Journal of Chromatography, 118 (1976) 331–335

CHROM. 8902

QUANTITATIVE DETERMINATION OF NALTREXONE AND BETA-NALTREXOL IN HUMAN PLASMA USING ELECTRON CAPTURE DETECTION

KARL VEREBEY, MICHAEL J. KOGAN, ANN De PACE and SALVATORE J. MULÉ
New York State Office of Drug Abuse Services, Testing and Research Laboratory, 80 Hanson Place, Brooklyn, N.Y. 11217 (U.S.A.)
(Received October 28th, 1975)

SUMMARY

A gas–liquid chromatographic method is described for the determination of naltrexone and beta-naltrexol in human plasma following derivatization with pentafluoropropionic anhydride using electron capture detection. The lower sensitivity of the method for absolute standards is 5–10 pg. Following an acute 100-mg dose to a subject, peak levels of naltrexone of 15 ng/ml at 2 h and of beta-naltrexol 84 ng/ml at 4 h were observed. The levels of both compounds decreased by 24 h after the dose: naltrexone to 2.9 ng/ml and beta-naltrexol to 25 ng/ml. Following chronic administration for two weeks of 100 mg per day the peak levels of naltrexone and beta-naltrexol increased to 26.9 and 131 ng/ml at 2 h, respectively, but by 24 h both compounds were at levels similar to those following a single dose. Thus no accumulation of either drug or metabolite in the plasma was seen following chronic naltrexone administration.

INTRODUCTION

Naltrexone was synthesized by Blumberg *et al.*[1] in 1965. It is a potent orally effective narcotic antagonist with a reasonably long time course of action and thus is considered useful in the out-patient treatment of opiate addicts following detoxification from either methadone or heroin[2,3]. The urinary excretion of naltrexone and its major metabolite beta-naltrexol[4,5] in man has been reported recently[6,7]. However, owing to the low concentrations in human plasma, quantitative determinations of naltrexone and beta-naltrexol have not been accomplished. In this report we describe a method for the quantitative determination of naltrexone and beta-naltrexol in the plasma of a subject taking an acute dose and chronic therapeutic doses of naltrexone.

MATERIALS AND METHODS

Chemicals and reagents

Beta-naltrexol·HCl (N-cyclopropylmethyl-7,8-dihydro-14-hydroxynormor-

phine) was a gift of Dr. R. Willette of NIDA (Bethesda, Md., U.S.A.), and naltrexone·HCl (N-cyclopropylmethyl-7,8-dihydro-14-hydroxynormorphinone) and naloxone·HCl, (N-allyl-7,8-dihydro-14-hydroxynormorphinone) were provided gratis by Endo Labs. (Garden City, N.Y., U.S.A.). The solvents used were glass-distilled, GC grade purchased from Burdick and Jackson (Muskegon, Mich., U.S.A.). All aqueous solutions, buffers and dilute acids, were prepared using double glass-distilled water. Pentafluoropropionic anhydride (PFPA) and glassware siliconizing fluid of Dri-Film® SC-87 were purchased from Pierce (Rockford, Ill., U.S.A.).

Stock solutions

Standard solutions of naltrexone (25 ng/ml), beta-naltrexol (200 ng/ml) and internal standard naloxone (250 ng/ml) were prepared in double glass-distilled water.

Calibration curves and quantitation

Naltrexone and beta-naltrexol standard curves were prepared by adding 1.25, 2.5, 12.5 and 25 ng of naltrexone, 5, 10, 20 and 40 ng of beta-naltrexol, 6.25 ng of naloxone to each of 1.0 or 2.0 ml of blank plasma. The samples for both standard curves were extracted according to the method described below, derivatized by PFPA and chromatographed. The peak height or area ratios were plotted against the respective concentration of standards and the slope determined. The unknown sample peak height or area ratio was divided by the slope to determine the concentrations of naltrexone and beta-naltrexol in the unknown samples.

