Comparison of Two Spectrofluorometric Procedures for Quinidine Determination in Biological Fluids

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Abstract □ Different methods for the spectrofluorometric determination of quinidine in plasma and urine were studied. The alkaline washings of plasma and urine extracts remove fluorescent metabolites, as shown by TLC analysis of urine extracts, and do not lead to a significant loss of alkaloids. The spectrofluorometric assays without alkaline washings of the benzene extract averaged 18% higher than the assays with alkaline washings. Since unchanged quinidine and hydroquinidine are responsible for antiarrhythmic activity, the method with alkaline washing is more appropriate for the control of quinidinemia than are other methods. The therapeutic plasma concentration range becomes 0.8–2.5 μg/ml with this method.

Keyphrases □ Quinidine—spectrofluorometric analysis, human plasma and urine, effect of alkaline washings □ Spectrofluorometry—analysis, quinidine, human plasma and urine, effect of alkaline washings □ Antiarrhythmics—quinidine, spectrofluorometric analysis, human plasma and urine, effect of alkaline washings

Quinidine, in spite of its well-known potential toxicity, remains a useful antiarrhythmic agent. The incidence of toxic reactions gets higher with increasing plasma drug concentration. Several spectrofluorometric methods were reported (1) for the estimation of quinidine in plasma, not only to prevent toxic levels but also to ensure adequate plasma concentrations of unchanged quinidine, which is responsible for activity.

The methods currently used involve either a protein precipitation with metaphosphoric acid and a direct spectrofluorometric determination on the filtrate (2–4) or an extraction with benzene followed by a transfer of the drug to sulfuric acid for spectrofluorometric determination (5). Although benzene is the most specific extraction solvent for unchanged quinidine, Armand and Badinand (6) found that alkaline washings of benzene extracts of dog urine removed an additional amount of metabolites without reducing significantly the amount of quinidine.

The purposes of this investigation were to study the effect of alkaline washings of the benzene extract of human plasma and urine samples containing known amounts of quinidine and to compare the data obtained with and without alkaline washing of the benzene extract of plasma and urine samples of patients treated with quinidine.

EXPERIMENTAL

Materials—Commercial quinidine base[1] was used without further purification. Benzene was spectrophotometric grade. All other chemicals were analytical grade.

Spectrofluorometric Determinations of Quinidine—Heparinized blood samples[2] from patients treated with quinidine were centrifuged. The plasma samples were kept at −20° until assayed by the method of Armand and Badinand (6) with and without alkaline washing of the benzene extract. In this procedure, 1 ml of plasma is alkalinized with 1 ml of 0.1 *N* NaOH. Then 30 ml of benzene is added. The resulting mixture is vigorously shaken (30 min), and the clear benzene extract is removed and washed twice by shaking for 5 min with 10 ml of 0.1 *N* NaOH. Twenty milliliters of this washed extract is mixed with 3 ml of 0.1 *N* H_2SO_4. The mixture is shaken for 10 min, and the sulfuric acid extract is analyzed by spectrofluorometry[3].

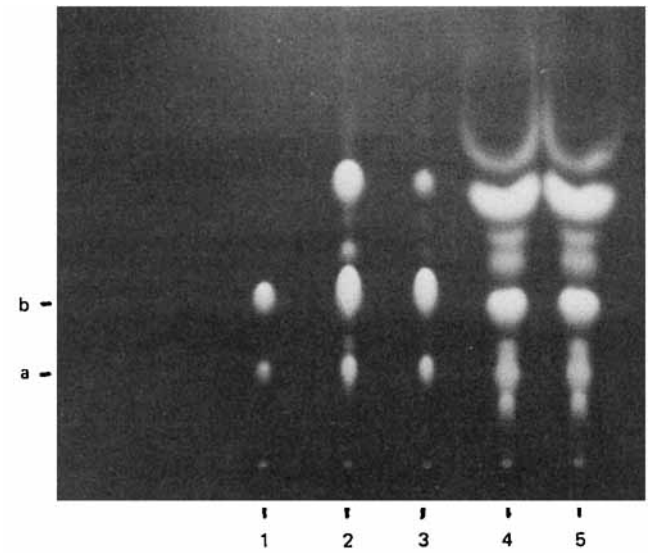

Figure 1—*Separation of hydroquinidine, quinidine, and their metabolites from urine by TLC. Key: 1, commercial quinidine containing hydroquinidine; 2, benzene extract; 3, benzene extract with alkaline washings; 4, pentanol–benzene (1:1) extract; and 5, pentanol–benzene extract with alkaline washings. Fluorescence bands for hydroquinidine and quinidine are designated by a and b, respectively (6).*

