Br. J. clin Pharmac. (1982), **14**, 363–368

ENALAPRIL MALEATE AND A LYSINE ANALOGUE (MK-521) IN NORMAL VOLUNTEERS; RELATIONSHIP BETWEEN PLASMA DRUG LEVELS AND THE RENIN ANGIOTENSIN SYSTEM

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1 Two single doses of 10 mg each of the converting enzyme inhibitor enalapril maleate or MK-421 and of its lysine analogue (MK-521) were administered p.o. to twelve male volunteers.

2 The active diacid metabolite of MK-421 and the lysine analogue were determined by radioimmunoassay and MK-421 by the active metabolite method following *in vitro* hydrolysis.

3 Peak serum levels of MK-421, active metabolite and lysine analogue were reached within 1, 3 to 4, and 6 h respectively. Practically all MK-421 had disappeared from serum within 4 h.

4 A close correlation between percent inhibition of plasma converting enzyme activity and the serum concentration of active metabolite was observed ($r = 0.98$, $n = 171$, $P < 0.001$). Similarly, converting enzyme blockade as expressed by the ratio plasma angiotensin II/angiotensin I was closely correlated with serum active metabolite levels ($r = 0.93$, $n = 15$, $P < 0.001$).

Introduction

Captopril, the first orally active converting enzyme inhibitor available, has been shown to be an effective antihypertensive drug (Horowitz, 1981) and has therefore been marketed recently in several countries of the world. Unfortunately, during the development phase of that drug, no assays to measure drug levels were set up and, therefore, pharmacokinetic studies have been severely hampered. Most information that became available was based on captopril's effect on the different components of the renin angiotensin system.

We have recently reported the results of initial studies evaluating three new converting enzyme inhibitors (Patchett *et al.*, 1980), enalapril maleate or MK-421, its parent diacid compound or 'active metabolite' (MK-422) and its lysine analogue (MK-521) in normal volunteers and subsequently in hypertensive patients. It has been shown that these compounds actively block the pressor response to exogenous angiotensin I (Biollaz *et al.*, 1981) decrease plasma converting enzyme activity, plasma angiotensin II and aldosterone levels (Brunner *et al.*, 1981) and also reduce blood pressure in hypertensive patients (Gavras *et al.*, 1981). Meanwhile, a radioimmunoassay to measure drug levels was developed (Hichens *et al.*, 1981). This has provided the opportunity to compare drug levels with the different components of the renin angiotensin system following the administration of these converting enzyme inhibitors. The results suggest that blood levels closely follow the degree of inhibition of plasma converting enzyme activity and also very well reflect the inhibition of total angiotensin I to angiotensin II conversion.

Methods

The study has been described in detail in a previous report (Brunner *et al.*, 1981). In short, twelve normotensive male volunteers aged 22 to 33 years were included in the study. Throughout the investigation, the subjects were maintained on a free salt intake. On the morning of day 1, the volunteers came to the hospital outpatient department at 07.00 h after an overnight fast. Upon arrival, they were installed in a comfortable armchair and an intravenous catheter was inserted into an antecubital vein. After a resting period of 30 min, blood samples for the measurement of plasma converting enzyme activity and drug levels were drawn immediately prior to drug administration at 08.00 h (time 0) and 15, 30, 60 and 90 min, 2, 3, 4, 6, 8, 10, 24, 48, 72 h later. In addition, blood samples for the measurement of plasma angiotensin I and II were drawn at times 0, 1, 4, 10 and 24 h in six subjects after

0306–5251/82/090363–06 $01.00

MK-421 as well as in six subjects after the lysine analogue MK-521. On the day of drug administration, the subjects remained seated in the armchair and were only allowed to rise for voiding and to eat lunch around noon. At 18.00 h the subjects left the hospital to return on the following mornings, up to the fourth day post-drug for the subsequent blood samples.

