Kidney International, Vol. 45 (1994), pp. 1497–1503

Bradykinin is a mediator of anaphylactoid reactions during hemodialysis with AN69 membranes

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Bradykinin is a mediator of anaphylactoid reactions during hemodialysis with AN69 membranes. Anaphylactoid reactions (AR) are the most feared complications of hemodialysis. Recently, a high incidence of AR has been reported during dialysis with AN69 membranes in patients treated with ACE inhibitors. Plasma levels of C3a, histamine and bradykinin were measured in 12 patients at the onset of AR during dialysis with AN69. We also investigated bradykinin generation in 10 symptom-free patients dialyzed with four different membranes. None of the 12 patients studied during AR displayed excessive complement activation or histamine release. In contrast, high bradykinin plasma levels (2392 ± 53 fmol/ml; mean ± SEM) were observed in all nine patients of whom bradykinin was measured. One patient developed two consecutive episodes of hypersensitivity on AN69 membranes even without taking ACE inhibitors. Bradykinin levels were high in both episodes (5280 and 10467.7 fmol/ml). Furthermore, this patient showed no symptoms and normal bradykinin levels (123.4 fmol/ml) when dialyzed with other membranes. The role of the membrane type in the AR is further substantiated by the observation that AN69 also provoked a significantly higher bradykinin generation (327.6 ± 18 fmol/ml; mean ± SEM) during symptom-free sessions compared to other membranes like Cuprophan^R (5.1 ± 7.3), Hemophan^R (17.2 ± 6.3) and Polysulfone^R (39.7 ± 6.6). Our findings strongly suggest that bradykinin is the principal mediator of AR during hemodialysis with AN69 membranes. To our knowledge it is the first time that data support the hypothesis of a more general role of bradykinin in shock-like symptoms. Furthermore, bradykinin generation must be regarded as a new marker of biocompatibility of extracorporeal treatments.

Exposure of blood to the extracorporeal circuit during hemodialysis may initiate a number of adverse reactions of which the life-threatening anaphylactoid reaction is the most feared one. Although the etiology of these reactions remains controversial, it is generally accepted that in many cases ethylene oxide hypersensitivity may have induced these anaphylactoid reactions [1]. Other etiological factors claimed to contribute to these reactions are complement activation [2] and passage of bacterial products across high permeable dialysis membranes [3–7].

Recently, a new mechanism of severe anaphylactoid reactions has been proposed to occur during hemodialysis with the synthetic AN69 polyacrylonitrile membrane [6–10]. Most of the reactions were observed in patients treated with angiotensin converting enzyme (ACE) inhibitors, though a few episodes have also been reported independent of ACE inhibition [9]. The pathogenesis of these anaphylactoid reactions is not elucidated, but the seeming involvement of ACE inhibitors in the vast majority of cases suggests a role for bradykinin. Bradykinin is generated via contact activation and thus, contact of blood with negative charges [11, 12] on the surface of AN69 membrane could cause release of bradykinin. Normally, the released bradykinin is effectively degraded by the action of angiotensin converting enzyme (which is identical to kininase II), but in the presence of an ACE inhibitor the degradation is inhibited and bradykinin can accumulate [13].

The purpose of the present study was to investigate whether the "bradykinin hypothesis" might be correct, first by evaluating plasma levels of C3a, histamine and bradykinin in patients who had shown severe clinical reactions during hemodialysis with AN69 membranes and then by additional, more indirect experiments.

Methods

Clinical studies

Anaphylactoid reactions were defined as a symptomatic blood pressure drop (nausea and faintness), flushing, swelling of tongue and/or throat, metallic taste, and paresthesiae in the extremities. Severe hypotension was defined as a systolic blood pressure drop of 50 mm Hg or more.

