Clinical Study

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Role of Bradykinin in Anaphylactoid Reactions during Hemodialysis with AN69 Dialyzers

Abstract
In vitro experiments have related anaphylactoid reactions in patients treated with angiotensin-converting enzyme (ACE) inhibitors during dialysis with AN69 membranes to excessive bradykinin generation using this negatively charged dialysis membrane. In the present clinical trial plasma bradykinin levels were followed during the early phase of dialysis in 10 patients, not being treated with ACE inhibitors, using AN69, cuprophane, and polysulfone membranes. Bradykinin was measured after extraction by radioimmunoassay. During this study one episode of anaphylaxis occurred during dialysis with the AN69 membrane. Blood samples were collected during the first 5 min of the adverse reaction and showed a more than 100-fold increase in the venous effluent of the AN69 dialyzer (baseline 40 ± 3 vs. 4,900 ± 130 fmol/ml after 5 min). Even though none of the patients received ACE inhibitors, there were 4 more asymptomatic individuals who displayed a more than two-fold increase in their plasma bradykinin concentrations in the venous effluent of the AN69 dialyzer. When these patients were treated either with cuprophane or with polysulfone dialyzers, no significant bradykinin formation was detected, nor were there any adverse events. Taken together, these findings show that anaphylactoid reactions with the AN69 membrane are due to excessive bradykinin generation which even may occur in the absence of ACE inhibitors.

Introduction

In 1990 two studies from Belgium appeared in the literature which reported on anaphylactoid reactions during dialysis with AN69 membranes in patients being treated with angiotensin-converting enzyme (ACE) inhibitors [1, 2]. These observations triggered a host of other publications, reporting similar events during dialysis with AN69 membranes [3–6]. Reports from the early 1980s had primarily addressed hypersensitivity reactions associated with cuprophane [7, 8] or ethylene oxide membranes [9], while the more recent reactions occurred exclusively with AN69 dialyzers with and without the simultaneous use of ACE inhibitors. In a survey of more than 500 dialysis

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patients in Europe, Verresen et al. [10] demonstrated that out of 72 patients treated with AN69 membranes and ACE inhibitors 42 suffered from hypersensitivity reactions, while 71 individuals treated with ACE inhibitors but dialyzed with other membranes were free of these side effects. Only 2 out of these 519 patients who were dialyzed with AN69 membranes but did not receive ACE inhibitors developed anaphylactoid reactions.

These observations provided strong empirical evidence that the combination of dialysis with AN69 membranes and ACE inhibition was involved in the evolution of these reactions. In terms of the underlying mechanism, it was hypothesized that the negatively charged AN69 membrane could activate Hageman factor (factor XII) which at least has been shown in vitro [11]. Active Hageman factor in turn would convert prekallikrein into kallikrein. This proteinase could then cleave bradykinin from high-molecular-weight kininogen [12].

As ACE inhibitors also block the degradation of kinins [13] by inhibition of kininase II (= ACE), bradykinin could readily accumulate during the first few minutes of dialysis, until the membrane is covered by plasma proteins.

Up to now, this hypothesis has only been tested in vitro. Lemke and Fink [14] compared cuprophane and AN69 membranes in terms of bradykinin generation in vitro. In the absence of ACE inhibitors there was little bradykinin formation, while in the presence of ACE inhibitors seven-fold higher plasma levels of bradykinin were found during incubation with AN69 as compared with cuprophane membranes. Comparable in vitro results were obtained by Schulman et al. [15] who observed, in comparison with cuprophane, 6- to 15-fold higher plasma levels of bradykinin during incubation with AN69 membranes. In terms of kallikrein formation, plasma levels of kallikrein-C1 inactivator complexes were 10- to 20-fold higher with the AN69 membrane.

In the present study, bradykinin generation was followed in vivo under standard hemodialysis conditions with AN69, cuprophane, and polysulfone membranes. For obvious ethical reasons, patients with concomitant ACE inhibitor therapy were strictly excluded from this clinical trial.

Methods

Patients

Ten long-term hemodialysis patients (3 females, 7 males) gave their informed consent to participate in this investigation. Their mean age was 57 years (range 21–68 years), and on average they were dialyzed for 36 months (range 3–112 months). Patients who were treated with ACE inhibitors or those who had a history of hypersensitivity reactions were excluded from the trial. The patients were normally treated either with cuprophane or polysulfone dialyzers. Since 1990 AN69 membranes were no longer routinely used in our dialysis center.

Dialyzers

Three different dialyzers were evaluated in this study: (1) cuprophane (GFE 12, Gambro, 1.3 m^2, ETO sterilized); (2) AN69 (Filtral 12, Hospal, 1.3 m^2, ETO sterilized), and (3) polysulfone (F60, Fresenius, 1.2 m^2, ETO sterilized).