Two compounds, MK-421 (*N*-[(S)-1-(ethoxycarbonyl)-3-phenyl-propyl]-L-alanyl-L-proline) and its lysine analogue (MK-521), were investigated. MK-421 is a pro-drug in ester form of the parent diacid-compound. *In vivo* it is hydrolysed to this active metabolite. Capsules containing 10 mg of MK-421 or its lysine analogue were prepared by the manufacturer. A single capsule was administered orally with 150 ml of tap water at 08.00 h. Each subject received both compounds at an interval of 1 week in a double-blind randomized crossover fashion.

Percent inhibition of plasma angiotensin converting enzyme activity was calculated using the formula:

$$\left(1 - \left(\frac{A_t}{A_o}\right)\right) \cdot 100$$

where A_o is plasma converting enzyme activity before MK-421 or lysine analogue administration and A_t plasma converting enzyme activity at a given time.

Plasma angiotensin II concentrations were quantitated using a modification of the method described by Düsterdieck & McElvee (1971) and plasma converting enzyme activity by a radioenzymatic assay. The characteristics of these methods in our laboratory have been described previously (Brunner *et al.*, 1981). Angiotensin I levels were determined using a method which is a modification of that described by Waite (1973). The serum drug concentrations were measured by radioimmunoassay (Hichens *et al.*, 1981). The radioimmunoassay measured the active metabolite MK-422 and the lysine analogue MK-521 directly, with intact MK-421 estimated as the difference between concentrations of MK-422 obtained after and before *in vitro* hydrolysis of MK-421.

Regression analysis was carried out according to the method of least squares after log transformation. To determine significant differences between groups of data, a Student's *t*-test was applied. All results are given as mean ± 1 s.e. mean.

Results

Figure 1 compares the time course of drug serum levels to that of percent inhibition of plasma converting enzyme activity following administration of 10 mg of MK-421. The unchanged drug reached its peak 1 h following drug administration and had practically disappeared after 4 h. The active metabolite MK-422 reached its peak later, i.e. between the 3rd and 4th hour which coincides with the peak inhibition of plasma converting enzyme activity. Seventy-two hours following drug administration, the level of active metabolite was very low whereas plasma converting enzyme activity was still inhibited by more than 20%.

In Figure 1b, a similar relationship for the lysine analogue is depicted. Peak levels of the drug are reached later, that is 6 h following drug administration, and this again coincides with peak converting enzyme inhibition. Longer duration of drug action is also illustrated 72 h following administration when converting enzyme activity is still blocked by more than 40%.

Figure 2 illustrates the relationship between measured drug levels and percent inhibition of plasma converting enzyme activity following administration of both drugs. Following a log transformation, an extremely close relationship between plasma drug levels and inhibition of converting enzyme activity was observed ($n = 171$, $r = 0.93$ and $n = 169$, $r = 0.94$ respectively). These relationships suggest that drug levels of at least 10 ng/ml should be reached to assure maximal blockade of plasma converting enzyme activity. With higher drug levels, apparently no significant increase in blockade can be gained.

In Figure 3, the ratio of measured plasma angiotensin II over plasma angiotensin I levels is related to the measured drug levels. This angiotensin II over angiotensin I ratio is used as an appreciation of total *in vivo* angiotensin conversion. A decrease in the ratio reflects blockade of conversion of angiotensin I to angiotensin II. A close inverse relation between this angiotensin II/angiotensin I ratio and measured drug levels was observed for both drugs, the correlation coefficient for the active metabolite of MK-421 being 0.94 and for the lysine analogue 0.88. Again it is apparent that drug levels of roughly 10 ng/ml should be reached in order to obtain close to maximal blockade of angiotensin conversion.

