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Dose-response relationships of ACE inhibitors and angiotensin II blockers

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It is difficult to establish dose-response relationships for ACE inhibitors in patients with hypertension or congestive heart failure. This has led to the widely held opinion that the effects of ACE inhibitors are hardly dose dependent. The purpose of this short discussion is to demonstrate that this class of compounds, as well as the more recent angiotensin II receptor antagonists, exhibit some very clear dose-response relationships when these are evaluated in normal volunteers based on the mechanisms for which they were designed. Characterization of these dose-response curves is important in order to use these drugs at their optimal dose and to obtain the maximal therapeutic benefit.

Introduction

Angiotensin converting enzyme (ACE) inhibitors are now widely used for the treatment of patients with hypertensive disorders[1–7] and with congestive heart failure[8–11]. Because of their efficacy, interest in these therapeutic agents has grown tremendously over the years; many new compounds have been developed, and many more are being investigated.

Most antihypertensive drugs were marketed at a time in their development when their optimal therapeutic dose was not yet established. A good example is captopril, which, at least in some countries, was used at daily doses of up to 450 mg, despite strong evidence that 25 mg twice or three times a day provides close-to-maximal efficacy[12,13]. A priori, it would appear that establishing the dose of an antihypertensive drug should be a relatively easy and straightforward task. In fact, this is not so. Indeed, many difficulties must be overcome in order to determine the optimal, i.e. lowest, maximally effective dose of an anti-hypertensive drug.

As pointed out by Sir George Pickering, hypertension is a quantitative rather than a qualitative disorder[14]. The mechanisms involved in the disease, even if all are not yet completely understood, probably also exist in normotensive subjects, albeit in a quantitatively different mixture. Accordingly, if the mechanism of action of an antihypertensive agent is well understood and directed specifically to one factor involved in blood pressure regulation, studies of its effect in normotensive volunteers will provide useful information to develop guidelines for the treatment of hypertensive patients.

Several years ago, it was demonstrated that the magnitude of blockade achieved by ACE inhibition, the minimum dose needed for maximal efficacy, and the onset and duration of action of the agents could be determined with a considerable degree of accuracy by challenging normotensive volunteers with repeated intravenous administrations of angiotensin (Ang) I[15]. The ability of ACE to cleave various natural and synthetic substrates has been used to measure its activity in vitro[16]. Furthermore, since ACE inhibition causes plasma Ang II to fall and Ang I concentration to rise, an alternative approach to estimating the degree of inhibition of ACE activity in vivo is to measure plasma concentrations of Ang I and Ang II and to use the ratio Ang II/Ang I as an estimate of in vivo ACE inhibition[17].

The inhibition of the pressor response to exogenous Ang I and II has also been used in normal volunteers who were given the angiotensin AT_1, receptor antagonist losartan orally[18,19]. Losartan produced a well-tolerated, long-lasting and clearly dose-dependent antagonism of Ang II. These studies in normotensive subjects included single increasing doses and repeated administration of oral losartan.

The following will provide a short review of some data from our laboratory which illustrate that there exist some well defined dose-response relationships for ACE inhibitors and AT_1 receptor antagonists in normal volunteers. There remains little doubt today that similar dose-response relationships must exist in patients with hypertension and even in those with congestive heart failure, even if these are difficult to demonstrate in large therapeutic trials.

Dose dependent effects of ACE inhibitors

It has been known for more than 20 years that the angiotensin converting enzyme is located in the endothelial cells of the cardiovascular system with a particular concentration in pulmonary capillaries[20]. As a consequence, it is not surprising that the bulk of the conversion of Ang I to Ang II takes place on the surface of the endothelial cells

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and particularly during the lung passage of the blood. There is, however, also some converting enzyme circulating in plasma. Even though it is well recognized that this circulating enzyme only plays a minor role in the conversion of Ang I to Ang II, it has still been used as a marker to measure enzyme activity in clinical situations where endothelial converting enzyme activity is not easily accessible for measurement. This use of plasma ACE activity as a substitute endpoint to assess the degree of converting enzyme inhibition has introduced an important potential source of erroneous judgement, and it has also raised a major question, whether plasma converting enzyme activity reflects the activity of endothelial converting enzyme.