The order of membranes was randomized for each patient. Prior to dialysis, the dialyzers were rinsed with 2 liters of heparinized (2,000 U/l) isotonic (0.9%) saline with an ultrafiltration rate of 500 ml/h for the cuprophane and of 1,000 ml/h for the high-flux membranes. Patient connection was performed with a blood flow rate of 200 ml/min.

Blood Sampling

Timed blood samples (2 ml) were drawn prior to and during the first 20 min of dialysis into chilled polypropylene syringes which contained 0.2 ml of a specific cocktail of proteinase inhibitors in order to block kallikrein (kinin production) as well as the kininases I and II (kinin degradation). This mixture consisted of aprotinin (10,000 KIU/ml), soybean trypsin inhibitor (0.8 mg/ml), Polybrene (4.0 mg/ml), 1,10-phenanthrolene (10.0 mg/ml), and EDTA (20.0 mg/ml). Samples were collected both before and after dialysis.

Bradykinin Assay

Plasma kinin levels were analyzed according to the method of Shimamoto et al. [16, 17]. Before the kinin measurement, a repeated extraction procedure had to be performed. Briefly, 0.8 ml of plasma was incubated with 1.6 ml of cold ethanol. After 15 min, the sample was centrifuged at 4°C for 10 min. The supernatant was then evaporated overnight at 40°C and redissolved in 0.6 ml of 66% acetone. Thereafter, the sample was washed with 1.4 ml of petroleum ether and centrifuged for phase separation at room temperature. The upper layer was aspirated and discarded, while the lower layer was again evaporated overnight.

Phosphate-buffered saline containing kininase inhibitors (30 mM EDTA and 30 mM 1,10-phenantroline) and 1% egg albumin was used as assay buffer. The antisynthetic bradykinin antibody (rabbit) was kindly provided by Dr. Shimamoto (Sapporo, Japan). Radioiodinated synthetic tyrosyl bradykinin (400 µCi/ug) was prepared by the chloramine-T method (3,000 cpm/tube). The second antibody was a donkey antirabbit IgG. The radioactivity of the precipitate was measured by gamma counting. The assays were evaluated by the spline approximation method using a commercially available computer program (RIALOG II; Zinser Analytic, Frankfurt, FRG). All samples were measured in triplicate. The reproducibility of the assay was determined routinely at concentrations of 15, 180, and 750 fmol/ml. The coefficients of variation at these levels were 19, 14, and 17, respectively.

Statistics

All results are expressed as mean values ± SEM. The significance was tested using the Student t test. Differences were considered significant when $p < 0.05$.
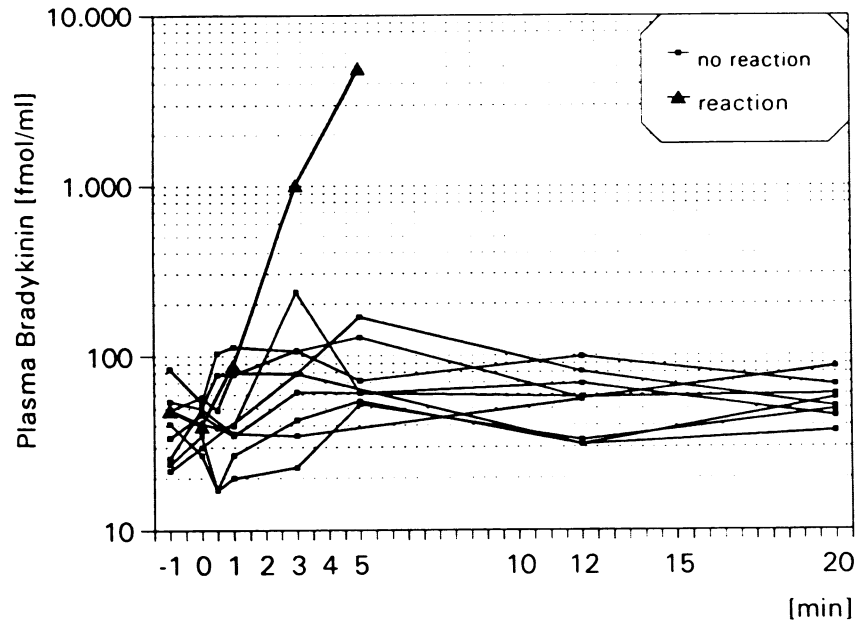
Fig. 1. Individual plasma bradykinin concentrations in the venous effluent during the early phase of AN69 dialysis. In the symptomatic patient data are only available for the first 5 min because the dialysis session had to be interrupted thereafter.

Results

Clinically, dialysis treatment with cuprophane and polysulfone membranes was tolerated well, and there were no adverse events in all patients studied. By contrast, one severe anaphylactoid reaction occurred in a patient who was treated with an AN69 dialyzer. The symptoms only abated when dialysis with the AN69 membrane was stopped and the patient switched to polysulfone.