Discussion

The results of the present study demonstrate that there exists a close relationship between plasma levels of active drug and some components of the renin angiotensin system. Since this is the first converting enzyme inhibitor for which a drug assay is available, such comparisons could not be done previously. In the case of MK 421, plasma converting enzyme activity closely reflects the measured levels of active drug. The inhibition of converting enzyme activity is complete when the active drug reaches its peak blood level. However, the recovery of converting enzyme activity is slower than one might expect from the rate

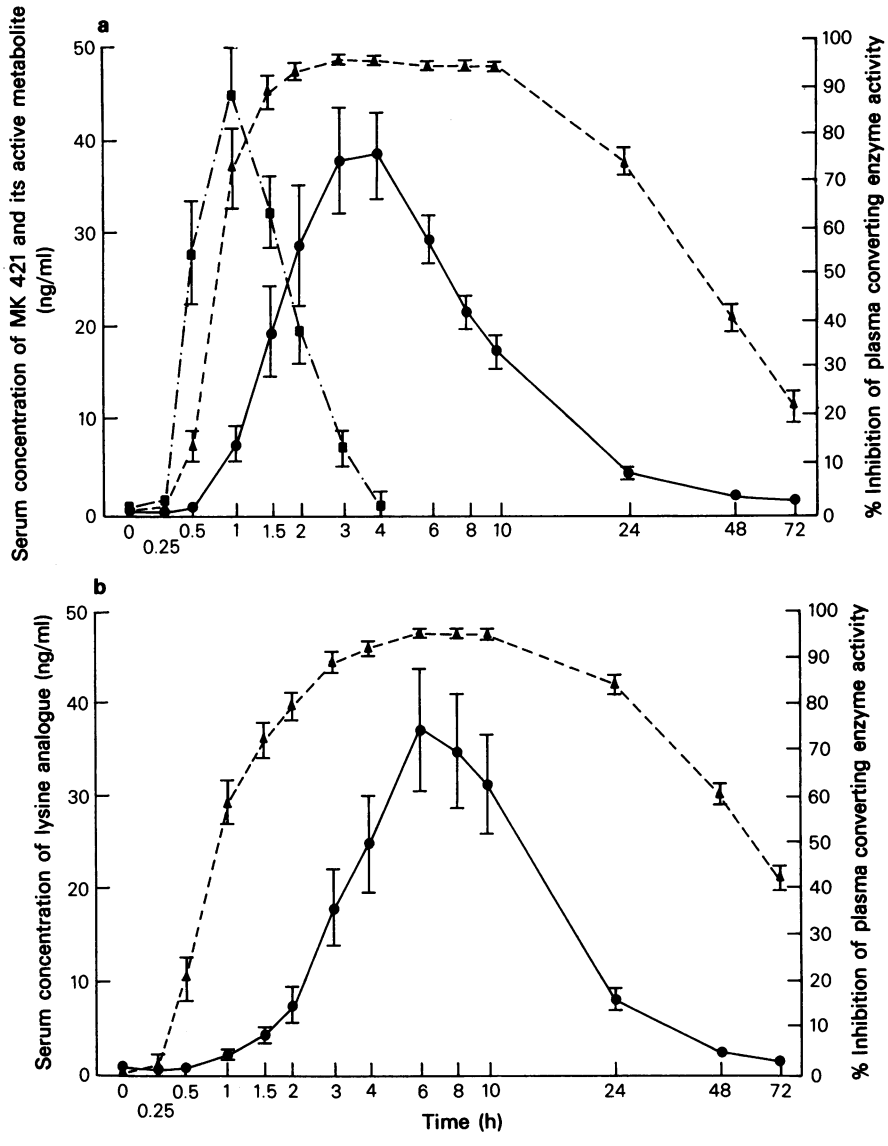

Figure 1 (a) Serum levels of MK-421 (■) and of its active metabolite (●) after a single oral dose of 10 mg MK-421. Time course of the inhibition of plasma converting enzyme activity following this single oral dose (▲). Mean ± 1 s.e. mean of 12 subjects.
(b) Serum levels of the lysine analogue compound (●) after a single oral dose of 10 mg. Time course of the inhibition of plasma converting enzyme activity following this single 10 mg oral dose (▲). Mean ± 1 s.e. mean of twelve subjects.

