International Journal of Pharmaceutics, 8 (1981) 81–87

Research Papers

THE DETERMINATION OF LIDOCAINE AND BENZOCAINE IN ISOPROPYL MYRISTATE

PAI-CHIE CHEN-CHOW * and SYLVAN G. FRANK **

College of Pharmacy, The Ohio State University, Columbus, Ohio 43210 (U.S.A.)

(Received August 14th, 1980)
(Revised version received January 9th, 1981)
(Accepted January 14th, 1981)

SUMMARY

An in vitro membraneless drug release model utilizing an isopropyl myristate (ISM) sink as the acceptor phase has been used for the study of the release of lidocaine and benzocaine from aqueous Pluronic F-127 gels. The use of this system necessitated the development of a quantitative determination of lidocaine and benzocaine in isopropyl myristate solution. Extraction processes were developed to prepare the samples for the appropriate assay procedures: benzocaine by ultraviolet absorption spectrophotometry and lidocaine by gas chromatography. Direct assay of aqueous acid extracts of ISM sink solutions was not possible for benzocaine due to interfering UV absorbing substances. In the case of lidocaine, variable results were obtained from direct injections of the viscous ISM solutions into the GC column. In the latter case, however, direct assay was useful for the estimation of lidocaine concentrations, providing qualitative results for establishing appropriate concentrations necessary for a more complicated, but quantitative method based on a double extraction procedure.

INTRODUCTION

Recent investigation in our laboratory has been directed at studying the release of the local anesthetics, lidocaine and benzocaine, from 20–30% aqueous gels of the nonionic surfactant, Pluronic [1] F-127. Such gels are transparent, of low toxicity, exhibit reverse thermal behavior, and appear to have the structure of a liquid crystal (Schmolka, 1972). For these reasons and others, they appear to have potential as topical drug

* Present address: American Cynamid Co., Pearl River, N.Y. 10965, U.S.A.
** To whom correspondence should be addressed.
[1] BASF-Wyandotte Corp.

delivery systems. In order to quantify the effects of various formulation parameters, a membraneless drug release model utilizing an isopropyl myristate sink, and similar to that employed by Poulsen et al. (1968) was adopted.

Isopropyl myristate has been used by several investigators as the receptor phase in in vitro models for studying the release of drugs from aqueous topical drug delivery systems (Poulsen et al., 1968; Ostrenga et al., 1971a and b). Since isopropyl myristate is immiscible with such vehicles, in vitro studies can be conducted in the absence of a membrane, and good correlations between in vitro release rates and in vivo results have been observed (Ostrenga et al., 1971a and b). Various techniques have been used to quantify the amount of drug transferred from the vehicle to the isopropyl myristate sink, including radioisotope methods (Poulsen et al., 1968; Ostrenga et al., 1971a and b) and thin-layer chromatography (Busse et al., 1969).

Both lidocaine and benzocaine have long been used as local anesthetic agents, and assay procedures for these substances in aqueous systems have been well established. Benzocaine is conventionally determined by UV absorption (Bottari et al., 1977), while GC is an analytical method of choice for lidocaine (Hucker and Stauffer, 1976; Irgens et al., 1976; Kline and Martin, 1978; Caille et al., 1977; Nation and Triggs, 1976; Holt et al., 1979; Reynolds and Beckett, 1968). In the former case for the present study, the presence of extraneous UV absorbing material in an initial aqueous acid extract of ISM necessitated their elimination by the development of a double extraction procedure. In the latter case a single extraction procedure is commonly employed for aqueous systems; however, multiple extraction procedures have been used in certain circumstances. Attempts were made at achieving a suitable direct assay for lidocaine from ISM sink solutions; however, due to the relatively high viscosity of isopropyl myristate, which introduced errors in injection volume measurement into the GC assay procedure, development of an appropriate method for lidocaine based on a double extraction procedure was also undertaken. As will be seen, however, the direct method is useful for concentration estimation purposes and both are reported herein. With appropriate modifications it is hoped that the methods established in this study can be applied to in vitro drug release studies of other local anesthetics from similar models.

MATERIALS AND METHODS

Materials

Benzocaine (98%, Aldrich Chemicals), lidocaine and ethylmethylglycinexylidide hydrochloride (gift from Astra Pharmaceutical Products), isopropyl myristate (≥95%, Tridom Chemicals), benzene (spectrograde, Burdick and Jackson Laboratories), and reagent-grade monopotassium phosphate and disodium phosphate (J.T. Baker Chemicals) were used as received. Phosphate buffer solutions of pH 8.15 ± 0.1 (25°C) were prepared by mixing 3.7 ml of 1/15 M monopotassium phosphate and 96.3 ml of 1/15 M disodium phosphate.