As a measure of bradykinin generation, the plasma bradykinin concentrations in the venous effluent from AN69 dialyzers are shown in figure 1. As can be seen, there was 1 patient who displayed extremely high bradykinin levels in the venous effluent 3 and 5 min after the onset of treatment (5 min: 4,900 ± 130 fmol/ml). This excessive bradykinin generation was clinically associated with a severe anaphylactoid reaction which only abated when dialysis was stopped after 5 min. There were 4 more patients with bradykinin levels in excess of 100 fmol/ml in the venous effluents during the first minutes of dialysis who were clinically asymptomatic (fig. 1). Arterial plasma bradykinin levels (samples drawn before the dialyzer) remained below 100 fmol/ml in all patients (data not shown) which held also true for the individual who suffered from the anaphylactoid reaction and displayed these excessive bradykinin levels in the venous effluent of the dialyzer.

That bradykinin generation during hemodialysis is a membrane-specific event is depicted in figure 2. The same cohort of patients was dialyzed also with cuprophane and polysulfone membranes. The mean plasma bradykinin concentrations in the venous effluent remained unchanged from baseline with both cuprophane and polysulfone membranes, but increased significantly when AN69 was used for dialysis. This difference was significant, even though the data from the symptomatic patient were not included, when mean values were calculated for the AN69 membrane.

Discussion

To our knowledge, the present report is the first to provide evidence in humans that AN69-related anaphylactoid reactions are in all likelihood based on excessive bradykinin generation within the dialyzer. By comparing arterial and venous plasma bradykinin concentrations (<100 vs. 4,900 fmol/ml), it was clearly shown that bradykinin was generated within the AN69 dialyzer. This finding also reveals how effectively bradykinin is degraded within the circulation, as long as kininases are not blocked by ACE inhibitors.

As this observation is merely based only on a single case, it might seem somewhat farfetched to postulate a
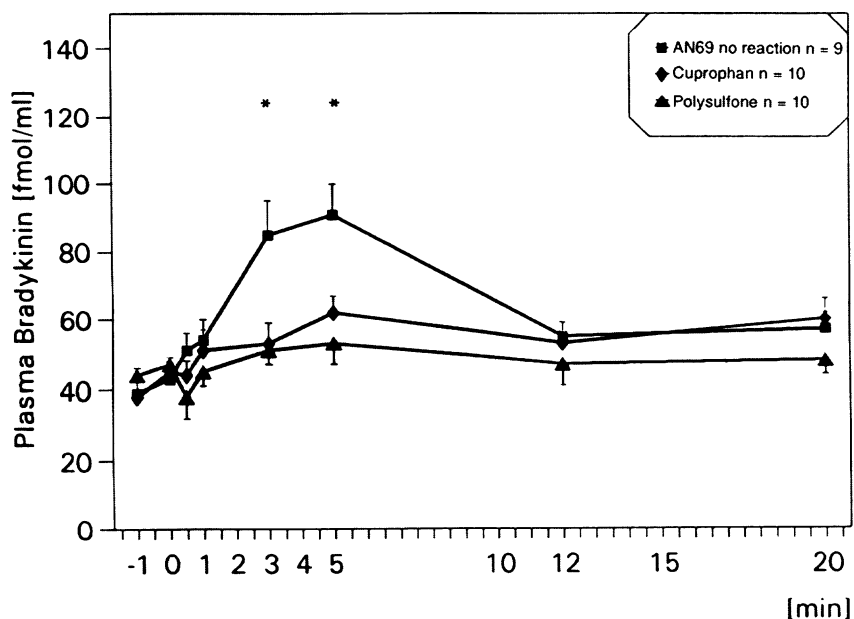
Fig. 2. Plasma bradykinin concentrations in the venous effluent of various dialyzers during the first 20 min of treatment. The AN69 curve does not include data from the symptomatic patient. Results are given as mean values ± SEM. $p < 0.05$ for AN69 versus other membranes.

general pathomechanism. However, besides the in vitro data reported by Schulman et al. [15] which fit well to our observations, there are preliminary data from experimental dialysis procedures with AN69 dialyzers in sheep that further strengthen the bradykinin hypothesis [18]. Both animals with (n = 8) and without (n = 8) captopril displayed significant bradykinin generation within the AN69 dialyzer which was more pronounced in sheep receiving ACE inhibitors. Anaphylactoid reactions were only observed in the group of captopril-treated animals dialyzed with AN69 membranes.

In our clinical study, bradykinin generation was strictly membrane specific, occurring only during dialysis with AN69, but not with cuprophane or polysulfone membranes. This pattern also has been observed in vitro with normal and uremic blood where significant activation of factor XII and kallikrein and subsequent bradykinin generation occurred only in the presence of AN69 membranes [15]. In addition, in experimental dialysis in sheep, significant bradykinin concentrations were only found in the venous effluent of AN69 dialyzers [18].

