

The inhibition of LP was performed using MDA method as described in the text.

* $P < 0.005$ compared with normal plasma.

** $P < 0.025$ compared with normal plasma.

Values for significance between normal and uremic plasma LP inhibitor activities are based on Student's t-test.

Thus, it appears that there is a significant reduction in LP inhibitor activity in plasma collected from uremic patients. A diminished level of plasma LP inhibitor activity is likely to result in an increased production of vasoactive LP products in plasma and tissues. Although there is no prima facie reason why LP inhibitor activity should decrease in uremia; two explanations may be envisaged. First, increased cell turn over as well as cell damage may favour LP activity. Secondly, there might be a relative or absolute decline in uremic plasma LP inhibitor activity as found in the present study. It is known that LP products inhibit prostacyclin biosynthesis (5), induce platelet aggregation, and blood clotting (6). The decreased LP inhibitor activity in uremic plasma may produce decrease in prostacyclin biosynthesis. Therefore, we would like to suggest that this may somehow, be related to increased bleeding in uremia. Work is in progress to define this relationship.

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15% homogenate based on the wet weight of the tissue. Whole homogenates were centrifuged at 1000 xg for 15 minutes at 4°C and the supernatant used as a source of LP activity. For assay purposes 0.2 ml of the supernatant (containing 4 mg of protein) was added to the buffer in the presence or absence of test plasma to give a final assay volume of 1.0 ml. After 1 h at 37°C with gentle shaking the reaction was terminated by adding 1 ml of TCA (200g/litre) and the precipitated proteins removed by centrifugation at 3000 xg for 10 minutes. Supernatant was heated with 1 ml of TBA (10g/litre) for 15 minutes at 100°C and the absorbance was measured at 535 nm. All determinations were performed in triplicate with suitable controls. Proteins were estimated by the Bio-Rad (U.S.A.) protein assay kit. LP inhibitor activity of plasma of the two groups (normal and uremic) at each of two plasma concentrations used in the assay was compared by using Student's t-test.

RESULTS AND DISCUSSION

Initially, the optimum conditions for the assay of LP activity in rabbit brain homogenate (1000 xg supernatant) were determined. Table 1 shows that LP activity (as measured by malondialdehyde (MDA) formation) increases with increasing amount of brain homogenate upto 10 mg protein/ml concentration. However, no further linear increase in MDA formation was observed indicating that MDA formation had reached maximum.

Table 1 Effect of rabbit brain homogenate on LP activity.

Rabbit brain homogenate (mg protein/ml)	MDA production (O.D. 535 nm) ± S.E.M.
1.0	0.11 ± 0.003
2.00	0.25 ± 0.003
4.0	0.55 ± 0
6.0	0.78 ± 0
12.0	1.2 ± 0

LP activity was determined by MDA assay as described

in text. Values are means ± SEM of three determinations. Protein was estimated using the Bio Rad (U.S.A.) assay kit.

Table 2 gives O. D. values for the production of MDA of different times of incubation. It is apparent that the formation of MDA is linear and increases with increasing time of incubation of rabbit brain homogenate. In our assay of LP activity in brain homogenate an incubation time of 1 hr was chosen.

Table 2. Effect of time of incubation of rabbit homogenate LP activity.

Time (Min)	MDA Production (O.D. 535 nm) ± S.E.M.
0	0.00
15	0.06 ± 0
30	0.12 ± 0
45	0.17 ± 0
60	0.20 ± 0
90	0.27 ± 0

LP activity was determined by MDA assay as described in the text. All experiments are means ± SEM of three experiments.

The inhibitory effects of human plasma at two concentrations 1% and 10% (v/v) against LP activity, in rabbit brain homogenate are shown in Table 3. In each group of subjects (with and without uremia) the plasma LP inhibitor activity displayed by uremic patients was absent or significantly reduced ($P < 0.25$) as compared to controls.

Table 3. Inhibition of lipid peroxidation by plasma.

Subject	Number of Subjects	Plasma (Conc. % v/v)	Mean Percentage Inhibition of LP ± SEM
Normal (Control)	20	1	21.78 ± 4.15
		10	75.5 ± 2.39
Uremia	19	1	6.85 ± 7.04*
		10	57.12 ± 13.71**

Decreased ability of human plasma to inhibit lipid peroxidation in uremia*

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ABSTRACT

Blood samples from normal healthy volunteers and uremic patients were obtained and plasma were prepared. The effects of these plasma samples on lipid peroxidation (LP) in rabbit brain homogenate were determined. Both plasma samples inhibited LP activity in our experiments. The percent inhibition of LP produced by normal plasma, concentration 1 and 10%, v/v, was 21.78 ± 4.15 and 75.5 ± 2.39 (Mean \pm S.E.M. $n = 20$) respectively. The corresponding values of LP inhibition obtained using the uremic plasmas, concentrations 1 and 10% v/v, were 6.85 ± 7.04 and 57.12 ± 13.71 respectively. These data show that there was a significant reduction in LP inhibitory activity in plasma of uremic patients. This suggests that there may be an increased production of vasoactive LP products in uremic patients. Since LP products profoundly affect blood clotting it can be inferred that aberrant levels of LP inhibitor in uremic plasmas could contribute to increased bleeding in uremia.

Key words : Lipid Peroxidation — uremia — bleeding

INTRODUCTION

A significant bleeding tendency and acquired platelet disorders are frequently found in patients with uremia¹

Accepted for publication on 4.9.1985.
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*This work was supported by research funds provided by the Aga Khan University, Faculty of Health Sciences Karachi — Pakistan.

It has been proposed that the prolonged bleeding times and abnormal bleeding tendency in uremic patients is related to a plasma factor which stimulates prostacyclin (PGI₂) release from the vessel wall². PGI₂ is a strong vasodilator and the most potent natural inhibitor of platelet aggregation yet described. We have recently reported that lipid peroxidation (LP) inhibits PGI₂ biosynthesis during pre-eclampsia³ and it has also been demonstrated that human plasma or serum contain endogenous factors that inhibit LP⁴. It is possible that an endogenous inhibitor of LP in plasma could constitute a local mechanism for regulating PGI₂ biosynthesis by the vessel wall during uremic bleeding. Using an assay method previously developed⁴ for measuring LP inhibitor activity in human plasma, we studied the LP inhibitor activity in the plasma of uremic patients.

MATERIALS & METHODS

Thiobarbituric acid (TBA), and trichloroacetic acid (TCA) was obtained from Sigma. Blood was obtained from uremic patients and normal healthy volunteers. The plasma from both groups was separated and tested against LP using the method as described below.

Plasma LP inhibitor activity was assessed by studying the effects of plasma on rabbit brain homogenate. The rabbits were sacrificed by stunning and the brains were quickly removed, washed in ice-cold 50 mM phosphate buffer, pH 7.4, also containing 150 mM NaCl (buffer). The brains were homogenized in the buffer to yield a