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*Short Communication*

# A comparison of the high-performance liquid chromatography and RIA measurement of medroxyprogesterone acetate

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## Introduction

Administration of high doses of medroxyprogesterone acetate (MPA) has been shown to be of value in the treatment of breast cancer, suggesting that the clinical response is related to the concentration of MPA in the body. Recent studies have attempted to correlate MPA concentrations with therapeutic effect [1, 2]. However, in these studies, most of the analyses were performed by immunoassay techniques. These assays are relatively non-specific, since the antiserum used in the analysis will not only detect MPA but also some of its metabolites [3]. Therefore most workers employ extraction techniques to separate MPA from its metabolites [4], but nevertheless some metabolites may be still measured [5]. Thus current pharmacokinetic studies, using RIA may not accurately reflect the concentration of unmetabolised, and therefore pharmacologically active, MPA in blood. Recently, chromatographic techniques for MPA have become available [6, 7] which selectively measure the parent drug. In order to compare various studies, it would seem useful to study the relationship between chromatographic techniques and RIA. Accordingly it was decided to compare the measured MPA concentrations by HPLC and RIA during a bioavailability study.

## Experimental

### *Materials and Methods*

Fourteen healthy, drug-free male volunteers (age 25–40 years) were administered 500 mg of medroxyprogesterone acetate (Provera, Upjohn) as a single oral dose. Blood

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samples were collected in heparinised tubes at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 26 and 32 h post-dose. Plasma was separated and the samples were stored at −20°C and assayed by RIA. They were refrozen and stored for a further 3 months and then assayed by HPLC.

The RIA method for measuring plasma MPA concentrations was based on the method described by Cornette *et al.* [8] using the same antiserum containing antibodies raised to conjugates linked to the 11 position of MPA [9]. The HPLC method was that described by Read *et al.* [6], except that the sensitivity of the assay was increased to 4 ng $ml^{-1}$ by using a more sensitive UV detector. Samples frozen at −20°C showed no deterioration in MPA concentration over a 3 month period.

The two sets of results were subjected to a simple regression analysis and the equation of the regression line calculated. The results at each time point also were compared by simple regression analysis. Pharmacokinetic analysis on the mean data from the two methods of analysis was performed using a one compartment oral model on the NONLIN program [10]. The mean peak plasma MPA concentrations were calculated by averaging each peak concentration for each volunteer.

## Results

A total of 147 samples were generated during the study and were assayed by both RIA and HPLC. The overall equation for the regression analysis of all RIA results ($x$) against the corresponding HPLC results ($y$) was $y = -7.1 + 0.34\,x$, which demonstrated a significant correlation ($P < 0.01$). Some 23 (6 at 0.5 h, 2 at 1.0 h, 2 at 12.0 h and the rest at 26 and 32 h) samples had no detectable MPA when measured by HPLC, but had detectable amounts when measured by RIA. These results were omitted from subsequent calculations and the remaining 124 concentrations were compared at each time interval. It was found that there were significant correlations between the MPA concentrations returned by RIA and HPLC at 2, 3, 4, 6, 8, 10 and 12 h (see Table 1), but not at other time points. The high negative intercept at 4 h is due to the one high HPLC reading, 63.4 ng $ml^{-1}$ with a corresponding RIA value of 125.2 ng $ml^{-1}$. If these values of MPA which were excluded because of the limit of sensitivity of the HPLC, were included as zero, there would be a loss of significance in these correlations only at the 12 h point. However there were differences between the methods as measured by the

