of 9:1 ratio of methylparaben and propylparaben.

Patients

The blood concentration of nalbuphine was determined in fourteen patients undergoing coronary artery bypass grafting (CABG) with mean age of 58 years (range 41—74) and seven patients having mitral valve repair or replacement (MVR) with mean age of 48.5 years (range 22—64), all of whom had given informed consent. The protocol was approved by the Human Investigation Committee of the University of Virginia School of Medicine. All patients were premedicated with nalbuphine 0.1 mg/kg and scopolamine 0.005 mg/kg intramuscularly 1.5 h prior to the study. After insertion of intravenous and intraarterial catheters, arterial blood was drawn for the control level of nalbuphine. The patients undergoing CABG then received nalbuphine 0.5 mg/kg body weight as a bolus through the central venous catheter. Blood was drawn after 5 min for determination of nalbuphine concentration. Additional increments of 0.5 mg/kg were given every 7 min to a total of 3 mg/kg and blood samples were taken 5 min after each increment. Blood samples were also taken at 2 min after skin incision and sternotomy, at the time of aortic cannulation for cardiopulmonary bypass, 30 and 60 min on cardiopulmonary bypass, immediately and hourly for 3 h postoperatively.

The patients having MVR received only 2 mg/kg total dose in increments of 1 mg/kg with determination of plasma levels 5 min after each dose. Otherwise, the sampling schedule was the same as for patients having CABG. No additional nalbuphine was given after the 2 mg/kg (MVR) or 3 mg/kg (CABG) dose. The amount of priming solution for the bypass circuit and the degree of hypothermia during bypass were noted.

Sample preparation

Plasma samples were prepared by centrifugation at 1040 *g* and frozen at −15°C until the time of analysis. Naloxone, 250 ng, was added to 1 ml of plasma as an internal standard. The sample was deproteinated with 0.25 *N* perchloric acid, vortexed, allowed to stand for 3—5 min, centrifuged and the supernatant collected. The supernatant was adjusted to pH 8 with 1 *N* sodium hydroxide and then extracted with 10 ml of ethyl acetate—2-propanol (9:1). After centrifugation the organic top layer was collected and evaporated to dryness with a gentle air stream. The sample residue was then redissolved in 1 ml of methanol and injected into the chromatograph using a 200-μl injection loop.

High-performance liquid column chromatography

An HPLC Bioanalytical Systems (West Lafayette, IN, U.S.A.), 25-cm Biophase ODS 5-μm chromatographic column and a Bioanalytical Systems LC-4A electrochemical detector in oxidation mode with a detector cell potential of 0.75 V were used. This voltage was chosen from the cyclic voltammograph obtained for nalbuphine (Fig. 1) on a Bioanalytical Systems Model CV-1B cyclic voltammeter. The mobile phase was 55% monobasic potassium phosphate (0.01 *M*) and 45% HPLC grade methanol which had been degassed and filtered through a 0.22-μm filter before use. The flow-rate was 0.8 ml/min resulting in

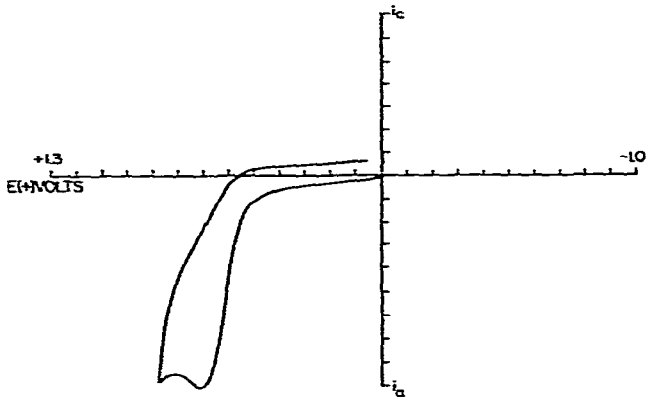

Fig. 1. Cyclic voltammograph of nalbuphine.

retention times of 400 sec for naloxone and 507 sec for nalbuphine. The plasma concentration of nalbuphine was determined from the ratio of the response of nalbuphine and naloxone in the samples when known amounts of naloxone had been added as the internal standard. A control plasma sample of nalbuphine containing 500 ng was analyzed daily in addition to the internal standard.

