The plasma kallikrein kinin system in severely ill and traumatised patients

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SUMMARY

Serial estimations of plasma prekallikrein in four critically ill patients in intensive care showed reduced values in each case, suggesting activation of the plasma kallikrein kinin system. In contrast, samples taken early from 15 patients attending an accident and emergency department with multiple trauma showed significantly elevated plasma prekallikrein concentrations; the significance of this observation is at present unclear.

INTRODUCTION

In 1905 Abelous described the preparation of an extract from human urine which when injected into dogs caused pronounced hypotension. The substances responsible for this effect were later isolated and given the name of kinins because of their ability to produce contraction of smooth muscle. There are two principal kinins, the nonapeptide bradykinin which predominates in plasma, and the decapeptide kallidin which is found primarily in urine (Levinsky, 1979). Kinins are very potent vasodilator substances, and bradykinin can prevent the vasoconstricting action of angiotensin II in equimolar concentrations. Kinins also increase capillary permeability and stimulate prostaglandin synthesis (Mills, 1979). Kinins are formed from inactive kininogen precursors by the action of kallikrein enzymes. There are two principal types of kallikrein. The plasma enzyme has a molecular weight around 100 000 and normally circulates as an inactive precursor, prekallikrein. Other kallikrein enzymes, collectively known as glandular kallikreins, are found in various tissues including pancreas, submandibular glands, and the kidney. These have molecular weights around 40 000 and have different substrate specificity from the plasma enzyme (Levinsky, 1979). The plasma kallikrein kinin system has important vasodilator and hypotensive effects and interacts with several

Fig. 1 The plasma kallikrein-kinin system.

other enzyme systems in blood (Kaplan *et al.*, 1977) (Fig. 1). The two principal steps are the conversion of prekallikrein to active kallikrein, which then acts on high molecular weight kininogen to form bradykinin. Active kallikrein is rapidly degraded *in vivo* and has a half-life in the circulation of around 5 min. The most important trigger of the plasma kallikrein kinin system is activation of Factor XII (Hageman Factor). Activation of Factor XII also triggers both the coagulation pathway and the fibrinolytic pathway; plasmin then digests the active Factor XII to form various fragments collectively known as prekallikrein activator. These fragments then convert prekallikrein into plasma kallikrein; the active kallikrein feeds back up the pathway to stimulate directly further activation of Factor XII, thereby amplifying the response. Plasmin can also directly activate the classical complement sequence and a further link with complement is seen in the fact that the most important inhibitor of plasma kallikrein is C1 esterase inhibitor. Active kallikrein has been shown using the Boyden chamber to be chemotactic for human neutrophils (Kaplan *et al.*, 1971).

The activity of the plasma kallikrein kinin system can be assessed by various methods. Circulating bradykinin can be measured by bioassay or by radioimmunoassay. Active plasma kallikrein can be measured either by enzymatic methods or by measuring the ability of plasma to liberate kinins from a known amount of kininogen precursor. The biological half-life of bradykinin and kallikrein in plasma is short and these assays are technically difficult (Levinson, 1979). An alternative approach is to convert all the plasma prekallikrein to kallikrein *in vitro* then measure activity against a specific substrate. A decrease in prekallikrein is taken to represent activation of the plasma kallikrein kinin system with consumption of precursors (Friberger *et al.*, 1978).

Bacterial endotoxins are known to be potent activators of the Factor XII dependent pathways (Kimball *et al.*, 1972) and there is good experimental and clinical evidence that the plasma kallikrein kinin system is activated either by the injection of endotoxin (Neiss *et al.*, 1968) or by Gram negative septicaemia (Webster *et al.*, 1959; O'Donnell *et al.*, 1976). Virtually all this work was done using either bioassays or enzymatic assays which measured total arginine esterase activity under alkaline conditions (Mason *et al.*, 1970). Recently, synthetic tripeptide chromogenic substrate assays have become available, in which specificity is achieved by copying the amino acid sequence of the preferred substrate of a given enzyme (Friberger, 1978). We, therefore, attempted to discover if these previous observations could be confirmed at a clinical level using such a contemporary type of assay. Previous studies were based on single point in time measurements. We have therefore performed serial estimations of prekallikrein in critically ill patients in an intensive care unit, some of whom had sepsis. Other substances known to activate Factor XII include collagen, vascular basement membrane, urate crystals and articular cartilage (Kaplan *et al.*, 1977). We have therefore measured plasma prekallikrein in patients with multiple trauma who were not septic.

PATIENTS AND METHODS

Serial prekallikrein estimations were performed in four critically ill patients in an intensive care unit who were receiving appropriate supportive therapy. Patient details are shown in Table 1. Single prekallikrein estimations were performed in 15 patients attending the Accident and Emergency Department, Royal Infirmary, Edinburgh with varying degrees of trauma. Blood samples were taken immediately on arrival in the Department, prior to treatment. Trauma was graded according to the standard injury severity score (Baker *et al.*, 1974). A normal range for plasma prekallikrein was established in 26 healthy adult control subjects.

Plasma prekallikrein estimations were performed by the method of Friberger *et al.*, (1978); 9 ml blood was taken into plastic tubes containing 1 ml 0.1 mol sodium citrate. Plasma was separated and stored at -20°C until assayed. Plasma (100 μl) was incubated at 37°C for 120 s with 800 μl prekallikrein activator (Cephotest) to convert prekallikrein to active kallikrein. The synthetic tripeptide chromogenic substrate H-D-Pro-Phe-Arg-pNA (S2302, Kabi Diagnostica, Stockholm, Sweden), for which plasma kallikrein is specific, was added to the incubation mixture (100 μl); the reaction was terminated after a further 40 s incubation at 37°C by the addition of 100 μl 50% acetic acid. The absorbance at 405 nm due to the pNA dye liberated from the substrate was measured by spectrophotometry. A standard pool of normal human plasma was prepared by mixing 3 ml of citrated plasma from each of 12 healthy adult subjects. Dilutions of this standard pool were used to prepare standard curves for each run of the assay; the absorbance for each sample was plotted on this standard curve and the prekallikrein concentration expressed as a percentage of the value for the standard pool. Statistical comparison between groups was performed by the Wilcoxon rank sum test; all results are expressed as mean $\pm$ standard deviation.