The precision and reproducibility of the alkaline washing procedure were evaluated by adding known amounts of quinidine to human plasma[2] and urine and by comparing the results obtained in the plasma with those of the protein precipitation method of Brodie and Udenfriend (2) in the same drug concentration range. The urine of one patient was also extracted with a pentanol–benzene (1:1) solvent mixture, which has been found optimal for the simultaneous extraction of quinidine, hydroquinidine, and their metabolites (7).

TLC—One urine sample from a patient receiving only quinidine was analyzed by TLC. Two 1-ml aliquots of this urine were alkalinized with 1 ml of 0.1 *N* NaOH; one was extracted with 25 ml of benzene and the other was extracted with 25 ml of pentanol–benzene (1:1). Ten milliliters of each organic extract was then washed twice with 5 ml of 0.1 *N* NaOH. Ten milliliters of the four washed and unwashed organic extracts was evaporated, and the residue was dissolved in 0.5 ml of the corresponding organic solvent.

Two hundred microliters of each resulting solution was applied on a silica gel plate[4]. Two hundred microliters of a benzene solution, containing 50 mg of commercial quinidine/liter, was also run for comparison. The chromatoplates were developed by the ascending technique with methanol (6) as the developing solvent. Fluorescent spots representing quinidine, hydroquinidine, and their metabolites were observed under UV light.

RESULTS AND DISCUSSION

The precision and reproducibility of the analytical procedure were verified by adding known amounts of commercial quinidine to human plasma and urine samples.

[1] K & K Laboratories, Plainview, N.Y.

[2] Supplied by M. Pierre Lemieux, Centre Hospitalier Notre-Dame of Montréal, and/or Dr. Michel Chabot, Institute of Cardiology of Montréal.

[3] Aminco-Bowman spectrofluorometer, American Instrument Co., Silver Spring, Md.

[4] Precoated TLC plates, Catalog No. 5765, Brinkmann Instruments, Rendale, Ontario, Canada.

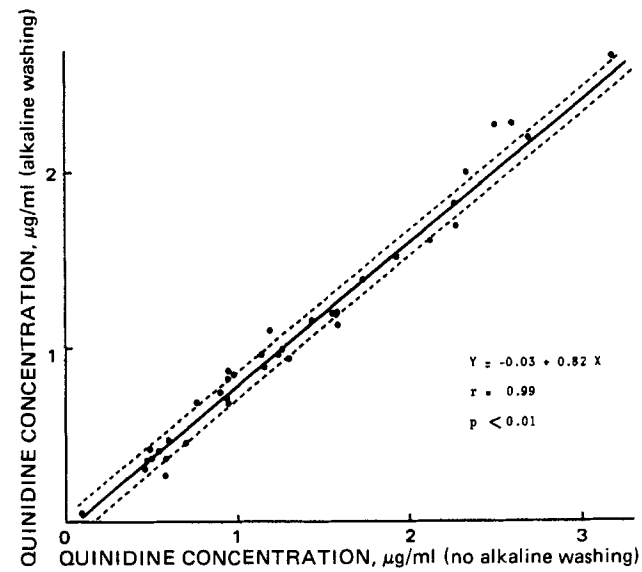

Figure 2—*Correlation of plasma quinidine concentration as measured by the method of Armand and Badinand (6) with (abscissa) and without (ordinate) alkaline washing of the benzene extract. The two dashed lines (- - -) parallel to the regression line (—) have a vertical distance from it that is equal to the standard error of estimate.*

When quinidine was added to eight plasma samples to obtain 0.1–5-µg/ml concentrations, the mean values of the percent found were 98.5 (*SD* = 4.1 and *CI*[5] = 3.4) and 100.9 (*SD* = 3.2 and *CI* = 2.6) for the protein precipitation method and benzene extraction procedure with alkaline washing, respectively. These results demonstrate that there is a good recovery of quinidine and hydroquinidine (8) with no significant loss of the alkaloids resulting from the alkaline washings of the benzene extract.

