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Effects of Angiotensin Converting Enzyme Inhibitors in Healthy Rats and in Rats With Carbon Tetrachloride-Induced Toxic Hepatitis

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Abstract: Sulphydryl-containing captopril (CPR) appears to act as a scavanger of oxygen derived free radicals. This is not present in other angiotensin-converting enzymes such as enalapril (EPR). The hepatotoxic effect of carbon tetrachloride (CCl4) may result from induction of reactive oxygen free radicals. The aim of this study was to analyse the effects of CPR and non-sulphydryl-containing enalapril (EPR) in healthy rats and in rats with CCL4-induced toxic hepatitis.

The rats were divided into two major groups. The first group consisted of healthy rats, and the second group consisted of rats with CCl4-induced toxic hepatitis. Each major group was sub-divided into 3 groups, where CPR, EPR, or a placebo was administered. The resulting 6 sub-groups were analysed for the hepatic effects of CPR and EPR in healthy rats and in rats with CCl4-induced toxic hepatitis.

Co-administration of CPR or EPR with CCl4 lead to an increase in hepatic enzyme levels, and to a greater level of liver damage in comparison with CCl4 alone.

In conclusion, both CPR and EPR may lead to hepatotoxicity, and sulphydryl-containing CPR does not appear to protect the liver from the toxic oxidant effect of CCl4.

Key Words: Captopril, enalapril, carbon tetrachloride, hepatotoxicity.

Introduction

The effects of most angiotensin-converting enzyme inhibitors (ACEI), and captopril (CPR) in particular, cannot be explained solely on the basis of their enzyme-inhibiting activity. Sulphydryl (-SH)-containing captopril appears to act as a scavanger of oxygen-derived free radical species, suggesting its potential importance in reversing post-ischaemic contractile dysfunction and myocyte necrosis. However, the free radical scavenging effects of $ACEI_s$, is probably not related solely to their sulphydryl content (1,2). Enalapril (EPR), and other ACEIs do not contain a sulphydryl group.

Various pharmacological and chemical substances are known to lead to liver damage. One of these chemicals is carbon tetrachloride (CCl4). CCl4 and its metabolites induce peroxidation of cell membrane lipids, regeneration of reactive oxygen free radicals, and hepatocellular fatty regeneration with centrolobular necrosis of the liver (3).

The aim of this study was to analyse the biochemical and histopathological effects of captopril (CPR) and enalapril (EPR) in healthy rats and in rats with CCL4-induced toxic hepatitis, and to investigate whether sulphydryl-containing CPR protects the liver from the toxic oxidant effect of CCl4.

Materials and Methods

Sixty male rats, each weighing an average of 150g, were used for the purpose of this study. The rats were divided into two major groups. The first group consisted of healthy rats, and the second group consisted of rats with CCl4-induced toxic hepatitis. Each major group was sub-divided into 3 groups (A;B;C and D;E;F), each of which contained 10 rats. The study groups in this experiment are summarised in Table 1. CCl4 was administered to the rats on the first day of the experiment. CPR was administered at 5mg/kg three times and EPR at 1mg/kg twice for the first three days of the study. The doses of CPR and EPR were chosen according to the recommendations of Przyklenk & Kloner (1). Sterile saline (SS) was used as the control.

Table 1. Study groups in the experiment

	Sub-Groups	Intraperitoneal Injection	Intragastric Administration
Healthy Rats	A	1 mL SS	1 mL SS twice daily for 3 days
	B	1 mL SS	15 mg/kg CPR thrice daily for 3 days
	C	1 mL SS	2 mg/kg EPR twice daily for 3 days
Rats with CC14-induced toxic hepatitis	D	0.5 mL/kg CCl4	1 mL SS twice daily for 3 days
	E	0.5 mL/kg CCl4	15 mg/kg CPR thrice daily for 3 days
	F	0.5 mL/kg CCl4	2 mg/kg EPR twice daily for 3 days