Study 1. From January 31, 1990 to April 30, 1990, 86 patients who required chronic hemodialysis were treated with an AN69 polyacrylonitrile parallel-plate dialyzer and with a dialysis solution containing acetate. The dialyzers were reprocessed with 0.45% (62.5 mmol/liter) sodium hypochlorite and reused five times. The dialyzers were reprocessed on a non-commercial microprocessor controlled unit which cleans five dialyzers at a time. It first rinses the blood compartment with reverse osmosis water at 2 liter/min for 10 minutes and the dialysate compartment by ultrafiltration, then flushes 0.45% sodium hypochlorite through both compartments at 300 ml/min for five minutes. After cleaning they were filled with 0.45% sodium hypochlorite and stored. Before reuse, the dialyzers were rinsed with a saline solution until negative (<1 ppm) for residual hypochlorite by a solution containing potassium iodate (20 g starch, 12.5 g potassium iodate, 1 g benzylalcohol, aqua ad 1 liter).

During this period 12 patients were treated with ACE inhibitors. All of them developed one episode of anaphylactoid

Received for publication August 9, 1993
and in revised form December 20, 1993
Accepted for publication December 20, 1993

reaction at the onset of hemodialysis on AN69. These 12 episodes of anaphylactoid reactions occurred during 28 hemodialysis sessions on AN69 in patients receiving ACE inhibitors. Thus, not during every "at risk" dialysis session was an anaphylactoid reaction observed. The anaphylactoid reactions resolved after discontinuation of ACE inhibitor therapy.

During the reactions blood samples were drawn into EDTA from the arterial (before the dialyzer) and venous (after the dialyzer) line. C3a and histamine were determined in these samples as described below and compared with samples drawn from 12 control patients without reactions but dialyzed on AN69 and not taking ACE inhibitors five minutes after start of dialysis (arterial line only).

Of only nine of the 12 patients showing reactions plasma from the samples drawn from the arterial line was available to measure bradykinin later on (June 1992). These samples were compared with the arterial samples of nine control patients without reactions but dialyzed on AN69 and not taking ACE inhibitors. Thus, these control samples were also stored for two years.

From ten of the 12 patients with anaphylactoid reactions the dialysate was analyzed for endotoxin content and five of them were also analyzed for the microbial count.

In all patients IgE antibodies against ethylene oxide were determined.

However, since we learned later (June 1992) that the reliable determination of bradykinin is normally done using a special inhibitor cocktail to block proteolytic as well as kininase activity (see below) we performed a control study. In this study samples (arterial line only) from nine patients on AN69 without reactions and not taking ACE inhibitors were drawn in parallel into EDTA and into inhibitor cocktail.

Study 2. In this study we examined the possible involvement of the dialysis membrane as a cause in the anaphylactoid reactions. For ethical reasons it was impossible to study the membrane effects with patients taking ACE-inhibitors. Since, however, according to the bradykinin hypothesis (see introduction) the ACE inhibitors are not directly involved in bradykinin generation but rather in bradykinin breakdown, we hypothesized that membrane differences must be detectable also in patients not taking ACE inhibitors. We therefore studied 10 asymptomatic patients in a prospective cross over trial using four different dialysis membranes (first use) in a single dialysis treatment. We used AN69 (Biospal 3000S; Hospal), Cuprophan (GFE 18; Gambro), Hemophan (GFS Plus 16; Gambro) and high flux polysulfone (F60; Fresenius). Five minutes after the tart of blood circulation and connection of the venous line to ıe patient, blood samples were drawn before and after the dialyzer for measurement of the bradykinin plasma levels.

During the course of this study a 26-year-old woman (not treated with ACE inhibitors) developed two episodes of anaphylactoid reaction three to five minutes after the start of hemodialysis only with AN69 but not with Cuprophan and Polysulfone. Ethylene oxide hypersensitivity was excluded by RAST. Blood samples were collected five minutes after the start of hemodialysis with AN69 (Biospal 3000S; Hospal), Cuprophan (GFE 18; Gambro) and Polysulfone (F60; Fresenius).

All subjects gave informed consent.

Laboratory methods

Plasma bradykinin assay. Plasma sampling and processing [14]. In study 1 blood was drawn into a syringe containing EDTA (1.5 mg/ml K_2EDTA) and centrifuged within 10 minutes at 4°C. The plasma was stored at −70°C till analysis. After thawing of plasma inhibitor cocktail was added (1:9 vol/vol). Two hundred microliters of this mixture were added to 800 μl ice cold ethanol and allowed to stand for 15 minutes at 4°C. Then the mixture was boiled for 15 minutes to 70°C. After centrifugation the supernatant was evaporated to dryness under a stream of air.