RESULTS

The analysis of nalbuphine was readily carried out using an electrochemical detector with HPLC. Plasma concentrations as low as 1 ng could be detected. The response of the detector was observed to be linear throughout the 1—400 ng range measured. Recovery from aqueous and plasma control samples was consistently 94% or greater. The daily control samples containing 500 ng of nalbuphine yielded concentrations of 496 ± 52.5 ng (mean ± S.D.) over a two-month period. The mean response factor ratio of nalbuphine to naloxone was 0.82 ± 0.02, an inter-day variation of 2.4% over 19 days. Within a given day the response factor varied 2.7%.

Fig. 2 is a chromatogram of human plasma containing internal standard naloxone. Fig. 3 is a typical chromatogram of nalbuphine extracted from human plasma. A small unidentified peak was seen after nalbuphine which may represent one of the metabolites. Since pure metabolites were not available, the source of this peak was not investigated. Plasma levels after premedication alone (control) were 14.6 ± 1.37 ng/ml in CABG and 14.6 ± 4.2 ng/ml in patients for MVR. There was an initial rapid increase in plasma nalbuphine as the 2 mg/kg (MVR) or 3 mg/kg (CABG) total doses were reached. Thereafter, a rapid decline occurred. Plasma levels in patients having MVR were consistently less than in patients undergoing CABG at all times. A small additional decline was associated with cardiopulmonary bypass. Blood levels decreased approximately 26% in both groups, with an approximately 50% increase in blood volume due to the use of the extracorporeal circuit in which the priming volume was 3042 ± 141 ml (CABG) and 2471 ± 245 ml (MVR) (mean ± S.E.M.). Systemic hypothermia to a mean of 25.3°C in both groups was accom-

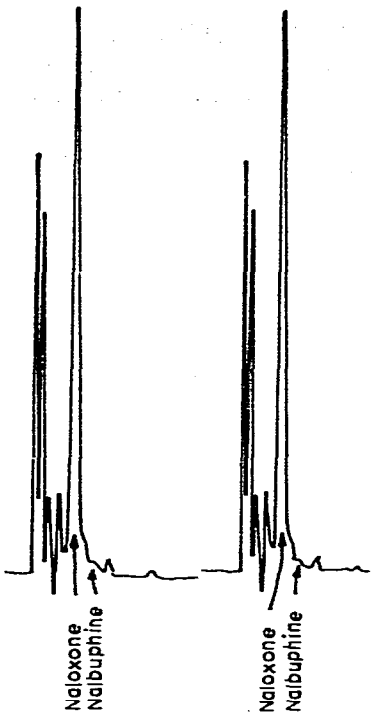

Fig. 2. Chromatogram of human plasma with naloxone internal standard.

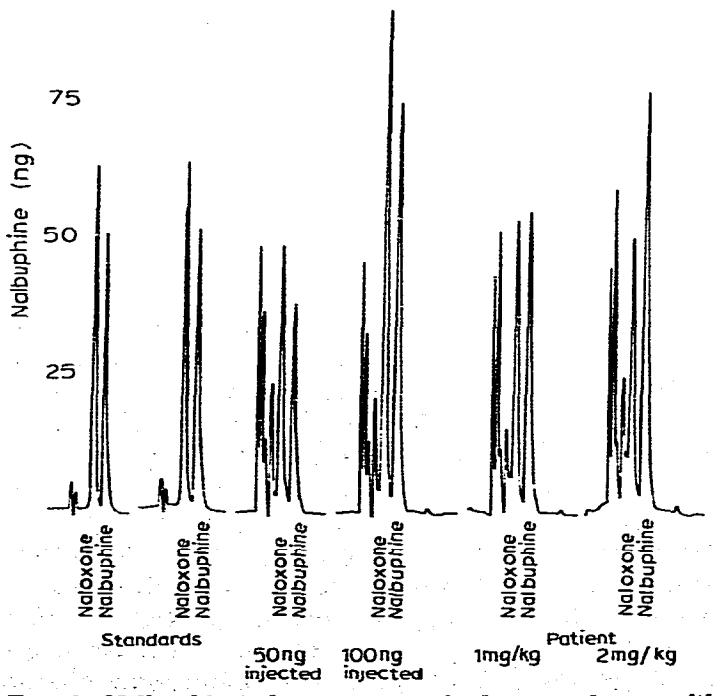

Fig. 3. Nalbuphine chromatogram in human plasma with naloxone as internal standard. Retention times: naloxone, 400 sec; nalbuphine, 507 sec.

panied by a further 76% decrease in plasma levels. Plasma levels of 45.5 ± 4.6 (MVR) and 72.6 ± 8.9 ng/ml (CABG), which exceeded or were comparable to known analgesic blood levels [2], were still present 3 h postoperatively (approximately 9 h from initial dose).