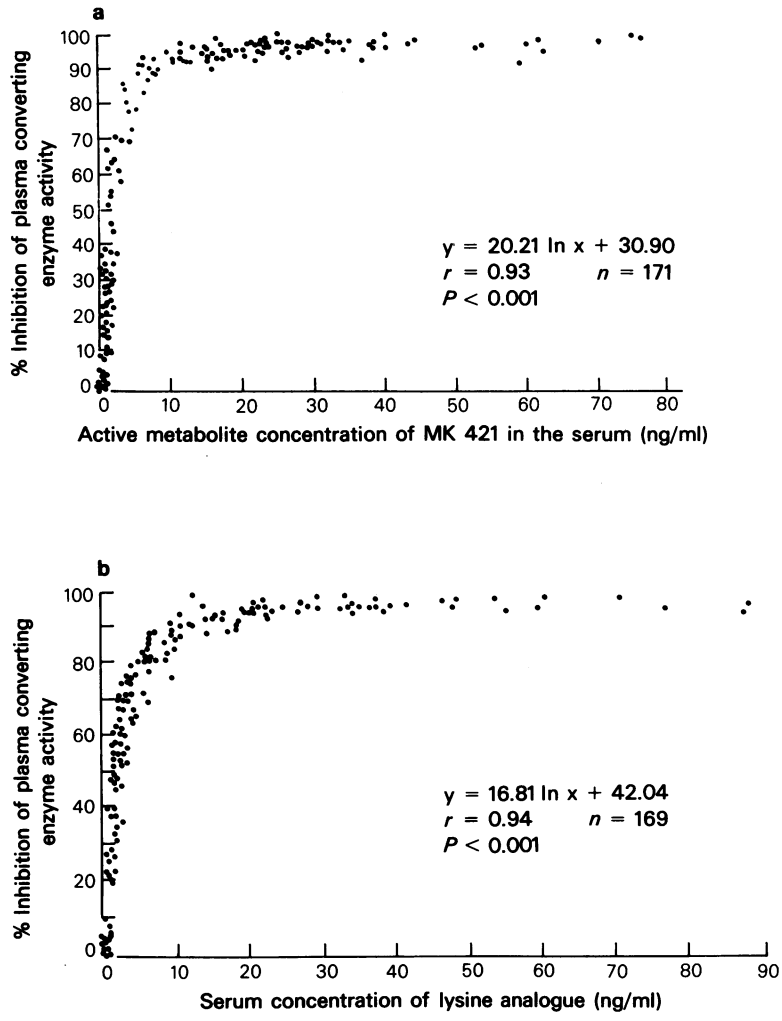

Figure 2 (a) Relationship between the serum levels of the active metabolite of MK-421 and the percent inhibition of plasma converting enzyme activity following a single oral dose of 10 mg MK-421.
(b) Relationship between the serum levels of the lysine analogue and the percent inhibition of plasma converting enzyme activity following a single oral dose of 10 mg lysine analogue.