In study 2, 900 μl of blood were collected into ice-cooled polypropylene tubes containing 100 μl inhibitor cocktail, mixed gently, cooled at 4°C and centrifuged within 15 minutes at 4°C. The inhibitor cocktail contained 10,000 KIU/ml aprotinin, 800 μg/ml soybean trypsin inhibitor, 4 mg/ml polybrene, 10 mg/ml 1,10 phenantroline and 20 mg/ml EDTA. Two hundred microliters plasma were added to 800 μl ice cold ethanol and allowed to stand for 15 minutes at 4°C. After centrifugation the supernatant was separated and evaporated under a stream of air. The dried residue was redissolved in 600 μl 66% acetone/water and washed with 1.4 ml petrol ether. After centrifugation at room temperature the upper layer was aspirated and discarded. The lower layer was evaporated under a stream of air.

Determination of kinins by radioimmunoassay. Buffer A. 0.01 M NaH_2PO_4, 0.14 M NaCl, 0.03 M EDTA, 3 mM 1,10-phenantroline, 0.5 g/liter NaN_3, 0.2 g/liter thimerosal, 0.4 ml/liter of Tween 20, pH 7.4.

Buffer B. Same as buffer A but containing polyethyleneglycol 6000, 60 g/liter instead of Tween 20.

Buffer C. Same as buffer A but containing ovalbumin, 10 g/liter instead of Tween 20.

Radioiodination. Tyrosyl-bradykinin was labeled with iodine-125 in a similar way as described by Shimamoto et al [15], diluted in buffer A to a concentration of 500,000 counts/min/ml and 70 mg/liter of rabbit immunoglobulin G were added.

Radioimmunoassay procedure. Standard dilutions of bradykinin (15,6 to 8000 pmol/liter) were prepared with buffer A. Antibody to kinin was provided by Dr. K. Shimamoto (Sapporo, Japan). The lyophilized antiserum was reconstituted in buffer C and diluted in buffer A to give a binding of the labeled antigen of 30 to 40% under the radioimmunoassay conditions. The dried samples were dissolved in 0.4 ml buffer A. A 50 μl sample (standard or unknown), 50 μl buffer A, 50 μl of a freshly prepared solution of the ^{125}I-tyrosyl-bradykinin and 50 μl of the antiserum dilution were mixed in 600 μl RIA tubes (Sarstedt, D-5223 Nümbrecht, Cat. No. 73.1055) and incubated for 16 to 24 hours at 4 to 10°C. Then 50 μl of the second antibody (diluted 1:32 according to the manufacturer recommendation) and 300 μl buffer B were added. The samples were centrifuged at 4600 g for 20 minutes at 9°C. The supernatant was aspirated, 500 μl buffer B were added and after centrifugation and aspiration of the supernatant the radioactivity of the precipitates was measured in a gamma counter. The assays were evaluated by the spline approximation method using the commercially available computer program RIALOG II (Zinsser Analytic, Frankfurt Germany).

Reproducibility of the RIA. The reproducibility of the assay is determined routinely at concentration levels of 15, 180, and 750

Table 1. Data of patients with anaphylactoid reactions during hemodialysis with AN69 membranes under ACE inhibition

