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Comparison of serum hydroquinidine determination by fluorescence polarization immunoassay and liquid chromatography

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Abstract: Hydroquinidine is a structural analogue of quinidine. It is used in the treatment and prevention of cardiac arrythmias and necessitates serum monitoring. Fluorescence polarization immunoassay (FPIA) of quinidine has been proposed and we have tested the performance of this assay for hydroquinidine using its cross-reaction with quinidine. Tracer (quinidine labelled with fluorescein) and anti-serum were purchased from Abbott S.A. Standard curves were obtained using specifically prepared hydroquinidine calibrators and within-run and run-to-run precision values (expressed as relative standard deviation) (RSD) lower than 5.3% (n = 10). In order to evaluate specificity of this assay in the clinical situation, FPIA and liquid chromatography results were compared.

Keywords: *Hydroquinidine; quinidine; fluorescence polarization immunoassay; liquid chromatography; therapeutic monitoring.*

Introduction

Hydroquinidine is an alkaloid derived from the saturation of the 10–11 double bond of quinidine. It is contained as an impurity (10%) in quinidine preparations [1] and is reported to have comparable or higher pharmacological activity than quinidine [2, 3].

It is also being increasingly used as oral treatment in the prevention of atrial and ventricular arrhythmia. Like quinidine it has a narrow therapeutic index and the therapeutic and toxic effects have been shown to be related to serum concentration (therapeutic range 1–2 mg l^{-1}) [4]. Patient response to treatment by hydroquinidine varies widely. This is due to variations in bioavailability, metabolism and elimination which are affected by congestive heart failure, hepatic or renal insufficiency and old age. Thus, to ensure therapeutic efficacy and to minimize toxicity, it is necessary to monitor hydroquinidine serum levels.

The ideal assay should be specific and sensitive enough to detect low concentrations accurately and rapidly. Various techniques have been proposed, including spectrofluorimetry [5] and liquid chromatography (LC) [1–6]. Recently, a fluorescence polarization immunoassay (FPIA) was developed for quinidine measurement. The Authors used the structural analogue of hydroquinidine with quinidine to evaluate its measurement with FPIA quinidine kits, using the cross-reaction of this drug with reagent antibodies. The methodology and the performance of this assay are described and the results obtained using patient serum samples are compared with those obtained by LC.

Materials and Methods

The 87 samples used in the study were obtained from 61 patients under hydroquinidine therapy. All blood samples were centrifuged and serum was stored at −20°C until analysis. Hydroquinidine (lot No. 5G1540) was donated by Roussel (Paris, France). Its purity measured by high-performance liquid chromatography (HPLC) was 99%.

HPLC

The HPLC assay was developed in a reversed-phase partition mode, using a μBondapak C_{18}, 10-μm column (300 × 3.9 mm, i.d.;

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Millipore). The mobile phase was a water–acetonitrile–tetrahydrofuran–acetic acid mixture (87:8:1:4, v/v/v/v) with a flow rate of 2 ml min^{-1}.

Under isocratic conditions, quantitation was performed at 250 nm based on the peak height ratio of hydroquinidine and quinidine used as internal standard. The calibration curve was carried out between 0–3 mg l^{-1}.

A 0.5-ml aliquot of patient serum or calibration standard sample was mixed with the internal standard, sodium hydroxide 0.1 M, and was extracted with a dichloromethane–isopropanol mixture (80:20, v/v). The organic phase was evaporated and the residue dissolved with 100 µl of methanol. 20 µl were injected into the chromatograph. Intra-run precision was measured on spiked serum samples containing 0.45 and 1.8 mg l^{-1}. Inter-run precision was determinated with serum samples containing 1.25 and 2.5 mg l^{-1}. This method is specific and allows separation of quinidine, hydroquinidine, 11-hydroxydihydroquinidine, 3-hydroxydihydroquinidine and dihydroquinidine 1-*N*-oxide, which are the three main metabolites of hydroquinidine [7].

FPIA

The principles of FPIA have been described elsewhere [8]. The technique was performed with an automated fluorescence polarization analyzer (TDX, Abbott Laboratories). Commercial reagents (lot No. 9506-20) were also obtained from Abbott.