CCl4: Carbontetrachloride
SS: Sterile Saline
CPR: Captopril
EPR: Enalapril

The CCl4 used in the study was a solution purchased from Sigma Laboratories (Cat no: C5331). The CPR and EPR were diluted in 1 ml of SS for groups B, C, E and F prior to administration. Intracardiac blood samples were collected from the rats, and their livers were removed on the fourth day of the experiment immediately after they were sacrificed by air embolus injection. Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, and conjugated bilirubin were determined with a Technicon RA-XT autoanalyser kits. The liver preparations were evaluated by a pathologist, who had not been informed of the particular groups of the specimens. Hepatic damage was graded according to the presence of histopathological characteristics on a scale of 1 to 7, as detailed in Table 2 (4). A hepatic damage score was obtained for each rat according to the presence of one or more of these characteristics, and the mean hepatic damage score for the 10 rats in each of the groups A, B, C, D, E and F was estimated (Table 3).

Biochemical parameters and hepatic scores were analysed by employing Kruskal Wallis and Mann-Whitney U tests, and values of $p<0.05$ were accepted as being statistically significant.

Results

The means of the biochemical parameters, and statistical comparisons of the groups are presented in Table 3.

ALP levels were found to be significantly increased ($p<0.05$) in comparison with the control group when ACEIs alone were administered. ALP levels were increased in all CCl4 containing groups in comparison with the control and ACEI-only groups. The highest increase in ALP levels was in the CCl4-with-CPR group.

The AST values for the ACEI-only groups and the control group were similar. AST values were significantly increased in the CCl4 group in comparison with the control and ACEI-only groups. Addition of CPR or EPR to the CCl4 led to even higher levels of AST, though this was only significant between the CCl4-with-EPR versus the CCl4-only groups.

Table 2. Hepatic damage scoring system.

Hepatic damage score	Histological finding
1	congestion of less than half of the liver lobule
2	congestion of more than half of the liver lobule
3	microvesiculation
4	focal necrosis around the central vein
5	necrosis of 1/3 of the distance between the central vein and portal tract
6	necrosis of 2/3 of the distance between the central vein and portal tract
7	necrosis of all of the distance between the central vein and portal tract

There was a slight increase in the levels of ALT in the ACEI-only groups in comparison with the control group, though this was only significant in the CPR-only group. There was a statistically significant increase in ALT in all CCl4-containing goups in comparison with the control and ACEI-only groups. The administration of CPR or EPR together with CCl4 led to a greater increase in ALT levels than with CCl4 alone.

There was no statistical difference between the control and ACEI-only groups with respect to the total bilirubin levels. The CCl4-containing groups led to a statistically significant increase in the total bilirubin level, but the effect of co-administration of ACEI with CCl4 was similar to that of CCl4 alone. The findings for conjugated bilirubin were similar to those for total bilirubin, with the exception that administration of ACEI with CCl4 led to a significantly higher increase in comparison with CCl4 alone.

Histological examination of the liver tissues in the control and ACEI-only groups revealed only minimal congestion. However, there was significant liver damage in all CCl4-containing groups, most of which exhibited congestion, microvesiculation and necrosis. Concurrent administration of CPR or EPR with CCl4 led to significantly greater liver damage in comparison with CCl4 alone, showing extension of the necrosis. The hepatic damage scores were in agreement with these findings, showing higher scores for groups E and F (Table 3).

Discussion

Various pharmacological and chemical substances which belong to the intrinsic or idiosyncratic group of hepatotoxins may induce a level of hepatic damage varying from asymptomatic hepatic functional disturbance to widespread liver necrosis. CCl4, which is an intrinsic hepatotoxin, was used for the purpose of inducing hepatic damage in this study (5). The sulphydryl content of the CPR did not translate into an advantage over EPR in terms of preventing CCl4-induced hepatic damage, which was similar in both groups.