Instrumentation

A Beckman model 26 UV/VIS spectrophotometer was used for the UV determination of benzocaine. The GC determination of lidocaine was performed on a Hewlett-Packard

model 5710 A gas chromatograph equipped with dual nitrogen–phosphorus (N-P) and flame-ionization (FI) detection. For FI determination, a 1.8 m X 4 mm (i.d.) glass coil column packed with 3% OV-17 on 80/100 mesh Gas Chrom-Q (Applied Science Laboratories) was used for the direct estimation of lidocaine concentration in isopropyl myristate (ISM). Operating temperatures were 212°C, 250°C and 250°C for the column oven, injector, and detector respectively, and flow-rates were 60, 30 and 240 ml/min for the carrier gas (helium), hydrogen and air, respectively. For N-P detection, a 1.8 m X 2 mm (i.d.) glass coil column packed with 3% OV-17 on 100/120 mesh Gas Chrom-Q was used. In this case, the operating temperatures for the direct estimation of lidocaine in ISM were 180°C, 250°C and 250°C for the column oven, injector and detector, respectively. Gas flow-rates were 60, 4 and 100 ml/min for the carrier gas (helium), hydrogen and air, respectively.

The operating temperatures for the determination of lidocaine in ISM through the double extraction procedure were 200°C, 250°C and 250°C for the column oven, injector and detector, respectively. The gas flow-rates were 30, 3 and 50 ml/min for the carrier gas (helium), hydrogen and air, respectively.

Procedures

Determination of benzocaine in isopropyl myristate by ultraviolet absorption spectroscopy. To each 5.0 ml sample of the ISM sink solutions of benzocaine, 4.0 ml of phosphate buffer was added and the systems mixed on a vortex device for 40 sec. The samples in this procedure and all others were contained in glass culture tubes closed with Teflon-lined screwcaps. Following mixing, the samples were centrifuged, and 4.0 ml of the upper ISM layers drawn out into new tubes and extracted with 3.0 ml of 0.5 N HCl. The aqueous acid solutions were then spectrophotometrically assayed at 223 nm. The concentrations of benzocaine were determined from an appropriate Beer's Law plot of absorbance as a function of benzocaine concentration in ISM.

Direct estimation of lidocaine in isopropyl myristate by gas chromatography with flame-ionization and nitrogen–phosphorous detection. One μl of ISM sink sample solutions of lidocaine were directly injected onto the GC columns at the operating conditions specified above. The concentrations of lidocaine in these solutions were estimated by comparison with external standard solutions consisting of known amounts of lidocaine in ISM.

Determination of lidocaine in isopropyl myristate by gas chromatography with nitrogen–phosphorous detection following double extraction procedure. To 0.5 ml or 1.0 ml samples of the ISM sink solutions of lidocaine, an appropriate amount of internal standard stock solution (1.0 mg/ml ethylmethylglycinexylidide hydrochloride) and 2.0 ml of 0.1 N HCl solution were added to each sample, following which they were shaken for 10 min. After centrifugation, the upper layers (ISM) were aspirated and discarded. 1.7 ml of the lower aqueous acid layers were then transferred to new tubes, and 0.5 ml of 0.5 N NaOH and 1.0 ml of benzene added, the samples mixed and centrifuged as before. The upper benzene layers were then ready for injection into the GC column. The concentration of lidocaine in the ISM sink solutions was determined by the customary procedure of constructing a calibration curve for ISM spiked with known amounts of lidocaine and plotting the peak height ratios as a function of lidocaine concentration.

RESULTS AND DISCUSSION

Determination of benzocaine in isopropyl myristate by ultraviolet absorption spectroscopy

A linear relationship was found between absorbance and benzocaine concentration, the sensitivity of the determination being very high, as concentrations of benzocaine as low as 0.5×10^{-5} M (0.83 μg/ml) could be measured.

The purpose of the initial extraction of the benzocaine/ISM solutions with the pH 8.15 phosphate buffer was to remove water-soluble low UV-wavelength absorbable materials in the solvent without loss of benzocaine (pK_a of benzocaine is 5.75). If a more basic solution (pH above 9) was used, a precipitate would form immediately. After the UV absorption interfering materials had been removed by extraction with phosphate buffer, the benzocaine peak could be differentiated from an ISM blank. Such differentiation allowed the quantitative determination of benzocaine, by subtracting the absorbance at 223 nm obtained from an ISM blank (processed through the same extraction procedure as the samples), from the total absorbance at the same wavelength.