The addition of 0.1–150 µg/ml of quinidine to 14 urine samples led to a mean percent of 98.1 (*SD* = 2.5 and *CI* = 1.5) when the benzene extract was washed with an alkaline solution. The reproducibility of the extraction procedures was also checked by five measurements on the patient urine sample used for TLC analysis. The mean values of urine concentrations found in the benzene extract without and with alkaline washing were 109.75 (*SD* = 3.75 and *CI* = 4.66) and 66.43 (*SD* = 3.08 and *CI* = 3.83) µg/ml, respectively. On the other hand, mean concentration values of 182.08 (*SD* = 3.53 and *CI* = 439) and 182.92 (*SD* = 4.80 and *CI* = 5.97) µg/ml were found in the unwashed and washed pentanol–benzene (1:1) extract, respectively, of the same urine sample. In this last extraction method, the concentration of fluorescent quinidine molecules (quinidine, hydroquinidine, and their metabolites) is not reduced by the alkaline washings because of their high solubility in the pentanol–benzene solvent system.

The importance of alkaline washings of the benzene extract is illustrated in Fig. 1 where quinidine, hydroquinidine, and their metabolites were separated by TLC. In this figure, the metabolite spots have nearly completely disappeared with this treatment. This figure demonstrates also that the high quinidine concentration values obtained with the pentanol–benzene extraction solvent mixture result from fluorescent metabolites which are not removed by alkaline washing of the extract.

Thirty-seven plasma samples obtained from patients treated with quinidine were examined to test the importance of the alkaline washing. The plasma quinidine concentrations found spectrofluorometrically from washed and unwashed benzene extracts are shown in Fig. 2. As indicated by the regression line, the unwashed benzene extract assay averaged 18% higher than the washed benzene extract assay. With this additional removal of fluorescent metabolites, the quinidine therapeutic plasma concentration range would then become 0.8–2.5 µg/ml instead of 1–3 (9) or 3–7 (10) µg/ml. Since unchanged quinidine and hydroquinidine are responsible for antiarrhythmic activity (1), the procedure of Armand and Badinand (6) should be considered more appropriate than other methods for the control of quinidinemia. Furthermore, there is always the possibility in clinical practice that the rate of quinidine metabolism can be different in some patients because of physiopathological conditions or drug interactions.

[5] Confidence interval of the mean at the 0.05 level of significance.

REFERENCES

(1) T. Huynh-Ngoc and G. Sirois, *J. Pharm. Belg.*, **29**, 152 (1974).

(2) B. B. Brodie and S. Udenfriend, *J. Pharmacol. Exp. Ther.*, **78**, 154 (1943).

(3) A. L. Edgar and M. Sokolow, *J. Lab. Clin. Med.*, **36**, 478 (1950).

(4) H. C. Archer, D. Weitzman, and H. L. Kay, *Br. Heart J.*, **17**, 534 (1955).

(5) G. Cramer and B. Isaksson, *Scand. J. Clin. Lab. Invest.*, **15**, 553 (1963).

(6) J. Armand and A. Badinand, *Ann. Biol. Clin.*, **30**, 599 (1972).

(7) G. Hartel and A. Korhonen, *J. Chromatogr.*, **49**, 70 (1968).

(8) T. Huynh-Ngoc and G. Sirois, *Pharm. Acta Helv.*, **49**, 37 (1974).

(9) G. Hartel, A. Louhija, A. Konttinen, and P. I. Halonen, *Br. Heart J.*, **32**, 57 (1970).

(10) L. Resnekov, D. Gibson, S. Waich, J. Muir, and L. McDonald, *ibid.*, **33**, 220 (1971).

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