It has to be remembered that to measure plasma ACE activity, plasma is incubated under standardized conditions in vitro. To do this, various reagents are used usually with synthetic, tripeptide substrates. The enzyme incubation product is still measured using a variety of methods. Depending on the conditions and the substrate that has been utilized, very different results are obtained. Indeed, in a given plasma obtained from a patient treated with an ACE inhibitor, the degree of inhibition of plasma ACE activity can be found anywhere between substantial and nil, depending on the method used to measure it[16,21]. Furthermore, one particular method may provide the most realistic results for one ACE inhibiting drug, where with another compound, one of the other methods may yield the most accurate results. Thus, with enalapril, a method using Hip-Gly-Gly as a substrate was found to be most accurate, whereas in the case of benazepril, the substrate Z-Phe-HisLeu had to be used to find the most accurate measurement of plasma ACE activity. It is thus understood that it is useless to report a degree of inhibition of plasma ACE activity obtained with a certain dose of a given ACE inhibitor without precisely describing the method used to reach that result.

A much better way to assess the degree of converting enzyme inhibition is to measure plasma Ang I and II and to calculate the ratio of Ang II to Ang I. This is what we call measurement of converting enzyme activity in vivo and has the advantage of reflecting the physiological situation, which also includes the action of the endothelial converting enzyme. Undoubtedly, this method yields the most meaningful results with any ACE inhibitor since it does not introduce any major artefacts due to dissociation of the compound from the enzyme in vitro, or due to synthetic substitute substrates. The prerequisites, however, are accurate methods to measure circulating Ang I and II.

Measurement of Ang I and II is needed for a precise assessment of the efficacy of converting enzyme inhibitors. These compounds have been designed with the goal of reducing Ang II levels. Only the measurement of Ang II can tell by how much the renin-angiotensin system has been inhibited. Angiotensin I, on the other hand, closely reflects plasma renin activity and circulating levels of active renin. Due to the suppressing effect of Ang II on renin secretion, falling Ang II levels, in the case of converting enzyme inhibition, induce a rise in plasma renin activity, circulating levels of active renin and closely associated plasma Ang I levels. These increased levels not only reflect the degree of the compensatory rise in renin secretion but also directly influence the circulating Ang II levels, even in the face of converting enzyme inhibition. Indeed, a very close correlation was found between measured Ang II levels and the Ang I concentration divided by the concentration of active drug in plasma[16] (Fig. 1).

This phenomenon becomes particularly relevant when studying the dose-response curve of converting enzyme inhibitors, which is known to be rather flat. It could be shown that increasing the dose of an ACE inhibitor resulted not only in dose-dependent converting enzyme inhibition but also in increasing levels of circulating active renin and Ang I levels. Though a 16-fold dose increase was clearly reflected in more complete converting enzyme inhibition, this effect was off-set by the ever-increasing Ang I concentration[21]. As a result, suppression of plasma immunoreactive Ang II was not increased with the high, as compared to the low, dose after a 10 day treatment course. As a consequence, the dose of converting enzyme inhibitor that should be chosen is that which provides a maximal degree of ACE inhibition with a minimal stimulation of renin release.

In a recent study we evaluated the dose-dependent effects of a new ACE inhibitor, temocapril, in normal volunteers[22]. The aim of the study was to evaluate the accuracy of different approaches to characterizing dose-dependent effects of ACE inhibitors, and particularly to compare the inhibition of the pressor response to Ang I with plasma ACE activity and the circulating levels of the converting enzyme inhibitor, i.e. its potent diacid active metabolite.