Patient	Treatment	Dialyzer use no.	C3a[a] *ng/ml* A	C3a[a] *ng/ml* V	Histamine[a] nM A	Histamine[a] nM V	Endotoxin *EU/ml*	CFU/ml
1	Enalapril 10 mg/day	2	444.4	886.7	1.9	3.47	0.44	20
2	Enalapril 20 mg/day	4	154.4	166.1	1.9	1.8	1.19	2500
3	Enalapril 2.5 mg/day	4	262.7	NA	0.23	NA	0.28	21
4	Enalapril 20 mg/day	1	199.7	NA	2.57	NA	0.31	10
5	Captopril 75 mg/day	1	258.3	250.6	1.61	4.91	0.03	4
6	Captopril 75 mg/day	2	1140.5	517.5	6.0	0.37	1.13	NA
7	Lisinopril 20 mg/day	2	472.2	481.7	5.78	1.6	0.33	NA
8	Enalapril 20 mg/day	2	296.3	229	2.55	0.48	1.6	NA
9	Enalapril 20 mg/day	1	805.9	359.8	1.79	1.9	0.09	NA
10	Enalapril 10 mg/day	2	668.7	502.1	5.91	1.34	0.14	NA
11	Enalapril 10 mg/day	1	340.3	392.9	1.42	1.88	NA	NA
12	Enalapril 10 mg/day	6	869.4	617.5	0.07	0.68	NA	NA
Median			392.4	437.3	1.9	1.7	0.31	
Mean			493.5	440.4	2.64	1.84	0.55	
± SEM			17.3	14.2	1.42	1.15	0.74	

Abbreviations are: A, arterial line; V, venous line; NA, not available.
[a] NS between arterial and venous values

fmol/ml; the coefficients of variation at these levels are 18.7, 14.3 and 16.6%, respectively.

Plasma histamine assay

Five milliliters of blood were collected into an ice cooled tube (Monoject) containing 7.2 mg K_2EDTA and centrifuged within 10 minutes at 2000 g at 4°C; the plasma was stored at −70°C until assayed. After thawing, the plasma samples were assayed with a commercial radioimmunoassay for measurement of histamine (Immunotech, AMAC Inc.)

Plasma C3a assay

Five milliliters of blood were collected into an ice cooled tube (Monoject) containing 7.2 mg K_2EDTA and centrifuged within 10 minutes at 2000 g at 4°C; the plasma was stored at −70°C until assayed. After thawing, the plasma samples were assayed with a commercial enzyme immuno assay for measurement of C3a (Progen).

Analysis for IgE antibodies against ethylene oxide

IgE antibodies against ethylene oxide were measured by a radio-allergo-sorbent-test (Pharmacia CAP System RAST RIA).

Endotoxin measurement

Ten milliliters dialysate were sampled in endotoxin-free glass tubes. This glassware was rendered endotoxin-free by heating for four hours at 180°C. Prior to analysis the samples were stored at −70°C. The Coatest Endotoxin method (KabiVitrum Diagnostica) was used for the quantitative, photometric determination of gram-negative bacterial endotoxins.

Quantitative bacteriology of dialysis solution

One hundred milliliters of dialysis solution were collected into a sterile container and treated by membrane filtration technique (Millipore 0.45 μ pore size). After filtration the membrane filter was inoculated on Tryptone soy agar (Lab M). After incubation at 37°C for 48 hours the number of colony forming units (CFU) was determined.

Statistical analysis

Data were analyzed by means of the Student's *t*-test and subsequent Tukey's Studentized range test (analysis of variance). As non-parametric test the Wilcoxon rank sum test was used. Differences were considered significant when a $P < 0.05$ was obtained.

Results

Study 1

In our first study we documented episodes of anaphylactoid reaction in 12 patients within few minutes after the start of dialysis (Table 1). All these patients were treated with ACE inhibitors. Reactions were observed with first-used (patients 4, 5, 9 and 11) and re-used (patients 1, 2, 3, 6, 7, 8, 10 and 12) AN69 dialyzers.

There was no statistically significant difference of the C3a plasma level in the arterial line between the patients with

Table 2. Symptoms and bradykinin plasma levels (arterial line; in fmol/ml) at the onset of anaphylactoid reactions during concomitant exposure to AN69 and ACE inhibition

Patient	Symptoms	Bradykinin plasma level *fmol/ml*
1	flushing, swelling of tongue and throat, nausea and paresthesiae	596
2	hypotension, flushing and abdominal pain	NA
3	hypotension, flushing	8212
4	hypotension, flushing	NA
5	hypotension, flushing, paresthesiae	NA
6	flushing, metallic taste	880
7	flushing, nausea	660
8	hypotension, dyspnea, paresthesiae and vulvodynia	6260
9	hypotension, flushing	1448
10	hypotension, flushing, swelling of throat, nausea and abdominal pain	1968
11	flushing, nausea and abdominal pain	608
12	hypotension, flushing, dyspnea and nausea	892
Median		1448
Mean		2392
± SEM		53.3

Abbreviation is NA, not available.