Finally, the present trial also demonstrates that bradykinin generation and anaphylactoid reactions do occur during AN69 dialysis even in the absence of ACE inhibitors. As can be seen from figure 1, there was a number of patients who displayed moderate bradykinin generation during dialysis with AN69 membranes without suffering

from anaphylaxis. It is well conceivable that these individuals would have become clinically symptomatic when their kininase II would have been blocked by ACE inhibitors. This is in agreement with the clinical results reported by Parnes and Shapiro [6] who observed AN69-related adverse reactions without concomitant ACE inhibition.

Taken together, this report, even though being based on a limited number of patients, provides good evidence for the notion that anaphylactoid reactions during dialysis with AN69 membranes are due to excessive bradykinin generation which even may occur in the absence of ACE inhibitors.

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References

1 Thielemans C, Madhoun P, Lenaers M, Schandene L, Goldman M, Vanderweghem JL: Anaphylactoid reactions during hemodialysis on AN69 membranes in patients receiving ACE inhibitors. Kidney Int 1990;38:982–984.

2 Verresen L, Waer M, Vanrenterghem Y, Michielsen P: Angiotensin-converting enzyme inhibitors and anaphylactoid reactions to high-flux membrane dialysis. Lancet 1990;336:1360–1362.

3 Jadoul M, Struyven J, Stragier A, van Ypersele de Strihou C: Angiotensin-converting enzyme inhibitors and anaphylactoid reactions to high-flux membrane dialysis. Lancet 1991;337:112.

4 van Es A, Henry FC, Lobatto S: Angiotensin-converting enzyme inhibitors and anaphylactoid reactions to high-flux membrane dialysis. Lancet 1991;337:112–113.

5 Alverez-Lara MA, Martin-Malo A, Espinoza M, Castillo D, Aljama P: ACE inhibitors and anaphylactoid reactions to high-flux membrane dialysis. Lancet 1991;337:370.

6 Parnes EL, Shapiro WB: Anaphylactoid reactions in hemodialysis patients treated with the AN69 dialyzer. Kidney Int 1991;40:1148–1152.

7 Ing TS, Daugirdas JT, Popli S, Ganghi VC: First-use syndrome with cuprammonium cellulose hemodialyzers. Int J Artif Organs 1984;6:235–239.

8 Villarroel F, Clarkowski AA: A survey on hypersensitivity reactions in hemodialysis. Artif Organs 1985;9:231–238.

9 Lemke HD, Heidland A, Schaefer RM: Hypersensitivity reactions during haemodialysis: Role of complement fragments and ethylene oxide antibodies. Nephrol Dial Transplant 1990;5:264–269.

10 Verresen L, Waer M,Vantenterghem Y, Michielsen P: Anaphylactoid reactions, haemodialysis, and ACE inhibitors. Lancet 1991;337:1294.

11 Margoles J: The interrelationship of coagulation of plasma and release of peptides. Ann NY Acad Sci 1963;104:133–145.

12 Griffin JH, Cochrane CG: Mechanism for involvement of HMW kininogen in surface dependent reactions of Hageman factor. Proc Natl Acad Sci USA 1976;73:2554–2558.

13 Zusman RM: Renin- and non-renin-mediated antihypertensive actions of converting enzyme inhibitors. Kidney Int 1984;25:969–983.

14 Lemke HD, Fink E: Generation of bradykinin in human plasma using AN69 and PAN-17DX membranes in the presence of an ACE inhibitor in vitro. XXIXth Congress EDTA Abstr., Paris, 1992, p 163.

15 Schulman G, Hakim R, Arias R, Silverberg M, Kaplan AP, Arbeit L: Bradykinin generation by dialysis membranes: Possible role in anaphylactic reaction. JASN 1993;3:1563–1569.

16 Shimamoto K, Ando T, Nakao T, Tanaka S, Sukuma M, Miyahara M: A sensitive radioimmunoassay method for urinary kinins in man. J Lab Clin Med 1978;91:721–728.

17 Shimamoto K, Ando T, Tanaka S, Nakahashi Y, Nishitani T, Hosoda S, Ishida H, Limura O: An improved method for the determination of human blood kinin levels by sensitive kinin radioimmunoassay. Endocrinol Jpn 1982;29:487–489.

18 Krieter DH, Lemke HD, Fink E, Bönner G, Nebendahl K, You HM, Eisenhauer T, Uhlenbusch KJ: Hemodialysis-related anaphylactoid reactions in sheep are associated with AN69 and not a new SPAN membrane (abstract). XIIth Int Congr of Nephrology, Jerusalem 1993.