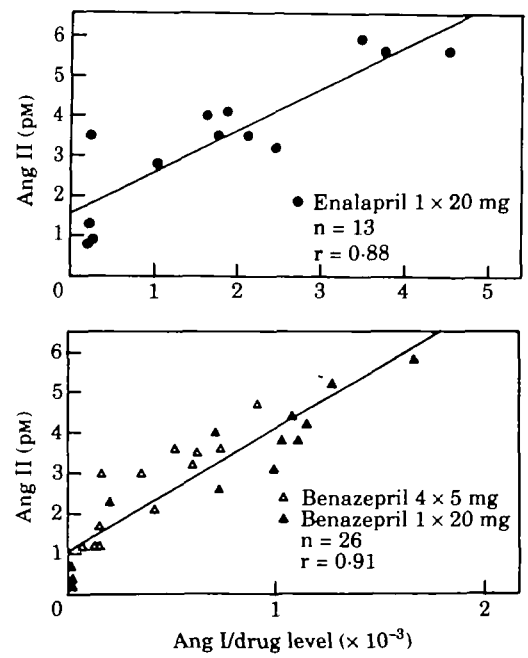

Figure 1 Scatter plots showing the relationship between venous plasma angiotensin II (Ang II) concentrations and the ratio of plasma angiotensin I (Ang I) to drug levels. Each point is the mean of determinations in six volunteers at a given time point. (From Juillerat *et al.*[16] with permission.)

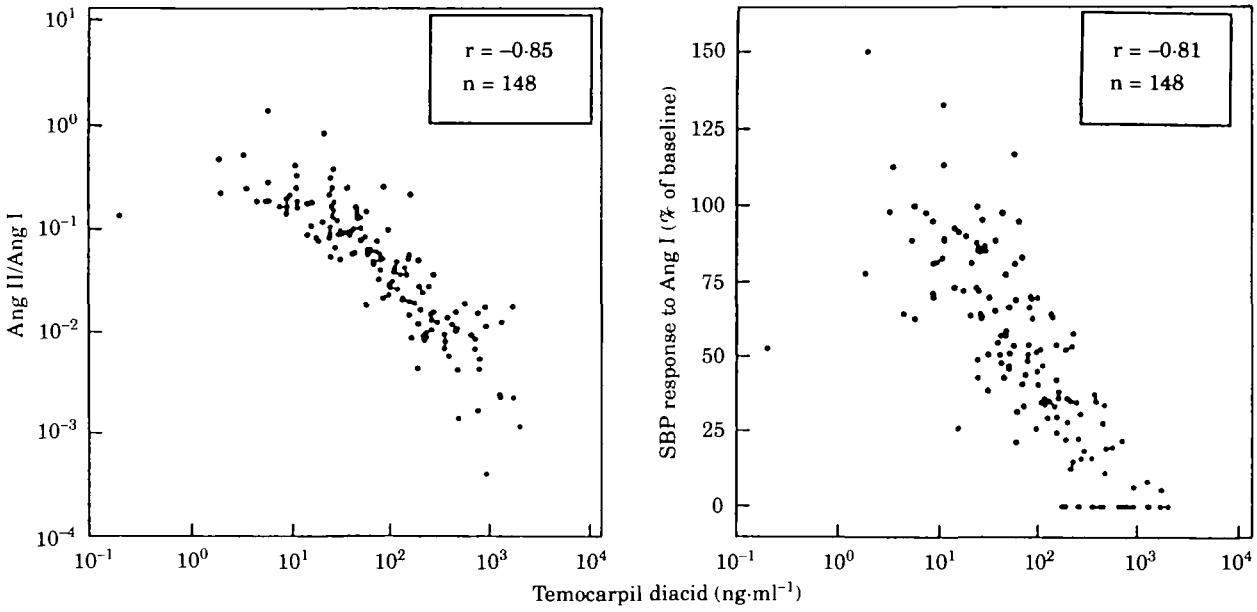

Figure 2 Correlations between plasma temocapril diacid and Ang II/Ang I ratio (left panel) and the systolic blood pressure (SBP) response to Ang I (right panel). (From Delacrétaz *et al.*[22] with permission.)

It has been shown for the first time that the blockade of the pressor response to Ang I with an ACE inhibitor is closely related to the plasma level of the active compound, i.e. the diacid metabolite (Fig. 2). In addition, the pressor response to Ang I was also found to correlate strongly with ACE activity in vivo, the parameter we consider to reflect most accurately ACE inhibition.

Consequently, if inhibition of plasma ACE activity is measured accurately, it closely predicts the reduction in Ang II dependent blood pressure induced by an ACE inhibitor. The correlation between plasma diacid levels and plasma ACE activity or the pressor response to Ang I was analysed based on a log-linear relationship. Indeed, these parameters are expected to exhibit a relationship best described by a sigmoidal E_{max} curve. However, since high and low extremes of drug concentrations were avoided in this study, only the linear part of such a curve was explored and linear regression calculated.

