Enalapril (MK421) activation in man: importance of liver status

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The *in vitro* conversion of enalapril (MK421) to enalaprilic acid (MK422) in human autopsy tissues was examined. MK422 was measured by radioimmunoassay. Human cadaver liver was the only tissue in which significant conversion was demonstrated. The esterase activity was stable after post mortem. Autopsy liver tissues from patients with elevated ante mortem liver function tests were found to have a reduced rate of deesterification.

Keywords radioimmunoassay enalapril (MK421) enalaprilic acid (MK422) deesterification liver

Introduction

Enalapril (MK421) is a recent addition to the family of orally active angiotensin converting enzyme (ACE) inhibitors which are proving of clinical use in the treatment of hypertension, congestive cardiac failure and ascites (Di Carlo *et al.*, 1983; Heel *et al.*, 1980; Johnston *et al.*, 1982; Romankiewicz *et al.*, 1983).

Enalapril is a prodrug whose pharmacological activity is dependent on deesterification to enalaprilic acid (MK422) (Biollaz *et al.*, 1982; Jackson *et al.*, 1982; Johnston *et al.*, 1983). The aim of the study was to examine the *in vitro* conversion of enalapril to enalaprilic acid by human tissue and to examine the influence of hepatic dysfunction on this metabolism. This may have clinical significance in the use of this drug in disease states where liver dysfunction is part of the clinical syndrome.

Methods

Enalaprilic acid radioimmunoassay

Enalaprilic acid was assayed using a specific radioimmunoassay (Hitchens *et al.*, 1981). The assay utilises a rabbit antibody raised against MK521, a lysine derivative of enalaprilic acid.

This antibody crossreacts with enalaprilic acid but not with enalapril. The radioactive ligand used was I¹²⁵ MK351A, a tyrosyl derivative of MK521. An 8 µl aliquot of each sample was added to tubes containing 20,000 counts/min I¹²⁵ MK351A; a 1:19,000 dilution of the MK521 antibody in a solution containing 0.005% w/v rabbit gamma globulin with 0.3125 units of anti-rabbit γ globulin goat antibody. These were contained in 0.1 M potassium phosphate buffer (pH 7.5) with 0.05 M sodium EDTA and 1 g/l bovine serum albumin to a total volume of 798 µl. After equilibration at 4°C for 48 h, bound radioactivity was separated from the free form by centrifugation. The interassay coefficient of variation was 6%, 8% and 5% at concentrations of 1.9, 6.2 and 19.3 ng/ml of enalaprilic acid respectively. The limit of detection was 0.1 ng/ml and enalapril crossreacted by a maximum of 0.2% in estimates of enalaprilic acid over the range of 0–200 ng/ml. The enalaprilic acid standards used ranged from 0.2 to 200 ng/ml.

Tissue conversion

The ability of tissues to hydrolyse enalapril to MK422 was assessed by measuring the generation of enalaprilic acid by tissue homogenates in the

presence of excess enalapril. A 500 µl aliquot of tissue homogenate (200 mg wet wt/ml) was incubated with 500 µl of enalapril (2.03×10^{-6} M in 0.1 M potassium phosphate buffer) at 37°C. Aliquots of 100 µl were pipetted at 0, 0.25, 0.5, 1, 2 and 3 h. Each aliquot was added to 900 µl of absolute ethanol to arrest further enzymatic activity. After centrifuging this solution for 5 min at 2,000 rev/min, 8 µl aliquots of each sample were then taken and blown down to dryness. The residue was reconstituted with 8 µl of assay buffer and enalaprilic acid estimated by radioimmunoassay. Recovery from tissue homogenate of standard concentrations of enalaprilic acid of 100 ng/ml, 50 ng/ml, 25 ng/ml and 12.5 ng/ml were 91.6 ± 1.5 ng/ml, 51.9 ± 2.3 ng/ml, 24.2 ± 0.5 ng/ml and 12 ± 0.7 ng/ml respectively, and was thus taken as 100% over the assay range. Results were converted to moles and standardised for unit protein concentration and unit time.

Experiments were carried out on post mortem tissue. A 1 g sample was cut and then homogenised in normal saline using a Polytron homogeniser (45 s at 15,000 rev/min) to produce a concentration of 200 mg wet weight/1 ml.

The protein content of each homogenate was determined (Lowry *et al.*, 1951) and the product of the bioactivation experiments expressed per mg of protein.

To establish that the esterases in the liver were stable after death, liver samples were obtained from three autopsies and studied serially. Tissue was stored at 20°C for 65 h, over which time portions were removed and frozen for subsequent analysis in a single assay. No difference in activity was demonstrated between homogenates of fresh and frozen tissues. Stable activity was demonstrated for up to 21 h at 20°C ($93 \pm 6.5\%$ of 0 h activity), after which a gradual deterioration in conversion rate was noted ($93.2 \pm 7.7\%$ at 27 h, $86.7 \pm 20.7\%$ at 44 h and $78.2 \pm 12.3\%$ at 68 h). Autopsy samples used in all other experiments were obtained within 22 h of death, frozen and stored at -20°C until required.