RESULTS

Plasma prekallikrein concentration in the 26 healthy controls was $104 \pm 23\%$ of the standard pool value (mean $\pm$ SD). The results of serial observations in the four critically ill patients are shown in Fig. 2; initial, mean and lowest prekallikrein values are shown in Table 1. Comparing the mean values in this group with the control group, plasma prekallikrein was significantly lower than in the controls ($51 \pm 13\%$, controls $104 \pm 23\%$, $p < 0.01$). In two patients prekallikrein values 30–80% of normal were observed over a five-day period; both patients subsequently recovered, when normal prekallikrein values were found. In the other two patients the plasma prekallikrein, initially in the same range, fell to undetectable levels. Both patients at that time experienced severe circulatory failure and one patient died (Fig. 2, Table 1).

Compared with controls the 15 trauma patients showed a significant increase in plasma prekallikrein (controls $104 \pm 23\%$, trauma patients $181 \pm 44\%$, $p < 0.05$) (Fig. 3). There was no correlation between plasma prekallikrein and the injury severity score (correlation coefficient 0.02, $p > 0.1$).

DISCUSSION

The reduction in plasma prekallikrein in critically ill patients in intensive care is in keeping with previous findings in septicaemic shock both in experimental animals (Neiss *et al.*, 1968), and in man (O'Donnell *et al.*, 1976; Mason *et al.*, 1970). Two of our patients (J.M. and R.M.) were obviously infected, although we cannot with certainty exclude sepsis in the others. A reduction in plasma prekallikrein could reflect decreased hepatic synthesis of prekallikrein, or alternatively, increased conversion of prekallikrein to kallikrein, which is then rapidly degraded. Decreased synthesis of prekallikrein is known to occur in severe hepatic dysfunction (O'Donnell *et al.*, 1976), but none of our patients had markedly deranged liver function tests. In two of the patients, rapid falls in

Table 1 Details of critically ill patients in intensive care (PK = prekallikrein)

Patient	Age	Sex	Diagnosis	PK (% of standard)			Outcome
				Initial	Mean	Lowest	
J.M.	45	M	necrotising fasciitis	68	67	37	survived
E.J.	24	F	stab wound of left ventricle	67	39	< 5	survived
A.K.	67	M	multiple trauma, flail chest	84	42	< 5	died
R.M.	54	M	perforated gastric ulcer, septicaemia	65	56	38	survived

Table 2 Injury severity score and plasma prekallikrein concentration in traumatised patients

Patient	Injury severity score	Prekallikrein (% standard)
D.B.	10	229
D.M.	14	234
J.F.	13	172
B.W.	29	203
M.J.	13	186
J.M.	29	174
G.A.	14	241
J.H.	17	64
L.D.	17	174
N.C.	9	166
P.M.	29	206
C.M.	21	175
E.D.	13	147
A.Q.	14	139
F.A.	13	204
n = 15	17 ± 6.8	181 ± 44%

plasma prekallikrein occurred (Fig. 2), at a rate which could not be explained purely by a decreased rate of synthesis. The results therefore suggest that activation of the plasma kallikrein kinin system occurs in critically ill patients and that in some cases such activation can be rapid and complete. Kallikreins are thought to play an important role in the conversion of prerenin to renin (Sealey *et al.*, 1978). Consumption of all the circulating prekallikrein might therefore inhibit subsequent activation of the renin-angiotensin system and interfere with this important compensatory response to hypotension. It may therefore be relevant that following disappearance of prekallikrein from the circulation one of our patients died in irreversible circulatory failure. The kinins formed as a result of activation of the kallikrein kinin system, in addition to causing vasodilation, will increase micro-vascular permeability, increase leakage of protein from blood to the interstitium, and thereby cause a reduction in plasma volume (McFarlane *et al.*, 1972). Indeed, the injection of either kallikrein or bradykinin into animals produces a state remarkably similar to septic shock (Mills, 1979; McFarlane *et al.*, 1972). The effects of kinins on micro-vascular permeability and the chemotactic properties of active kallikrein for neutrophils (Kaplan *et al.*, 1971) might contribute to the subsequent development of the adult respiratory distress syndrome. It therefore seems likely that activation of the plasma kallikrein kinin system will adversely affect the outcome in severely ill patients. Currently available inhibitors of plasma kallikrein, such as aprotonin, are poorly specific and not completely effective (Sumida, 1979). More potent and specific inhibitors might prove to have beneficial effects in septic, shocked and critically ill patients.

The significance of the increased prekallikrein concentration in traumatized patients is unclear. The subjects were studied immediately on arrival in the emergency department and it seems unlikely that any alteration in hepatic synthesis could be

Fig. 2 Serial plasma prekallikrein concentrations in critically ill patients in an intensive care unit.

responsible. It seems more likely that prekallikrein is released into the circulation from an extravascular pool in response to trauma. Further studies are in progress to establish how much of this prekallikrein is converted to active kallikrein and to clarify how this newly observed phenomenon correlates with other aspects of the response to injury.

Fig. 3 Plasma prekallikrein concentrations in patients attending accident and emergency department with trauma.

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