Dose dependent effects of angiotensin II antagonists

About 20 years ago, Ang II analogs were used as the first specific probes to block the renin-angiotensin system in animals and humans. It could be demonstrated for the first time with those probes that blockade of the renin-angiotensin system, at least in certain patients, can reduce the blood pressure of hypertensive patients[23,24]. These early compounds were later abandoned because of their peptidic nature, which made parenteral administration mandatory and also because of their partial agonistic Ang II like action.

The recent reports of the synthesis and experimental evaluations of highly active non-peptidic Ang II antagonists has reactivated this approach to block the renin-angiotensin system[25–30]. It is likely that blockade at the receptor level provides the most specific access to inhibition. Furthermore, receptor blockade leaves the possibility open of specific and selective inhibition of subtypes of Ang II receptors with different functional targeting. Indeed, subtypes of Ang II receptors have already been reported[31] and it is probable that further subtypes will be discovered in the near future.

The Ang II antagonist losartan (DuP 753 or MK 954) has been extensively evaluated in experimental models and found to be highly efficacious[25–30]. We have had the opportunity to evaluate, for the first time, the efficacy of this compound in blocking the Ang II induced pressor effect in humans[18]. To this end, in an initial study, single rising doses up to 40 mg were administered orally. In a second investigation the same doses were given once a day for 8 days to normal volunteers. A clear dose-dependent inhibition of the pressure response to exogenous Ang I or II could be demonstrated with a degree of inhibition of about 70% with the 40 mg dose. At the same time it became evident that the effect was quite long lasting since with the 40 mg dose, the Ang II pressure response was still partially inhibited 24 h after drug administration[18] (Fig. 3). Taking into account the peak blocking effect after the 40 mg dose on the 8th day of administration, there was no apparent cumulative effect of the drug.

Meanwhile it was demonstrated in animals that the drug produced an active metabolite (Exp 3174) which exhibits an at least 10-fold higher affinity to the Ang II receptor subtype I and a longer half-life than the mother compound losartan[32]. Therefore, the purpose of the next study was not only to investigate the effect of higher doses of losartan on the pressure response to exogenous Ang II but also to characterize this response as a function of the concentra-

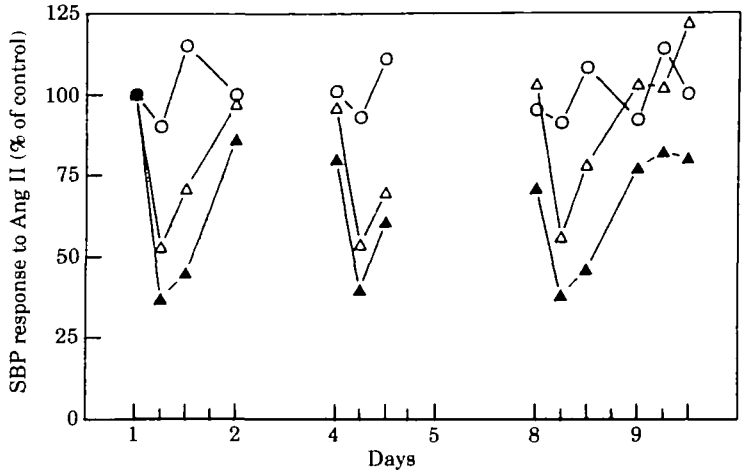

Figure 3 Effects of 8 consecutive days of treatment with daily single oral doses of losartan (20 mg, 40 mg or placebo) on systolic blood pressure (SBP) response to test doses of angiotensin II (Ang II) in healthy volunteers. 0–0 = placebo (n = 6); △–△ = 20 mg (n = 5); ▲–▲ = 40 mg (n = 6) (From Christen *et al.*[18] with permission.)

tion of losartan and of its active metabolite. Six subjects received in an open, non-randomized, cross-over fashion, placebo and 40, 80 and 120 mg losartan in a single oral dose at one-week intervals[19].

The parent compound was readily absorbed with a peak reached at the first post-dose blood collection, i.e. after 1 h. The active metabolite was eliminated much more slowly than the parent compound and it remained detectable in plasma for up to and more than 24 h. Dose-dependent inhibition of the blood pressure response to exogenous Ang II lasting for more than 24 h was again observed. During the 1 to 6 h post-drug period, 80 and 120 mg provided a higher degree of inhibition than the 40 mg dose. There was no difference between the results obtained with either 80 or 120 mg.