Calibrators used to standardize the TDX were prepared at concentrations of 0, 0.5, 1.0, 2.0, 4.0 and 8 mg l^{-1} for hydroquinidine and quinidine. These standards were prepared by diluting an aqueous stock solution (hydroquinidine or quinidine) with drug-free pooled human serum. The hydroquinidine calibration curve was stored digitally in the analyser memory using the "assay 56 program". Three serum specimens containing 0.5, 1.25 and 2.5 mg l^{-1} of hydroquinidine were used to evaluate the intra-run and inter-run precision. Inter-run values of RSD were based on single standards included in each run.

Cross-reactions between hydroquinidine and quinidine antibodies (TDX reagent) were evaluated by using standard serum samples spiked with hydroquinidine. These specimens were analysed using the quinidine calibration curve.

Data analysis

The Wilcoxon test was used to compare mean polarization of the quinidine and hydroquinidine calibration curves. Linear least-squares regression analysis was used to determine the correlation between FPIA and HPLC. The mean of absolute differences for the 87 paired values of hydroquinidine concentration in serum determined by FPIA and HPLC was compared with zero, using Student's *t*-test. A *p*-value of <0.05 was considered statistically significant. An allometry curve was determined according to Vassault *et al.* [9].

Results and Discussion

The aim of this study was to evaluate the possibility of using an FPIA quinidine kit to monitor hydroquinidine therapy. It was necessary to answer three main questions:

(1) Is there a total cross-reaction between hydroquinidine and quinidine with reagent antibodies?
(2) Is any such cross-reaction constant and is it possible to measure hydroquinidine with the quinidine calibration curve?
(3) Does the proposed method correlate well with HPLC?

The relationship between quinidine or hydroquinidine concentrations (in mg l^{-1}) and fluorescence polarization (in millipolarization units) was non-linear and is best described by a log/logit curve. Comparison of polarization values obtained with identical concentrations (in the mg l^{-1} range) of quinidine and hydroquinidine (summarized in Table 1) shows that polarization is significantly higher for hydroquinidine. This observation indicates that the affinity of hydroquinidine for antibodies is slightly less than that of quinidine. This point is confirmed in Figs 1 and 2. Figure 1 shows the

Table 1
Comparison of the polarization values obtained with quinidine and hydroquinidine calibrators

Concentration (mg l^{-1})	Polarization (mP)* Quinidine	Hydroquinidine
0	189.17	187.17
0.5	171.64	172.08
1.0	149.33	150.16
2.0	110.19	122.98
4.0	73.48	84.21
8.0	51.69	55.19

*mP = millipolarization units.

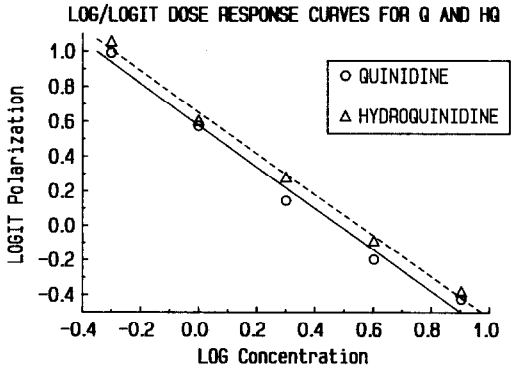

Figure 1
The log/logit dose response curves for quinidine (Q; —) and hydroquinidine (HQ; ---).

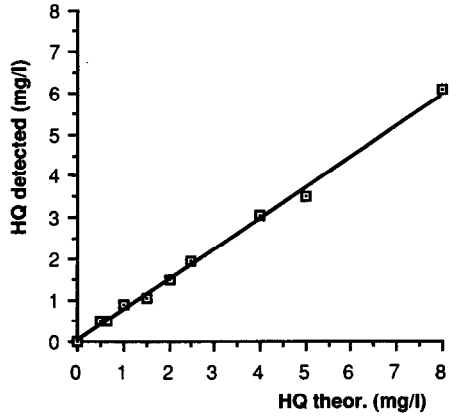

Figure 2
Values obtained with serum spiked with hydroquinidine (HQ), using the quinidine calibration curve from Abbott laboratories: x = hydroquinidine theoretical value; y = hydroquinidine value detected.

log/logit dose response curves for quinidine and hydroquinidine. Linear regression gave the following relationships:

logit $P = 0.58 - 1.20 \log C$ for quinidine ($r = 0.9927$), and

logit $P = 0.65 - 1.19 \log C$ for hydroquinidine ($r = 0.9973$),

where P is the polarization and C the concentration. Comparisons of the values are significant for the intercepts but not for the slopes, demonstrating parallelism between the log/logit curves.