The human autopsy tissues examined were kidney, brain, spleen, heart, lung and liver. The activity of human serum obtained from blood donors (500 µl neat) was also examined. Liver autopsy tissues were classified as normal or abnormal according to their histological assessment (*viz*; no abnormalities or minor abnormalities *vs* major histological abnormalities of necrosis, fatty change and cirrhosis) and ante-mortem liver function tests which were significantly different (Table 1). The results of these experiments were calculated per mg of protein. In addition, to estimate the cellular content of the abnormal and normal liver homogenates,

Table 1 Ante mortem liver function tests and post mortem histology in the group of patients with normal liver function compared to the group with liver disease

	Liver function tests				Histology
	Alkaline phosphatase (55-260 u/l)	γ -glutamyl transpeptidase (M 15-60 u/l) (F 15-45 u/l)	Aspartate aminotransferase (15-45 u/l)	Bilirubin (3-20 µmol/l)	Albumin (M 36-48 g/l) (F 33-43 g/l)
Normal group <i>n</i> = 8	168 ± 16	25 ± 5	32 ± 4	17 ± 3	38 ± 3
Abnormal group <i>n</i> = 4	861 ± 428*	463 ± 336*	116 ± 63	86 ± 53*	27 ± 2*
					Normal, minor congestion and fatty change
					Severe congestion with centrilobular necrosis and marked fatty change and/or micro nodular cirrhosis

**P* < 0.05

the liver cellular enzymes—aspartate amino-transferase, alkaline phosphatase and γ -glutamyl transpeptidase—were measured per mg of homogenate protein and were not found to be significantly different. The rate of conversion of enalapril to enalaprilic acid was found to be temperature dependent and all assays were standardized at 37°C. The optimal pH was found to be pH 7.5.

Experiments with human liver homogenate showed increasing enalaprilic acid yield with increasing protein content. The rate of conversion over the 3 h incubation period using 500 μ l of liver homogenate (200 mg wet wt/ml) and 500 μ l of enalapril (2.03×10^{-6} M) was found to follow approximately first order (linear) kinetics with the correlation coefficient of variation for enalaprilic acid generated against time being greater than 0.96 with all human liver homogenate samples tested.

In addition to comparing the conversion rates, comparisons were also made of the amount of enalaprilic acid generated at 3 h.

Between group comparisons were by student unpaired *t*-test and within groups by paired *t*-tests. Group results were expressed as mean $\pm$ 1 s.e. mean. Linear regression coefficients were calculated by the least squares method.

Results

Liver was the only tissue that produced appreciable conversion of enalapril to enalaprilic acid, with moderate activity detected in lung and minor activity in heart and splenic tissue (Figure 1). No

activity was demonstrated in serum nor in kidney or brain tissue.

Autopsy tissue samples of eight normal and four abnormal livers were analysed, with the rate of conversion of enalapril to enalaprilic acid in the normal group being significantly greater than it was in the abnormal group, when comparing the conversion rates calculated from the slope of the linear regression of each subject (enalaprilic acid mol mg^{-1} protein h^{-1} —normal mean = $2.24 \times 10^{-8} \pm 0.18 \times 10^{-8}$, range 3.10×10^{-8} to 1.78×10^{-8} , abnormal mean = $0.58 \times 10^{-8} \pm 0.06 \times 10^{-8}$, range 0.74×10^{-8} to 0.4×10^{-8} , $P < 0.005$, $n = 12$). The difference was also significant whether the enalaprilic acid formed was expressed per mg protein or per unit of liver enzyme activity.

Discussion

In these experiments we have developed an assay system to measure the deesterification of enalapril to enalaprilic acid using tissue obtained at post mortem. The data with liver homogenates suggests that the esterase activity is stable for some considerable time post mortem. Using post mortem tissues we have also demonstrated that liver tissue is a major site for this conversion with moderate conversion occurring in lung tissue. Only minor conversion was detected in heart and splenic tissue and no conversion was detected in serum or kidney or brain tissue. There was a significant difference in the *in vitro* conversion rate of enalapril to enalaprilic acid with normal and abnormal liver tissue. Liver function may thus be extremely important in the *in vivo* pharmacokinetics of this drug. This is of potential clinical importance as the current clinical use of enalapril is in hypertension, congestive cardiac failure and resistant ascites, diseases in which abnormal liver function may also be present. These experiments suggest that enalapril may be more slowly deesterified to enalaprilic acid in patients with severe liver impairment. It is possible therefore that the onset of the acute therapeutic effects may be delayed in these patients due to a reduced rate of bioactivation of enalapril to enalaprilic acid. Chronic dosage levels of enalapril may need to be higher in these patients than those patients with normal liver function.

Studies on the pharmacokinetics and pharmacodynamics of enalapril in patients with liver disease have yet to be assessed. The clinical relevance of the findings in this paper are thus yet to be confirmed.

Figure 1 Conversion rates of enalapril to enalaprilic acid mol mg^{-1} protein $\text{h}^{-1} \pm$ s.e. mean, for various human autopsy tissues.

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