A SPECTROFLUOROMETRIC METHOD FOR THE DETERMINATION OF AJMALINE IN PLASMA

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- 1 Ajmaline was found to have maximum fluorescence at neutral pH with 300 nm excitation and 365 nm emission wavelengths (corrected).
- 2 The fluorescence intensity had a linear relationship to concentration up to $50 \mu\text{g ml}^{-1}$ and the recovery of ajmaline after extraction from plasma was $92.5 \pm 3\%$.
- 3 Extraction of drug-free plasma and of samples containing known concentrations of ajmaline showed that drug levels in the range found clinically could be measured accurately by fluorimetry.
- 4 Serial plasma ajmaline concentrations were measured in a subject after intravenous injection of ajmaline (50 mg). The rates of plasma clearance of the drug were found to be similar to those obtained in previous studies.

Introduction

Ajmaline, an alkaloid base obtained from *Rauwolfia Serpentina* has antiarrhythmic properties (Arora & Madan, 1956) which have proved useful clinically (Bianchi & Beccari, 1966; Soler, Casellas & Trilla, 1966; Mendez & Kabela, 1970). Several methods have been developed for the measurement of ajmaline in plasma based on polarography, thin-layer chromatography or colorimetry (Tsarenko, Orlov & Schraiber, 1963; Yalcindag, 1969; Sobiczewska & Borkowski, 1970; Kabela, Jalife & Pastclin, 1974). A more sensitive and specific method for the measurement of ajmaline and its derivative 17-mono-chloroacetyljmaline (MCAA) was developed by Dombrowski, Crain, Browning & Pratt (1975) using thin-layer chromatography and fluorescence detection. However, in the absence of MCAA we have found that ajmaline concentrations can be determined by direct measurement of the fluorescence of plasma extracts adjusted to pH 6.5.

The method described here is rapid, technically simple and sensitive. It is suitable for the measurement of plasma ajmaline levels in the absence of MCAA and could be adapted for the measurement of ajmaline in the presence of this derivative.

Methods

All reagents were analytical grade. Solutions were prepared in deionised water and all glassware was washed with deionised water before use. A sample of

plasma (2.0 ml) was mixed with 2.0 ml sodium bicarbonate (1 mol litre^{-1}) and extracted in a 50 ml glass-stoppered tube with 20 ml diethylether by shaking for 5 min. The ether layer was removed to a centrifuge tube and the plasma was shaken with a further 20 ml ether. The combined ether extracts were centrifuged at 3000 g for 5 min and the ether layer was washed with 5.0 ml deionised water in an extraction tube by shaking for 5 min followed by centrifugation. Ajmaline was extracted from the ether by shaking for 10 min with 2.0 ml HCl ($0.1 \text{ mol litre}^{-1}$). The solvent phase was aspirated and 0.2 ml NaHCO_3 (1 mol litre^{-1}) was added to the acid layer to adjust the pH to 6.5. The samples were shaken well to remove bubbles of CO_2 , and the fluorescence was measured in a Farrand spectrofluorimeter using 300 nm excitation and 365 nm emission wavelengths (corrected). A reagent blank was prepared from 1 vol NaHCO_3 and 10 vol HCl. A standard solution of ajmaline was extracted with each batch of plasma samples. Extracted blanks, using 2 ml water instead of plasma, showed the same relative fluorescence and emission spectra as the reagent blanks and were not normally included with a batch of samples.

Results and Discussion

1 Emission spectra

Figure 1 shows the emission spectra of pure ajmaline hydrochloride, the reagent blank, extracted drug-free plasma and an extracted plasma sample taken from

Figure 1 UV-emission spectra at 300 nm excitation of (a) reagent blank (b) standard ajmaline hydrochloride (equivalent to $1 \mu\text{g ml}^{-1}$ free base) (c) extracted drug-free plasma and (d) extracted plasma from same subject 4 min after i.v. injection of ajmaline base (50 mg).

the same subject 4 min after an intravenous infusion of ajmaline (50 mg). These spectra were obtained using 300 nm excitation by scanning the emission wavelengths from 300 nm to 500 nm. The emission wavelength selector was changed automatically at a constant rate and the photomultiplier output was connected to a Servoscribe recorder. It can be seen that the extract from drug-free plasma had similar fluorescent emission to the reagent solution, while the characteristics of the ajmaline fluorescence were identical for both the pure drug and the drug extracted from plasma. The emission spectra for ajmaline hydrochloride and ajmaline base supplied as Cardio-rythmine Injectible (Servier, Orleans, France), were also found to have the same emission spectra and the same relative fluorescence at all concentrations.

2 Effect of pH on ajmaline fluorescence

Ajmaline was found to have maximum fluorescence in $\text{NaHCO}_3\text{-HCl}$ solutions of pH 6.0 to 7.0. Figure 2 shows the relative fluorescence obtained from solutions of $1 \mu\text{g ml}^{-1}$ ajmaline at various pH. It can be seen that the fluorescence ajmaline was suppressed

Figure 2 Fluorescence intensity of ajmaline hydrochloride (equivalent to $1 \mu\text{g ml}^{-1}$ free base) in $\text{NaHCO}_3\text{-HCl}$ solutions of various pH.