The inhibition of the pressure response to the exogenous Ang II challenge in individual subjects was then analysed vs the respective concentrations of the active metabolite of losartan. The Hill equation was fitted to the data of each individual subject. To take into account the duration of the drug effect, the integral of the percentage of inhibition over 9 h was related to the area under the curve of the active metabolite during the same period (Fig. 4). The six individual points are plotted in three subgroups according to the dose received. It is clearly apparent that increasing the dose from 80 to 120 mg did not provide any enhancement of the time integrated inhibition.

Preliminary studies in hypertensive patients using doses between 50 and 150 mg p.o. once daily have already demonstrated some clear antihypertensive efficacy which,

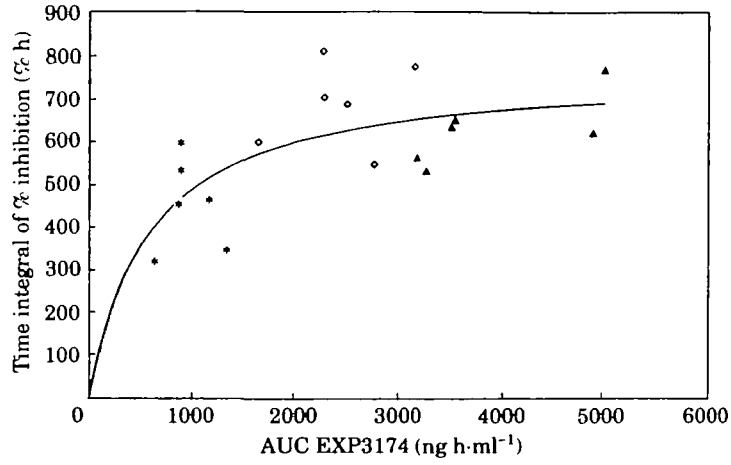

Figure 4 Time integral of the inhibition of the pressor response to Ang II challenge vs the area under the concentration-time curve (AUC) of the active metabolite of losartan (Exp 3174), up to 9 h in 6 volunteers. The solid line represents a Hill equation fit to the data. * = 40 mg; ◇ = 80 mg; ▲ = 120 mg. (From Munafo *et al.*[17] with permission.)

on the 5th day, was actually comparable to that of enalapril[33]. As expected, Ang II antagonism also induced a compensatory rise in plasma renin activity, Ang I and II[18]. Whether these elevated Ang II levels can actually attenuate the antihypertensive effect of losartan remains to be further investigated.

Clearly Ang II antagonism can reduce blood pressure in hypertensive patients. Losartan, the very potent, orally active Ang II antagonist, is now available for investigational therapeutic use. Whether this approach will turn out to be as effective as converting enzyme inhibition in reducing blood pressure in hypertensive patients and improving the haemodynamics of patients with congestive heart failure will be known within the next 2 to 3 years. In addition, it will be equally important to learn whether side effects can be further reduced when changing from ACE inhibitors to Ang II antagonists, and this is particularly interesting in the case of ACE inhibitor induced cough or angioneurotic oedema.

Conclusions

There remains little doubt that ACE inhibitors and Ang II receptor antagonists exhibit distinct and well characterized dose-response relationships for the specific effect for which they were designed, i.e. blockade of Ang II synthesis or Ang II receptor interaction. Even these direct and straight forward relationships are already attenuated and modified by the compensatory rise in renin release induced by the pharmacological agents.

However, when it comes to assessing the clinical effect expected in patients with hypertension and/or congestive heart failure, everything is much more difficult. For instance, blood pressure reduction is not only dependent on the induced reduction in Ang II but also on the reactive response of the sympathetic nervous activity, on sodium intake and renal sodium handling, on structural changes of the vascular wall, on tissue penetration of the drug, on blood pressure variability, etc. Considering all the variables and possible compensatory mechanisms it is rather surprising that dose-dependent effects can sometimes be shown in patients with cardiovascular discorders. However, distinct dose-response relationships exist for ACE inhibitors and Ang II receptor antagonists. They are extremely relevant for the therapeutic use of these compounds since inadequate dosing can become a source for treatment failure and side effects.

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