Extraction procedure

All glassware was siliconized with a 5% (v/v) solution of Dri-Film in toluene. The following substances were added to 20-ml round-bottom centrifuge tubes: Antifoam A (A.H. Thomas, Philadelphia, Pa., U.S.A.) 0.5 to 2 ml of plasma and 25 μl of aqueous internal standard containing 6.25 ng of naloxone. The pH was adjusted to 8.5 with 1.0 ml of saturated sodium bicarbonate and the aqueous phase was shaken with 12 ml of chloroform. After 10-min shaking and 5-min centrifugation the chloroform layer was transferred into clean centrifuge tubes containing 5 ml of 0.5 *N* HCl. After back extraction into acid the chloroform layer was discarded and 4.5 ml of the HCl phase was transferred into clean centrifuge tubes and neutralized with 2.5 *N* NaOH. The pH was adjusted to 8.5 with saturated sodium bicarbonate and the aqueous phase extracted with 7 or 8 ml of chloroform. After shaking and centrifugation the chloroform was transferred to a clean 15-ml test tube and evaporated to dryness on a rotary flash evaporator (Evapo-o-mix, Buchler). To the dry residue 100 μl of PFPA were added, the tube was tightly stoppered (00 size hollow nalgene stoppers; A.H. Thomas) and placed in a heating block at 110° for 40 min. After the reaction the samples were stored overnight at −16° and usually analyzed the following day. The anhydride was evaporated at room temperature under a stream of nitrogen. The dried sample was taken up in 50–100 μl of ethyl acetate and 1–2-μl aliquots were injected into the gas chromatograph. Fig. 1 shows a chromatogram for two standards of beta-naltrexol and naltrexone extracted by the method described above.

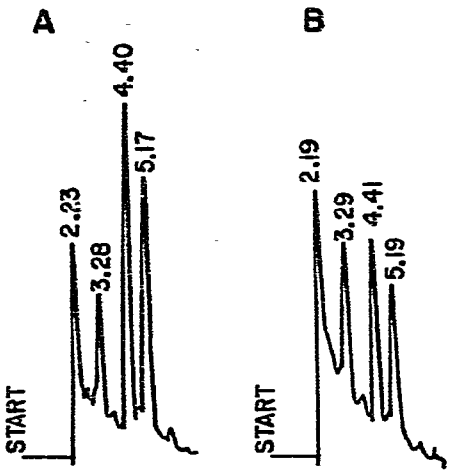

Fig. 1. Chromatogram A represents 6.25, 40, and 25 ng of naloxone, beta-naltrexol and naltrexone, respectively, extracted from 1 ml of plasma. Chromatogram B represents half the above concentration of naltrexone and beta-naltrexol while using the same amount of internal standard, naloxone. The retention times for naloxone, beta-naltrexol, and naltrexone are 3.28, 4.40, and 5.17 ± 0.02 min, respectively. The signal is automatically suppressed over the first 2.2 min by time programming. The first apparent peak at 2.20 ± 0.03 min is the time the signal is attenuated to scale.

Gas–liquid chromatographic conditions

Analysis was performed under isothermal conditions on a Hewlett-Packard Model 5830A gas chromatograph, equipped with a ^{63}Ni linear electron capture detector operating at 1×10^9 mA standing current. The column was 6 ft. × 2 mm I.D. packed with 3% OV-22 on Supelcoport, 80–100 mesh. The carrier gas was a mixture of 10% methane in argon at a flow-rate of 35 ml/min. The injection port temperature was 250°, the detector temperature 300° and the column oven temperature 215°.

Subject

The subject in this study was a paid volunteer from the New York State Office of Drug Abuse Services. He was informed of the study protocol and signed an informed written consent prior to the study. Blood (12–15 ml) was drawn 1, 2, 4, 8, 12 and 24 h after drug administration. The blood was centrifuged and the plasma separated from the cells and stored at —17° until analysis.

RESULTS AND DISCUSSION

A solvent extraction method and derivatization process was developed for the electron capture analysis of naltrexone and beta-naltrexol in human plasma. The recovery of naltrexone and beta-naltrexol from plasma within the concentration range of the standards was 77 ± 2% and 54 ± 2%, respectively. When derivatization was carried out under milder conditions (70° for 25 min), in the presence of solvent (benzene–PFPA, 4:1), pentafluoro-derivatives different from those shown in Fig. 1

were obtained for naloxone, beta-naltrexol, and naltrexone. Their retention times were 4.45, 3.24 and 7.27 min, respectively. Absolute standard curves and standards extracted from plasma and derivatized either neat or in benzene provided linear relationships between the concentration and either peak height or area ratios of naltrexone, beta-naltrexol, and the internal standard naloxone.

The detector response for beta-naltrexol was greater than that for naltrexone when compared with the derivatization moieties obtained under neat conditions. Since in the clinical plasma samples naltrexone levels were extremely low, the neat derivatization condition was used. Both naltrexone and beta-naltrexol are stable at −16° for at least one week in excess PFPA either before or after reaction. However, once the derivatizing agent is removed and the derivatives reconstituted in solvent, it is necessary to inject them onto the gas chromatograph as soon as possible since the derivatives rapidly decompose in solvent. The derivatives were found to be equally soluble in either ethyl acetate or acetonitrile but less soluble in non-polar solvents such as benzene.