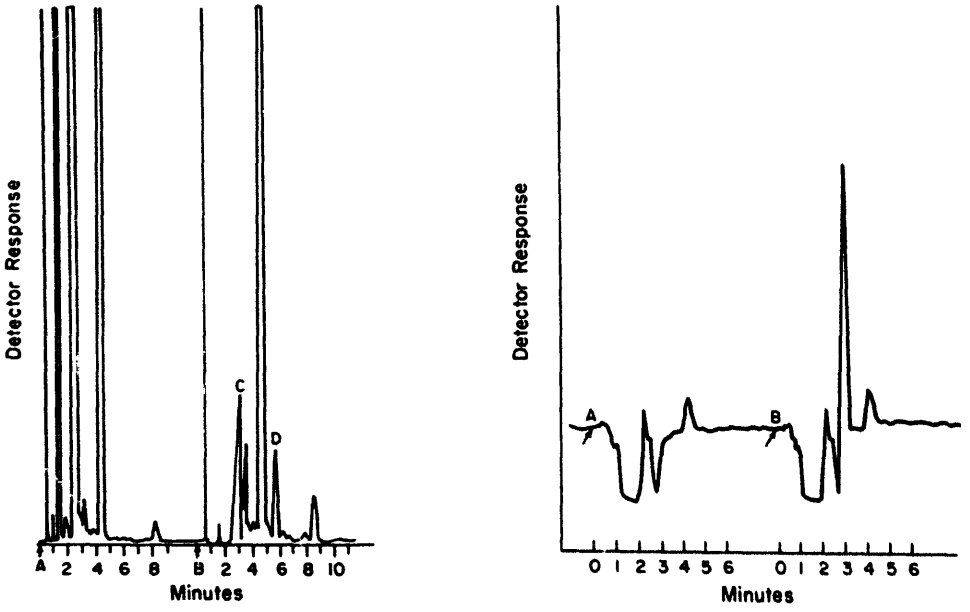

Fig. 1. GC chromatograms of lidocaine (2.5 mg/ml) resolved from isopropyl myristate (ISM) by flame-ionization detector at different recorder attenuations (ATT). Key: A, 1 μl ISM at ATT 10 × 512; B, 2 μl lidocaine in ISM at ATT 1000 × 512; C, ATT changed to 10 × 512 and D, lidocaine peak.

Fig. 2. GC chromatograms of lidocaine resolved from isopropyl myristate (ISM) with nitrogen–phosphorous-sensitive detector (recorder attenuation 1 × 32). Key: A, 1 μl of ISM; B, 1 μl of lidocaine in ISM (8.5 μg/ml).

Direct estimation of lidocaine in isopropyl myristate by gas chromatography with flame-ionization and nitrogen–phosphorous detection

A chromatogram of lidocaine resolved from ISM by flame-ionization detection, is shown in Fig. 1. The primary ISM peaks appeared within 4.8 min and the retention time of lidocaine was 5.2 min. As can be seen from Fig. 1, the ISM peaks were very high and dominant compared to those of lidocaine. With the nitrogen–phosphorous detector, however, the chromatogram (Fig. 2) was much cleaner, since the retention time of lidocaine was 3 min. As was expected, this detector is more sensitive to lidocaine than the FI detector under the conditions employed. In addition, one of the features of a N-P detector is that its response to most solvents including ISM is negative and low (Hewlett-Packard, 1977). Thus, the nitrogen–phosphorous detector was the detector of choice for the quantitative determination of lidocaine.

Unfortunately it was not possible to develop a direct assay procedure due to problems in finding a satisfactory internal standard, and in inconsistencies of injection volumes. Monoethylglycinexylidide, ethylmethylglycinexylidide-HCl, glycinexylidide, aminopyrine and aminophenazine were tried, but none were successful as internal standards when in ISM solution. On the other hand, non-proportionalities of peak heights to injection volumes, and changes in drug retention time resulting from variations in injection volumes made an external standard method unreliable. These 3 factors permitted only a qualitative estimation of lidocaine concentration, and necessitated an extraction procedure in order to achieve quantitative results. The direct injection process could, however, be used to estimate the concentration range of a series of lidocaine-containing ISM samples withdrawn during the drug release period (6 h). This procedure provided a quick determination of the concentration range involved, allowing the selection of the appropriate calibration curve before proceeding with the more tedious double extraction procedure for the quantitative determination of lidocaine.

Determination of lidocaine in isopropyl myristate by gas chromatography with nitrogen–phosphorous detection, following double extraction procedure

Lidocaine solutions in ISM at concentrations ranging from 0.1 to 10 μg/ml (0.1, 0.5, 1.0, 2.5 and 10.0 μg/ml) and from 6 to 60 μg/ml (6, 15, 30, 45 and 60 μg/ml) were subjected to the double extraction procedure described earlier, and the resulting benzene solutions assayed by GC equipped with N-P detection. Linear relationships were found between the peak height ratios lidocaine: ethylmethylglycinexylidide (EMGX) and lidocaine concentration for the representative concentration ranges. A typical gas chromatogram is shown in Fig. 3. No interfering peaks were present in the blank ISM extract, and the retention times of the internal standard and of lidocaine were 2.1 and 2.6 min, respectively. In addition, a test of sensitivity and linearity of the nitrogen–phosphorous detector to lidocaine indicated that this method could detect lidocaine concentrations as low as 0.02 μg/ml, and that a linear relationship existed from 0.1 μg/ml to 100 μg/ml of lidocaine in benzene. A slightly split peak appeared upon the injection of 1 mg/ml of lidocaine in benzene because of overloading.