Table 3. Effect of the use of an inhibitor cocktail on bradykinin plasma levels (arterial line; in fmol/ml) 5 minutes after the start of hemodialysis on AN69 in patients without ACE inhibition

	1990	1992	
	IC−	IC−	IC+
	198	568	0
	543	324	92
	116	44	44
	174	68	52
	580	64	32
	278	144	44
	856	280	72
	160	92	0
	248	664	208
Median	248	144	44
Mean	350	250	62.7
± SEM	15.9	15.2	7.9

Abbreviations are: IC−, blood drawn without inhibitor cocktail; IC+, blood drawn with inhibitor cocktail.

NS between IC− of 1990 and 1992.

$P < 0.05$ between IC− and IC+.

$P < 0.01$ between IC− of 1990 and bradykinin plasma levels of patients showing anaphylactoid reactions (Table 2).

reactions (493.4 ± 17.3 ng/ml, Table 1) and control group 1 (458.9 ± 16.9 ng/ml, individual data not shown). No significant differences were observed between the C3a plasma levels from the arterial and venous lines of both the cases and the controls.

There was no statistically significant difference of the histamine plasma level in the arterial line between the patients with reactions (2.64 ± 1.42 nM) and control group 1 (1.54 ± 0.89 nM). No significant difference has been observed between the histamine plasma levels from the arterial and venous lines of both the cases and the controls.

In ten episodes of anaphylactoid reactions, endotoxin content was measured in the dialysate. The values ranged from 0.03 to 1.6 EU/ml (3 to 160 pg/ml). During five episodes dialysate was sampled for the counting of colony-forming units (CFU). Only once a high number (2,500 CFU/ml) was found but at the occasion of the other reactions much smaller numbers were measured (<21 CFU/ml).

Bradykinin plasma levels in the arterial line of the patients during reactions (2392 ± 53.3 fmol/ml; mean ± SEM; Table 2) were found significantly higher compared to the controls of 1990 (350.3 ± 15.9; Table 3). Since during the reactions we were not aware of the correct sampling technique for bradykinin plasma our data were validated later [1992] by comparing our technique with an established one (**Methods** section, compare Table 3). In this control study the use of an inhibitor cocktail (IC+) resulted in about a fourfold lower bradykinin plasma level (62.7 ± 7.9 fmol/ml) compared to sampling without inhibitor cocktail (250 ± 15.1; IC−). We conclude that the absolute values of bradykinin plasma levels measured in blood samples obtained during the reactions were likely to be too high but the differences between case sessions and non-case sessions were valid and indicative of bradykinin generation during the anaphylactoid reactions, since the differences caused by inappropriate sampling were much lower. The results of the 1990 control group also demonstrate that the storage for two years and subsequent sample treatment cannot be responsible for the high bradykinin levels in the plasma of patients with anaphylactoid reactions as the comparison with the 1992 IC− control group shows (Table 3). Interestingly, there is some correlation between the intensity of elevation of bradykinin plasma levels and the degree of symptomatology: patients without severe hypotension (patients 1, 6, 7, 11) had lower bradykinin plasma levels than patients presenting with severe hypotension (Table 2).

None of the patients showed IgE antibodies against ethyleneoxide.

Study 2

In a second study bradykinin plasma levels of 10 chronic hemodialysis patients not taking ACE inhibitors were measured in a cross over design before and five minutes after the start of dialysis following the first-use of four different dialysis membranes: AN69 (Biospal 3000S; Hospal), Cuprophan (GFE 18; Gambro), Hemophan (GFS Plus 16; Gambro) and high flux polysulfone (F60; Fresenius) (Table 4). Bradykinin plasma levels in hemodialysis patients before dialysis ranged from 0 to 212 fmol/ml (26.5 ± 28, mean ± SEM; data not shown). With the use of polysulfone and AN69 an increase of the plasma bradykinin level in the arterial line was observed five minutes after the start of dialysis (91.5 ± 12.1 and 61.2 ± 7.5). Only with the AN69 membrane was a significant bradykinin generation across the dialyzer noted.