In order to make a simultaneous determination of naltrexone and beta-naltrexol in all plasma samples over a 24-h period, the choice of sample aliquot becomes critical. This occurs because in some samples the concentration of beta-naltrexol is as much as 15 times higher than naltrexone. For plasma samples taken after 12 h, aliquots of 0.6–2.0 ml were found adequate, whereas for plasma samples taken earlier than 12 h 0.2–0.4 ml aliquots were used.

Plasma levels of naltrexone and beta-naltrexol

To ascertain the practical application of the method developed, plasma levels of naltrexone and beta-naltrexol were determined in a subject following single and multiple doses of naltrexone. Serial blood samples were taken after an acute dose of 100 mg naltrexone at 1, 2, 4, 8, 12 and 24 h after the dose. Following the 24-h sample, the same subject received 100 mg naltrexone daily for 14 days and then blood samples were taken following the last 100-mg dose to evaluate the plasma levels of naltrexone and beta-naltrexol following chronic naltrexone treatment. Fig. 2 shows that in the acute study naltrexone reached a peak at 2 h of 15 ng/ml and by 24 h the level declined to 2.9 ng/ml. The rapid rise of beta-naltrexol in plasma following a single dose indicates a rapid rate of biotransformation of naltrexone to beta-naltrexol. A peak level of 84 ng/ml of beta-naltrexol was found at 4 h and the levels decrease to 25 ng/ml by 24 h after the dose.

Following chronic treatment the peak concentration at 2 h of both naltrexone and beta-naltrexol were higher in the plasma, 26.9 ng/ml and 131.0 ng/ml, respectively. However, by 24 h after the dose the levels of these compounds were approximately at the same concentration as in the acute study. If this pattern of naltrexone disposition is confirmed in other subjects and is representative of the population, no substantial accumulation of naltrexone or beta-naltrexol would be expected following chronic naltrexone treatment.

In conclusion, the method described in this report provides sufficient sensitivity for the quantitative determination of naltrexone and beta-naltrexol in human plasma following single and multiple therapeutic doses. As compared to the bis(trimethylsilyl)trifluoroacetamide derivatives of naltrexone and beta-naltrexol measured by flame ionization detection, the PFPA derivatives using electron capture detection

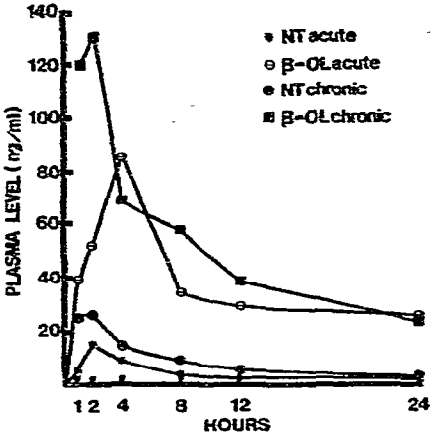

Fig. 2. Plasma levels of naltrexone (NT) and beta-naltrexol (β-OL) in a subject following the administration of an acute 100-mg oral dose of naltrexone (NT and β-OL acute) and following chronic doses of 100 mg naltrexone for 14 days (NT and β-OL chronic).

increases the sensitivity of the method of absolute standards approximately a thousand-fold and for plasma extracted materials approximately a hundred-fold.

ACKNOWLEDGEMENTS

These studies were supported in part by NIDA Contract No. ADM-45-74-133. The authors wish to express their gratitude to Drs. R. Resnick and J. Volavka (New York Medical College) for the clinical plasma samples.

REFERENCES

1 H. Blumberg, I. J. Pacher and Z. Matossian, *U.S. Pat.*, 3,332.950, July 25, 1967.
2 W. R. Martin, D. R. Jasinski and P. A. Mansky, *Arch. Gen. Psychiat.*, 28 (1973) 784.
3 R. B. Resnick, J. Volavka, A. M. Freedman and M. Thomas, *Amer. J. Psychiat.*, 131 (1974) 646.
4 E. J. Cone, *Tetrahedron Lett.*, 28 (1973) 2607.
5 N. Chattergie, J. M. Fujimoto, C. E. Inturrisi, S. Roerig, R. I. Wang, D. W. Bowen, F. H. Field and D. D. Clarke, *Drug Metab. Dispos.*, 2 (1974) 401.
6 E. J. Cone, C. W. Gorodetzky and S. W. Yeh, *Drug Metab. Dispos.*, 2 (1974) 506.
7 K. Verebely, S. J. Mulé and D. Jukofsky, *J. Chromatogr.*, 111 (1975) 141.