Figure 2 shows the concentrations measured with hydroquinidine spiked serum and expressed as quinidine concentration using a quinidine calibration curve. The relation appears linear with a high correlation ($r = 0.9998$) and with a slope <1.

Thus, the constant cross-reaction and the lower affinity of hydroquinidine are confirmed, allowing the use of a correction factor to measure hydroquinidine with a quinidine calibration. Nevertheless, owing to the lower therapeutic concentration range of hydroquinidine (1–2 mg l^{-1}) compared to quinidine (2–5 mg l^{-1}), it seems desirable to use a calibration curve in the 0–4 mg l^{-1} range with specifically prepared hydroquinidine calibrators: 0, 0.4, 0.8, 1.5, 2.5 and 4 mg l^{-1}.

Table 1 shows also that the polarization values at zero concentration were not identical in both calibration curves. However, the difference is only 1.07% and is not considered to be statistically significant; measurement of several drug-free human serums gave values for the mean ±SD of 188.50 ± 1.85 ($n = 10$). This observation implies that results can be considered as serum independent. The within-day and between-day precision of the assay is shown in Table 2. RSDs ranged from 1.1 to 5.3% ($n = 10$) for low and medium concentrations.

Specificity was tested by analysing 20 serum samples from cardiology patients not receiving hydroquinidine treatment. All results were

Table 2
Precision data for LC and FPIA

	Target hydroquinidine concentration (mg l^{-1})	Mean	RSD (%)
FPIA			
Within-day analysis ($n = 10$)	0.5	0.46	4.1
	2.5	2.63	1.1
Between-day analysis ($n = 10$)	0.5	0.49	5.3
	1.25	1.24	2.8
	2.5	2.51	2
LC			
Within-day analysis ($n = 7$)	0.45	0.44	3.6
	1.8	1.84	2.3
Between-day analysis ($n = 7$)	1.25	1.28	6.2
	2.5	2.46	6.1

lower than the detection limit of the method (0.05 mg l^{-1}). A response (0.1 mg l^{-1}) was obtained only with serum spiked with a quinine concentration higher than 160 mg l^{-1}.

The influence of intrinsic factors such as protides, bilirubin and lipids were not evaluated but are well documented in the literature.

Table 2 shows that the precision of FPIA is comparable to that of HPLC. Eighty-seven serum samples were assayed by both FPIA and HPLC. Figure 3 shows good correlation between the two methods ($r = 0.96$). This is not surprising since the concentration of the hydroquinidine metabolites are much lower than that of hydroquinidine itself [7]. Moreover, the cross-reactivity of these metabolites toward quinidine antibodies is probably low, as shown for quinidine metabolites. This results in good specificity of FPIA for the determination of hydroquinidine.

For the paired concentration values in serum, a mean difference (HPLC–FPIA) of -0.044 ± 0.027 was observed. This mean was not significantly different from zero. The equation of the allometry curve, which takes into account the variability of both HPLC and FPIA estimates of the true hydroquinidine concentration, was: $y = 1.04x + 0.01$, and demonstrates that the two assay methods are in close agreement.

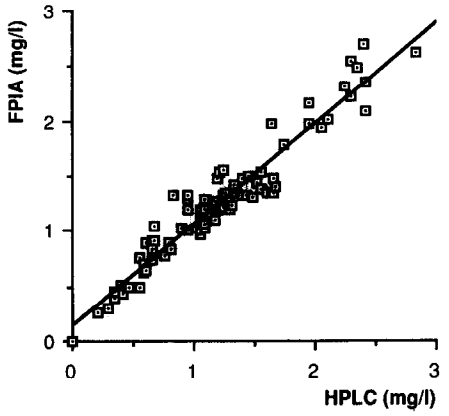

Figure 3
Hydroquinidine values of 87 patient serum samples assayed by LC and FPIA: $y = 0.963x + 0.077$; $r = 0.96$.

This study confirms the feasibility of monitoring hydroquinidine with FPIA, using hydroquinidine calibrators and quinidine reagents. Although HPLC is specific, it requires an extraction step prior to analysis. FPIA was found to be precise, easier and faster, and is thus a good alternative to HPLC for this purpose.

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