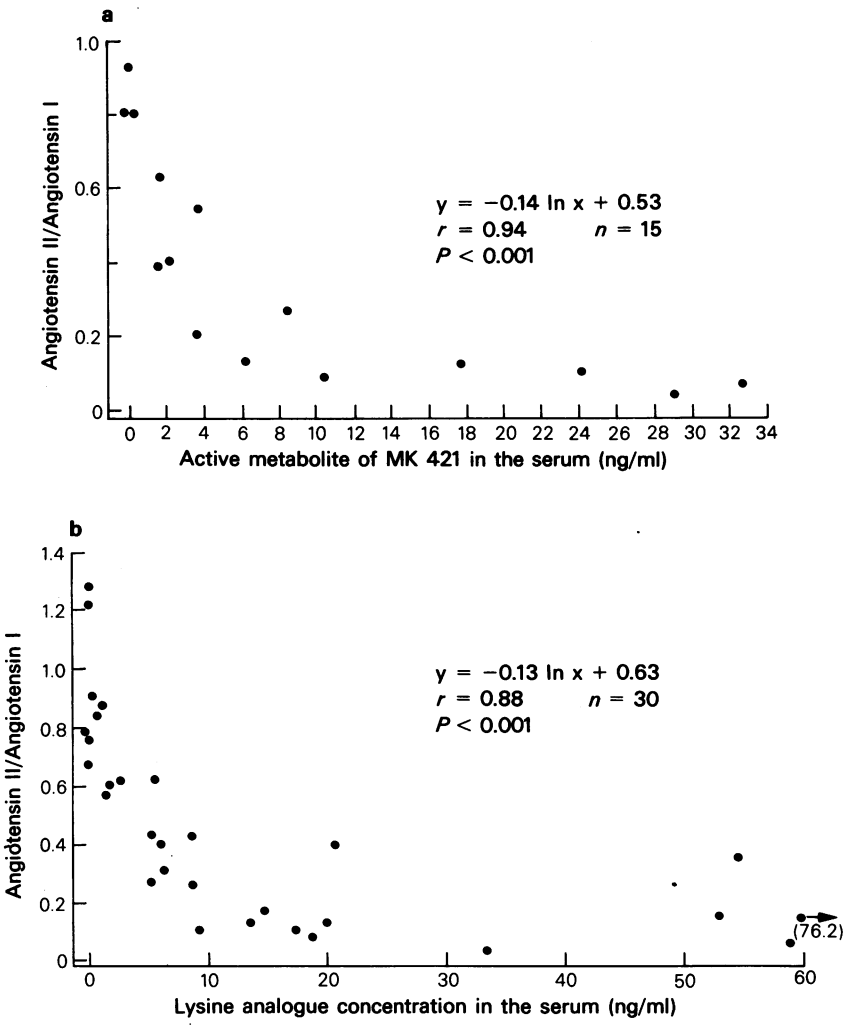

Figure 3 Correlation between the serum concentration of the active metabolite of MK-421 and the angiotensin II/angiotensin I ratio.
(b) As in the upper panel but for the lysine analogue.

of disappearance of the drug from the plasma (Ulm *et al.*, 1982). There is also a close relation between plasma levels of the lysine analogue and the inhibition of converting enzyme activity; the duration of action of this compound seems to be longer than that of MK-421 since 72 h after drug administration, the inhibition of converting enzyme activity is still about 40%. The observation that the angiotensin II/angiotensin I ratio also exhibits a close inverse correlation with the active drug levels in the serum provides good evidence that measurement of plasma converting enzyme activity is a reliable index of total converting enzyme activity.

In the case of captopril, the effect of the drug could be related to changes of components of the renin angiotensin system. Though the measurement of plasma converting enzyme activity turned out to reflect quite well the inhibiting action of captopril, it was only so if the measurement was carried out with certain precautions. Particularly, plasma converting enzyme had to be measured almost immediately following blood drawing since levels tend to increase

during the storage (Roulston *et al.*, 1980). Blood pressure response to captopril seems to reflect only incompletely converting enzyme inhibition, since a marked discrepancy between captopril's effect on blood pressure and on the various components of the renin angiotensin system could be demonstrated (Waeber *et al.*, 1980).

Based on the present observations, it appears that in the case of MK-421 or its lysine analogue, the measurement of plasma converting enzyme activity provides an excellent indicator of whether a sufficient amount of drug has been administered to a patient. This kind of 'bioassay' will very much help to monitor compliance of patients treated with these drugs. This is all the more so that plasma converting enzyme activity has been shown to remain stable for many weeks (Brunner *et al.*, 1981) during storage of the plasma at −20°C.

We wish to thank Dr P. Lutterbeck of MSD in Zürich for providing the drugs and for general discussions.

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(Received January 22, 1982, accepted June 9, 1982)