During our study, a 26-year-old woman who was not treated with ACE inhibitors developed two episodes of anaphylactoid reactions during two sessions on first-use AN69 membranes (Biospal 3000S; Hospal). Bradykinin plasma levels were measured and compared to sessions remaining uneventful on Cuprophan (GFE 18; Gambro) and high-flux polysulfone membranes (F60; Fresenius). The use of AN69, but not the other two membranes, resulted in extremely high bradykinin plasma levels both in the arterial and venous lines (Table 5).

Table 4. Bradykinin plasma levels (fmol/ml) 5 minutes after the start of hemodialysis: Comparison between several membranes

Patient No.	Cuprophan		Hemophan		Polysulfone		AN69	
	A	V	A	V	A	V	A	V
1	168	0	0	0	175	47.3	0	136
2	0	0	164	128	0	41.1	92	444
3	0	0	0	0	0	0	44	144
4	0	0	28	44	0	0	52	0
5	0	0	0	0	0	125.6	32	1212
6	0	0	56	0	24.4	18.6	44	328
7	0	0	0	0	0	0	72	340
8	0	0	44	0	211	64.2	0	56
9	0	0	0	0	469.7	100.6	68	252
10	0	46	0	0	34.7	0	208	364
Median	0	0	0	0	12.2	29.8	48	290
Mean	18.7	5.1	29.2	17.2	91.5	39.7	61.2	327.6
± SEM	± 7.3	± 7.3	± 7	± 6.3	± 12.1	± 6.6	± 7.5	± 18

Abbreviations are: A, arterial line; V, venous line.
$P < 0.05$ for arterial/venous differences between AN69 and all other membranes.

Table 5. Bradykinin plasma levels (fmol/ml) at the onset of anaphylactoid reactions during hemodialysis with AN69

	0 min	5 min A	5 min V
AN69 (1st episode)	212	2112	5280
AN69 (2nd episode)	76.1	2106.6	10467.7
Cuprophan (one uneventful treatment)	0	36	0
Polysulfone (one uneventful treatment)	28.7	0	123.4

Abbreviations are: A, arterial line; V, venous line.

Discussion

Our patients with anaphylactoid reactions neither displayed a IgE-mediated sensitization against ethylene-oxide, nor excessive release of histamine or complement fragments during the reactions. Therefore, these mechanisms are obviously not involved in our cases. In contrast, the bradykinin plasma levels of the patients were significantly elevated (Table 2). Though the sample collection and sample treatment were different from generally recommended procedures, we were able to exclude that the bradykinin plasma levels were simply due to inappropriate sample handling but reflected, indeed, bradykinin generation during hemodialysis. Interestingly, some correlation could be found between the intensity of elevation of bradykinin plasma levels and the severity of anaphylactoid reaction; patients showing severe hypotension had higher bradykinin plasma levels than patients who did not.

Two effects seem to be responsible for the bradykinin accumulation observed: an increased release of bradykinin caused by the AN69 membrane and a reduced breakdown of bradykinin due to the inhibition of the ACE (=kininase II) by ACE inhibitors. This hypothesis is supported by *in vitro* data indicating that the accumulation of bradykinin formed via blood contact with the AN69 membrane is strictly ACE inhibitor dose-dependent [16].

Very high plasma levels of bradykinin at the onset of the anaphylactoid reaction during hemodialysis with AN69 membranes were observed in a 26-year-old patient who was not treated with ACE inhibitors (Table 5). She remained symptom-free and showed normal plasma levels of bradykinin during hemodialysis with Cuprophan and high flux polysulfone. Importantly, in this setting a methodological flaw in the collection of samples was excluded as blood was drawn into an inhibitor cocktail. Therefore, these data strongly suggest that bradykinin is the principal mediator of anaphylactoid reactions during hemodialysis with AN69 membranes. On the other hand, this case demonstrates that special properties of the AN69 membrane must be responsible for the bradykinin release. However, the development of an anaphylactoid reaction must in this case also be due to an as yet unrecognized inability of the patient to degrade bradykinin efficiently.