Thus, this method consisting of a double extraction process plus nitrogen–phosphorous GC detection permitted specific monitoring of lidocaine release into the isopropyl myristate sink.

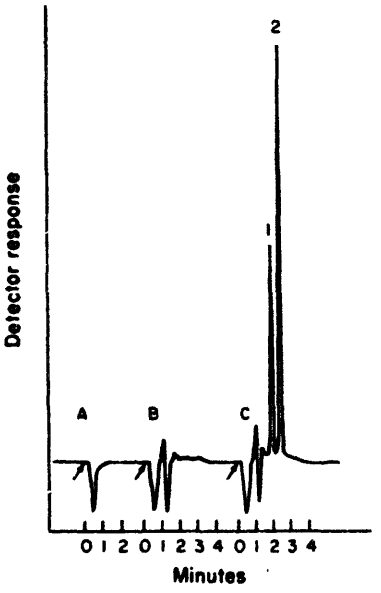

Fig. 3. GC chromatograms by nitrogen–phosphorous-sensitive detection (recorder attenuation 32 × 1). Key: A, benzene; B, benzene extract of isopropyl myristate; C, benzene extract of isopropyl myristate containing an internal standard, ethylmethylglycinexylidide (peak 1), and 3 μg/ml of lidocaine (peak 2).

ACKNOWLEDGEMENT

The authors wish to thank Astra Läkemedel AB, Södertälje, Sweden, for their support of this project.

REFERENCES

Bottari, F., DiColo, G., Nannipienri, F., Saettone, M.F. and Serafini, M.F., Release of drugs from ointment bases II: In vitro release of benzocaine from suspension type aqueous gels. J. Pharm. Sci., 66 (1977) 926–931.

Busse, M.J., Hunt, P., Lees, K.A., Maggs, P.N.D. and McCarthy, T.M., Release of betamethasone derivatives from ointment – in vivo and in vitro studies. Br. J. Dermatol., Suppl. 4, 81 (1969) 103–112.

Caille, G., Lelorier, J., Latour, Y. and Besner, J.G., GLC determination of lidocaine in human plasma. J. Pharm. Sci., 66 (1977) 1383–1385.

Hewlett-Packard Gas Chromatography Instrumental Manual, 5840 A, 1977, Operating Note.

Holt, D.W., Flanagan, R.J., Hayler, A.M. and Loizou, M., Simple gas–liquid chromatographic method for the measurement of mexiletine and lignocaine in blood-plasma or serum. J. Chromatogr., 169 (1979) 295–301.

Hucker, H.B. and Stauffer, S.C., GLC analysis of lidocaine in plasma using a novel nitrogen-sensitive detector. J. Pharm. Sci., 65 (1976) 926–927.

Irgens, T.R., Henderson, W.M. and Shelver, W.H., GLC analysis of lidocaine in blood using an alkaline flame-ionization detector. J. Pharm. Sci., 65 (1976) 608–610.

Kline, B.J. and Martin, M.F., Simplified GLC assay for lidocaine in plasma. J. Pharm. Sci., 67 (1978) 887–888.

Nation, R.L. and Triggs, E.J., Gas chromatographic method for the quantitative determination of lidocaine and its metabolite monoethylglycinexylidide in plasma. J. Chromatogr., 116 (1976) 188–193.

Ostrenga, J., Halebian, J., Poulsen, B.J., Farrell, B., Mueller, N. and Shastri, S., Vehicle design for a new topical steroid, fluocinonide. J. Invest. Dermatol., 56 (1971a) 392–399.

Ostrenga, J., Steinmetz, C. and Poulsen, B.J., Significance of vehicle composition I: Relationship between topical vehicle composition, skin penetrability, and clinical efficacy. J. Pharm. Sci., 60 (1971b) 1175–1179.

Poulsen, B.J., Young, E., Coquilla, V. and Katz, M., Effect of topical vehicle composition on the in vitro release of fluocinolone acetonide and its acetate ester. J. Pharm. Sci., 57 (1968) 928–933.

Reynolds, F. and Beckett, A.H., The determination of bupivacaine, lignocaine and mepivacaine in human blood. J. Pharm. Pharmacol., 20 (1968) 704–708.

Schmolka, I.R., Artificial skin I. Preparation and properties of Pluronic F-127 gels for treatment of burns. J. Biomed. Mater. Res., 6 (1972) 571–582.