The $t_{½\beta}$ was determined from a log concentration plot against time for three samples drawn at hourly intervals postoperatively (Figs. 4 and 5) and was 3.0 h (MVR) and 3.5 h (CABG).

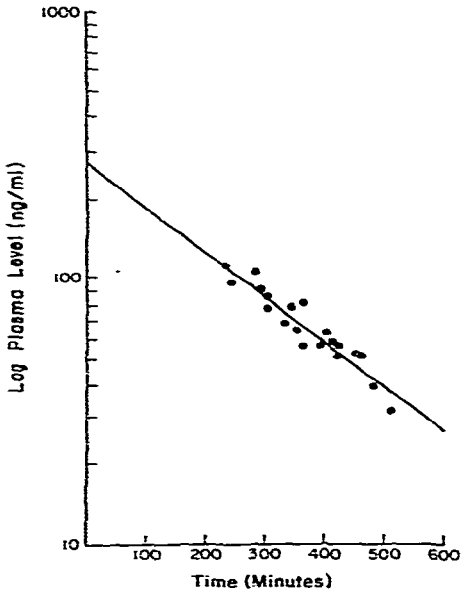

Fig. 4. Determination of $t_{½\beta}$ of nalbuphine in MVR.

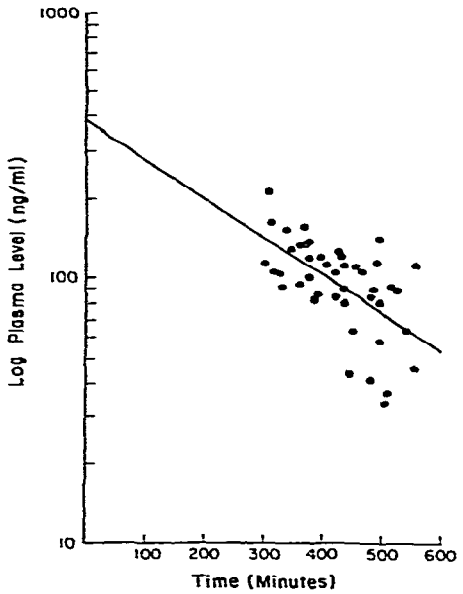

Fig. 5. Determination of $t_{½\beta}$ of nalbuphine in CABG.

DISCUSSION

Nalbuphine is metabolized in the liver to two metabolites, 14-hydroxy-7,8-dihydronormorphine and 14-hydroxy-7,8-dihydro-N-cyclobutyl-methylnormorphine (Fig. 6). It is excreted in the urine as unchanged nalbuphine, its conjugates, and the two metabolites [3] accounting for 71% of a dose [3]. The remaining drug is probably eliminated in the feces as a result of biliary excretion [3]. The assay described here was used for detection of nalbuphine and not for its metabolites. Our control samples were taken about 90 min following a premedication dose of 0.1 mg/kg which accounts for the lower plasma level than reported by previous investigators at 60 min after the dose [3]. Redistribution and metabolism of administered nalbuphine appears similar to that of other narcotics administered to cardiac patients [4]. Administration of heparin before bypass appeared to have no measurable effect on the nalbuphine plasma concentration. The nalbuphine level decreased with cardiopulmonary bypass due to dilution as has been reported with fentanyl [4]. Despite the use of