To further investigate the contributory role of AN69 in the generation of bradykinin, we compared four different dialysis membranes. Only during dialysis with AN69 membranes did we observe a significant generation of bradykinin within the dialyzer. The cause of bradykinin generation by AN69 membrane can be understood by taking into account that the membrane surface carries negatively charged groups [12]. These negative groups may activate the Hageman factor which is known to convert prekallikrein to kallikrein [12]. Kallikrein will release bradykinin from high molecular weight kininogen. Despite a significant generation of bradykinin within the AN69 dialyzer, the bradykinin plasma levels in the arterial line remained rather low. This can be explained by the pulmonary clearance of bradykinin mediated by angiotensin converting enzyme activity and which might be estimated to be 95 to 97% of the released amount within the AN69 dialyzer [17].

Our *in vivo* data are supported by *in vitro* experiments [16, 18]. Incubation of blood with AN69 membrane fragments resulted in severalfold higher bradykinin levels than when incubated with Cuprophan membrane fragments [16, 18]. Surprisingly, incubation longer than five minutes resulted in higher bradykinin levels with Cuprophan, whereas with AN69 the generated bradykinin disappeared [18]. With additional experiments these findings could be explained by adsorption of bradykinin to AN69 [18]. On the other hand, persisting generation of bradykinin is not expected during clinical dialysis with Cuprophan as the early formation of a protein layer on the membrane surface will block further activation of the Hageman factor and therefore also the subsequent release of bradykinin.

The association of anaphylactoid reactions with hypochlorite

(reprocessed but not with formaldehyde) reprocessed AN69 dialyzers [19] is in agreement with the hypothesis of surface activation of the kallikrein-kinin pathway. Hypochlorite, a strong oxidizing agent, removes the protein coat from AN69 and is therefore likely to activate the Hageman factor. In contrast, the use of formaldehyde fixes the protein layer on the surface and reduces the chance for further activation of the Hageman factor. A recent report has associated anaphylactoid reactions with reuse of hollow fiber hemodialyzers and ACE inhibitors [20]. However, the relative contribution of the membrane, the reuse procedure, or the reuse agent was not well defined. On the other hand, our findings did not suggest any association between the number of dialyzer uses and the risk of developing an anaphylactoid reaction [6] (Table 1).

Practical experience shows that, generally, the amount of bradykinin released within the dialyzer will not be enough to exceed the threshold level necessary to trigger clinical reactions. However, in particular cases, like the one reported above or when bradykinin degradation is blocked by ACE inhibitors, bradykinin may increase above a critical level to provoke anaphylactoid reactions. Bönner et al, who have investigated the vasodepressor effect of bradykinin in normotensive and hypertensive subjects by injection of bradykinin intravenously and intra-arterially, found a correlation between the increase in arterial plasma levels of bradykinin and the corresponding fall in systolic blood pressure [17]. Arterial bradykinin plasma levels must increase approximately 250 fmol/ml in order to achieve a drop in blood pressure of 50 mm Hg [17].