Figure 3 Relative fluorescence intensity of ajmaline hydrochloride at various concentrations of the free base.

in strongly acid or alkaline solutions. The pH obtained by adding 0.2 ml Na HCO_3 to the acid extract was consistently 6.5 so that it was considered unnecessary to check the pH routinely before measuring the fluorescence.

3 Recovery, sensitivity and reproducibility

The mean recovery of ajmaline added to ten aliquots of pooled drug-free plasma and carried through the extraction procedure was $92.5 \pm 3\%$ ($\pm$ s.d.). Similar recovery values were obtained when the drug was extracted from water. All plasma drug levels were therefore calculated on the fluorescence of standards extracted along with the samples. The fluorescence of ajmaline was found to be linear up to concentrations of $50 \mu\text{g ml}^{-1}$ as shown in Figure 3.

The sensitivity of this method is limited by the sensitivity of the type of fluorimeter used and by the 'back-ground' fluorescence of the extracted plasma.

Figure 4 Plasma concentrations of ajmaline after i.v. injection of ajmaline base (50 mg).

With the system used in this study as little as $0.01 \mu\text{g ml}^{-1}$ could be detected and the mean ($\pm$ s.d.) fluorescence of twenty drug-free plasma extracts from different subjects was equivalent to $0.038 \pm 0.013 \mu\text{g ml}^{-1}$ ajmaline. Thus, the sensitivity of this method is well within the sensitivity ($0.1 \mu\text{g ml}^{-1}$) of the chromatographic method (Dombrowski *et al.* 1975).

Repeated measurements of plasma ajmaline levels in the range 0.1 to $1.0 \mu\text{g ml}^{-1}$ gave the values which varied by no more than 8%.

No attempt has been made to determine whether other fluorescent drugs may interfere with this method for measuring ajmaline. It is possible that 17-monochloroacetylajmaline (MCAA), which is extracted from plasma by the same technique may also contribute to the fluorescence at these wavelengths. However, Domrowski *et al.* (1975) have reported that MCAA can be extracted selectively and quantitatively from the acid phase by methylene chloride. Thus, it should be possible to measure ajmaline in plasma from patients given the MCAA derivative by including this extra extraction step in the method.

The plasma levels of ajmaline measured in a subject given ajmaline base (50 mg) intravenously are shown in Figure 4. The initial rapid decline of the plasma ajmaline concentration, with a half-life of 1–2 min is followed by a less rapid phase of elimination. These findings are in agreement with the results of other studies (Spilker, Shargel, Koss & Mintoya, 1975) in which more time-consuming methods were employed for the determination of ajmaline concentrations. The rapid method described here may be of value in acute clinical studies in which attempts are made to correlate the electrophysiological effects of anti-arrhythmic drugs with their plasma levels (Curry, 1975).

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References

- ARORA, R.B. & MADAN, B. (1956). Antiarrhythmics; Ajmaline and serpentine in experimental cardiac arrhythmias. *J. Pharmac. exp. Ther.*, **117**, 62–67.
- BINXHI, C. & BECCARI, E. (1966). L'ajmaline nella terapia delle aritmie cardiache. Sperimentazione policentrica pianificata. *Minerva Med.*, **57**, 1273–1281.
- CURRY, P.V.L. (1975). *Fundamentals of arrhythmias: Modern methods of investigation in cardiac arrhythmias; The modern electrophysiological approach*, eds Krikler, D.M. & Goodwin, J.F., pp. 39–80. London: W.B. Saunders.
- DOMBROWSKI, L.J., CRAIN, A.V.R., BROWNING, R.A. & PRATT, E.L. (1975). Determination of 17-monochloroacetylajmaline and its metabolite in plasma by TLC fluorescence detection. *J. pharm. Sci.* **64**, 643–645.
- KABELA, E., JALIFE, J. & PASTCLIN, G. (1974). The actions of Antiarrhythmic agents on experimental arrhythmias. In *Concepts on the Mechanisms and Treatment of Arrhythmias*, ed. Gensini, G.G., pp. 199–218. New York: Futura.
- MENDEZ, R. & KABELLA, E. (1970). Cardiac pharmacology. *Ann. Rev. Pharmac.*, **10**, 291–312.
- SOBICZEWSKA, M. & BORKOWSKI, B. (1970). Bestimmung des Ajmaline. *Arzneiformen. Pharmazie*, **25**, 334–335.
- SOLER, J., CASELLAS, A. & TRILLA, E. (1966). Action de la ajmalina en el sindrome de Wolff-Parkinson-White. Utilidad diagnostica. *Arch. Ins. Card. Mex.*, **36**, 68–73.
- SPIPKER, B., SHARGEL, L., KOSS, R.F. & MINATOYA, H. (1975). Cardiovascular effects and blood concentrations of ajmaline and its 17-monochloroacetyl ester in cats. *Arch. int. Pharmacodyn.*, **216**, 63–78.
- TSARENKO, N., ORLOV, Y. & SHRAIBER, M. (1963). Poliarograficheskii metod opredeleniia ajmalina. *I. Med. Prom. USSR*, **17**, 38–40.
- YALCINDAG, O.N. (1969). Ajmalin'in idantifikasyon Reaksiyonlari. *Turk. Hij. Tecr. Biyol. Derg.*, **29**, 121–127.

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