**Table 1**
The correlation of the results, from the two LC and RIA methods, obtained during the analysis of plasma samples in man following an oral dose of 500 mg medroxyprogesterone acetate

| Time after dose (h) | *N* | *r* | 2*P* | Regression |
|---|---|---|---|---|
| 0.5 | 8 | 0.4318 | n.s. | |
| 1 | 12 | 0.4083 | n.s. | |
| 2 | 13 | 0.8679 | 0.01 | HPLC = −8.27 + 0.35 RIA |
| 3 | 13 | 0.5899 | 0.05 | HPLC = 5.73 + 0.15 RIA |
| 4 | 13 | 0.7897 | 0.01 | HPLC = −36.45 + 0.67 RIA |
| 6 | 14 | 0.9523 | 0.001 | HPLC = −3.68 + 0.21 RIA |
| 8 | 12 | 0.7254 | 0.01 | HPLC = −2.50 + 0.18 RIA |
| 10 | 13 | 0.8509 | 0.001 | HPLC = −1.16 + 0.16 RIA |
| 12 | 9 | 0.8026 | 0.01 | HPLC = −3.63 + 0.21 RIA |
| 26 | 9 | 0.3110 | n.s. | |
| 32 | 8 | 0.4889 | n.s. | |

**Table 2**
Mean plasma concentrations (ng $ml^{-1}$) of medroxyprogesterone in 14 subjects, measured by the two methods following an oral dose of 500 mg

| Time after dose (h) | HPLC (ng $ml^{-1}$) | RIA (ng $ml^{-1}$) |
|---|---|---|
| 0.5 | 2.3 ± 0.6 | 7.3 ± 1.0 |
| 1 | 5.7 ± 1.0 | 25.8 ± 4.3 |
| 2 | 12.2 ± 3.5 | 58.4 ± 8.0 |
| 3 | 16.9 ± 2.4 | 76.0 ± 9.0 |
| 4 | 17.1 ± 4.0 | 89.5 ± 10.3 |
| 6 | 14.9 ± 4.0 | 87.2 ± 17.8 |
| 8 | 9.1 ± 2.2 | 64.7 ± 8.6 |
| 10 | 6.9 ± 1.3 | 52.9 ± 6.1 |
| 12 | 4.9 ± 1.3 | 49.4 ± 4.6 |
| 26 | 4.4 ± 1.0 | 32.9 ± 3.1 |
| 32 | 2.5 ± 0.7 | 21.1 ± 1.0 |

slope of the regression lines of each correlation ($P < 0.001$). When the regression lines were forced through the origin, measurement of the slopes revealed that the HPLC values were about one fifth those of RIA at each time point.

The mean data from the two methods (Table 2) were then subjected to pharmacokinetic analysis and fitted to a one-compartment model. The mean elimination half-lives were not significantly different from each other, being 33.8 and 39.7 h respectively when measured by HPLC and RIA. The mean peak plasma MPA concentrations following HPLC was approximately one fifth that following RIA, being 23.8 ± 5.0 and 109.6 ± 15.7 ng $ml^{-1}$ respectively. The median times to peak however were not significantly different, being 4 h using both methods.

## Discussion

Plasma concentration determinations of MPA have previously relied on RIA techniques for measurement. Although these techniques are very sensitive, lack of absolute specificity has complicated the interpretation of blood concentration data. The present results show that RIA, without prior extraction, appears to over-estimate the HPLC values by about 5 times. Since RIA is not specific, the increase in concentration is probably due to cross-reacting MPA metabolites. Nevertheless there is a good correlation between the two sets of values, especially for times after 2 h. MPA is very rapidly metabolised during absorption, due to the extensive first-pass effect of MPA, so that following absorption, metabolite formation may be complete. Thus, over the present study period (less than one half-life) there will be an almost constant relationship between drug and metabolites, so that MPA plasma concentrations measured by both methods of analysis, peak at the same time as metabolites, and decline virtually in parallel, with the resulting elimination half-life estimations being similar. The lack of correlation at the very late time points probably reflects loss in sensitivity in the HPLC measurement since values were close to its limit of sensitivity. Thus, since the lower limit of sensitivity for HPLC is 4 ng $ml^{-1}$, its usefulness may be limited in some studies where very low concentrations are expected. Nevertheless, for "steady-state" values HPLC offers a useful analytical alternative to RIA. With increased sensitivity, HPLC would be

the desired method for pharmacokinetic purposes. Now that the relationship between RIA and HPLC has been established, the utility of RIA, with its ease of use and its sensitivity, in therapeutic drug monitoring and bioavailability assessment is worth considering. Where MPA specificity becomes a problem, the addition of a solvent extraction procedure is all that would be required.

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