The hepatotoxicity of CCl4 was confirmed in this study with significant elevations of ALP, AST, ALT, total bilirubin, conjugated bilirubin and hepatic damage scores. CPR or EPR alone also led to an elevation in ALP levels. CCl4 hepatotoxicity was more marked when it was used concurrently with ACEIs. There are two possible mechanisms for this. Firstly, it is known that ACEIs can result in greater hypotension in the presence of hepatoxicity (6). Therefore, it is possible that hypotension secondary to ACEI administration decreases liver perfusion, causing secondary hypoxia and exacerbating the hepatotoxicity caused by CCl4. The healthy rats may have tolerated a degree of reduction in liver perfusion, but it is unlikely that the rats with impaired liver function could have tolerated this. Secondly, ACEIs may have a direct cytotoxic effect on the liver (7,8), and this may have exacerbated the CCl4 toxicity. The blood pressure of the rats could not be studied in this experiment due to

Table 3. Biochemical and Histopathological findings.

Para-Meter	A	B	C	D	E	F	Statistical Significance
ALP (U/L)	65 ± 11	105 ± 10	102 ± 37	171.5 ± 33	342 ± 148	179 ± 47	A<B*, A<C*, A<D*, A<E**, A<F*, B<D*, B<E**, C<D*, C<F*, D<E*, E<F*
AST (U/L)	178 ± 70	155 ± 13	176 ± 70	8686 ± 6536	10700 ± 7662	12246 ± 7864	A<D†, A<E†, A<F†, B<D†, B<E†, C<D†, D<F†
ALT (U/L)	28±11	42 ± 12	36 ± 16	1873 ± 1107	3375 ± 2738	3144 ± 1408	A<B*, A<D†, A<E†, A<F†, B<D†, B<E†, C<D†, C<F†
Total bilirubin (µmol/K)	2.42 ± 1.17	2.30 ± 1.7	2.52 ± 1.7	10.0 ± 8.5	12.3 ± 5.1	13.7 ± 8.5	A<D*, A<E*, A<F*, B<D*, B<E*, C<D*, C<F*
C. bilirubin (µmol/L)	0.51 ± .0.17	0.34 ± 0.13	0.40 ± 0.17	6.80 ± 0.51	8.8 ± 5.1	9.3 ± 6.8	A<D†, A<E†, A<F†, B<D†, B<E, C<D, C<D†, C<F†, D<E*, D<F*
Hepatic Damage	1.2 ± 0.4	1.1 ± 0.3	1.3 ± 0.4	7.5 ± 2.8	9.5 ± 3.1	8.9 ± 2.1	A<D†, A<E†, A<F†, B<D†, B<E†, C<D, C<F†, D<E*, D<F*

*p<0.05, **p<0.01, †p<0.001,

Values are presented as mean ± standard deviation.

Only relationships of statistical significance are presented in the table.

T bilirubin: Total bilirubin, C bilirubin: Conjugated bilirubin, ALP: Alkaline phosphatase, AST: Aspartate Aminotransferase,

ALT : Alanine aminotransferase

A: Intraperitoneal + oral sterile saline (SS),

B: Intraperitoneal SS + oral 15 mg/kg/day CPR

C: Intraperitoneal SS + oral 2 mg/kg/day EPR

D: Intraperitoneal 0.5 ml/kg CC14 + oral sterile saline

E: Intraperitoneal 0.5 ml/kg CC14 + oral 15 mg/kg/day CPR

F: Intraperitoneal 0.5 ml/kg CC14 + oral 2 mg/kg/day EPR

technical difficulties. In addition to the mechanisms of toxicity described above, CPR can, rarely, cause cholestatic liver damage in humans, which is reversed in most patients upon withdrawal of the drug (9,10). However, jaundice may continue for several weeks in a number of patients, and finally lead to widespread liver necrosis, hepatic coma, and death (11). EPR is also known to lead to hepatoxicity, albeit less frequently than CPR (12).

In conclusion, both CPR and EPR may lead to hepatotoxicity, and sulphydryl-containing CPR does not appear to protect the liver from the toxic oxidant effect of CCl4.

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