Although there is increasing evidence of the central pathogenetic role of the AN69 membrane in the induction of the reported new type of anaphylactoid reaction, the unpredictability of the occurrence of these reactions remains intriguing. Despite combination of hemodialysis with AN69 and ACE inhibition some patients do not develop anaphylactoid reactions while others do so only episodically [6]. It is possible that this is related to the dose and timing of the intake of the ACE inhibitors in relationship to the start of the dialysis session. The *in vitro* data provided by Lemke and Fink might support this hypothesis [16]. The rinsing procedure of the dialyzer and/or the anticoagulation mode might be invol ved with the contributory role of some dialysate factors: microbial contamination, chemical composition and/or temperature. A central pathogenetic role of the microbial contamination of the dialysate in the induction of anaphylactoid reactions during dialysis with AN69 membranes has been suggested [6,7]. However, anaphylactoid reactions when using AN69 membranes have been described during isolated ultrafiltration [19] and during hemofiltration [10], and hemodiafiltration using sterile pyrogen-free fluids [21]. Secondly, our finding of a very low endotoxin content and microbial count in the dialysis solution at the onset of several episodes of anaphylactoid reactions equally argues against a central pathogenetic role of a poor bacteriological quality of the dialysis solution. However, these findings do not exclude that in particular settings massive transfer of negatively-charged bacterial substances (such as endotoxins) from the dialysate to the AN69 membranes might contribute to increased bradykinin generation. It has been demonstrated *in vitro* that endotoxin may activate the kallikrein-kinin pathway [22]. A recent report documented the preventive role of alcalin rinsing of the AN69 dialyzers [23], suggesting that the negative charge of the membrane may have been masked by adsorption of electrolytes on the membrane resulting in a decreased activation of the kallikrein-kinin pathway.

In addition to these variable exogenous factors, interindividual differences in bradykinin generating and degrading capacities might play a role. Variable tissue and circulating bioactivity of kallikrein inhibitors (C1 inhibitor, antithrombin III and α-2-macroglobulin) may lead to differences in bradykinin generation. For instance, patients with hereditary angioedema lacking C1 inhibitor have been shown to have an activated kallikrein-kinin pathway during attacks of mucocutaneous swellings [24]. On the other hand, interindividual differences in degradation of bradykinin may exist. In addition to angiotensin converting enzyme (kininase II), carboxypeptidase N (kininase I) is an important enzyme for bradykinin degradation. Rare genetic deficiencies in carboxypeptidase N have been reported, and it is interesting to speculate that patients who are heterozygous for carboxypeptidase N deficiency might be at greater risk [25]. This hypothesis has also been put forward to explain why only few non-dialysis patients (0.1%) treated with ACE inhibitors develop angioedema [26]. On the other hand, patient susceptibility might be related to the pathophysiological mechanism of vasodilation of bradykinin. Vasodilation is an indirect effect of bradykinin, exerted by nitric oxide generated from L-arginine by nitric oxide synthase [27]. The release of nitric oxide might be decreased in the presence of hypercholesterolemia [28] and severe atherosclerosis [27]. Interestingly, accumulation of asymmetric dimethylarginine, an endogeneous competitive inhibitor of nitric oxide synthase, has been found in the circulation of patients with chronic renal failure sufficient to inhibit nitric oxide synthesis [29]. It is also conceivable that some dialysis patients develop deficiency of L-arginine, which is a small molecule easily dialyzed and found in high concentrations in fish, poultry and beans, the intake of which by dialysis patients is restricted because of the high phosphorus content. Therefore, it is tempting to speculate on the role of the plasma levels of L-arginine and asymmetric dimethylarginine on the patients' susceptibility to develop anaphylactoid reactions.

In conclusion, our findings strongly suggest bradykinin to be the mediator of the anaphylactoid reactions in patients dialyzed with AN69 membranes. Of all dialysis membranes studied only AN69 membranes provoked a significant generation of bradykinin. Simultaneous treatment with ACE inhibitors highly enhance the risk of development of anaphylactoid reactions by reducing bradykinin breakdown. However, as we observed in one patient dialyzed with AN69 membranes, anaphylactoid reactions with high systemic bradykinin plasma levels can develop even without treatment with ACE inhibitors. Therefore, bradykinin generation must be regarded as a new marker of biocompatibility.

Acknowledgments

Part of this paper was presented in 1991 in abstract form at the 2nd Benelux Dialysis Symposium in Kampen, The Netherlands, and in 1993 at the 4th Benelux Dialysis Symposium in Amsterdam, The Netherlands, and at the XIIth International Congress of Nephrology in Jerusalem, Israel. We are indebted to Dr. Shimamoto for providing anti-bradykinin antibody to us and to Dr. Reuten for his advice regarding the statistical analysis. The authors thank Mr. Marc Dekens and Mrs Gudrun Godec for technical assistance.

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