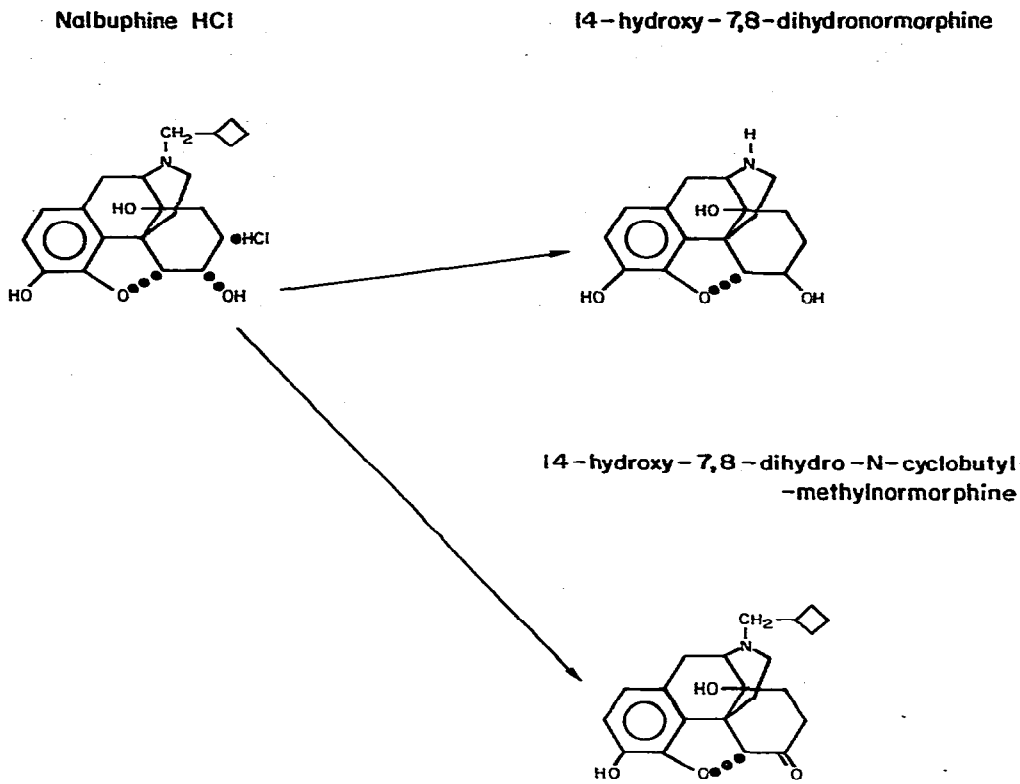

Fig. 6. Nalbuphine and its metabolites.

moderate systemic hypothermia, redistribution, metabolism, or excretion occurs during bypass. Because of the water-induced diuresis which usually occurs during cardiopulmonary bypass, the more rapid decline of plasma nalbuphine may be the result of greater urinary excretion of unchanged drug rather than metabolism. Since urinary levels of nalbuphine were not measured we can only speculate that this occurred. The presence of lower plasma levels in MVR at all sampling intervals was surprising and may be the result of a smaller total dose (2 mg/kg), greater urinary output due to diuretic administration, increased surgical bleeding, or the younger average age of patients [5]. Plasma concentrations, of more than 20 ng/ml, which are known to be analgesic [2], were present at 9 h after the initial dose. This is considerably longer than the 5 h reported by Weinstein et al. in dogs receiving 1 mg/kg doses [1]. It suggests that doses of nalbuphine in humans be spaced at longer intervals than the 3—6 h currently recommended.

The alpha or distribution half-life of nalbuphine has not been previously reported, but the beta or metabolism half-life of 3—3.5 h determined in this study is consistent with the duration of analgesia reported in humans by Tammisto and Tigerstedt [6]. The alpha or distribution phase of the plasma concentration curve could not be analyzed since single bolus dosing was not used and plasma sampling was not continued immediately after dosing.

The assay previously described for nalbuphine is a gas—liquid chromato-

graphic method using an electron-capture detector [1]. This method is time-consuming, requires dry conditions (relative humidity less than 50%) to derive the labile heptafluorobutyrate derivative of nalbuphine, and results in a recovery of only 70%. This paper describes a method which permits rapid assay of nalbuphine unaffected by moisture, and documents its applicability in clinical pharmacology. HPLC has been similarly used for the analysis of agonist narcotic drugs such as morphine [7] and oxymorphone [8]. It has also been used for analysis of the narcotic antagonists naloxone and naltrexone [8]. In the method used in the present study for the analysis of nalbuphine (a drug with mixed agonist and antagonist properties), naloxone was used as the internal standard. Common to all of these drugs is a phenolic hydroxy group. A dihydroxyphenolic residue common to the catecholamines epinephrine, norepinephrine, and dopamine allows them to be analyzed by this method and suggests that any drug containing this structure can be quantitatively analyzed by HPLC with an electrochemical detector.

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