

# Kinin generation by hemodialysis membranes as a possible cause of anaphylactoid reactions

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1. Severe anaphylactoid reactions have been reported in some patients treated with angiotensin converting enzyme (ACE) inhibitors during hemodialysis with a polyacrylonitrile (PAN) membrane. Generation of bradykinin via contact activation at the negatively charged membrane surface and reduced bradykinin breakdown due to ACE inhibition have been suggested as possible causes. This hypothesis was evaluated in the present study.

2. PAN or cellulose dialyzer membranes were incubated with plasma at different concentrations of ACE inhibitor. The rate and extent of kinin accumulation was dependent on the ACE inhibitor concentration.

3. Bradykinin levels were determined in "historical" plasma samples drawn from patients treated with ACE inhibitor at the onset of anaphylactoid reactions during hemodialysis with PAN dialyzers. The kinin levels were significantly higher ( $2.4 \pm 0.05$  pmol/ml) than in samples from a group of control patients ( $0.29 \pm 0.02$  pmol/ml).

4. Plasma kinin levels were measured in patients who developed anaphylactoid reactions during dialysis with a PAN membrane though not being treated with ACE inhibitor. At the onset of the reaction, kinin levels increased to 2.1 pmol/ml in the line entering the dialyzer and to 10.5 pmol/ml in the line leaving the dialyzer compared to not more than 0.12 pmol/ml upon dialysis with other membranes.

5. These *in vitro* and *in vivo* results demonstrate that contact of blood with a polyacrylonitrile membrane leads to the generation of kinins which accumulate if ACE is inhibited. It is very likely that kinin accumulation in the circulation is the cause of anaphylactoid reactions during hemodialysis with PAN membranes in patients treated with ACE inhibitors and, in some cases, in patients not receiving ACE inhibitor medication.

**Key words:** angiotensin converting enzyme (ACE) inhibitors, cellulose membranes, hemodialysis, kinins, polyacrylonitrile membranes.

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## Introduction

During hemodialysis there is extensive contact between blood and artificial membrane surfaces which in relatively rare cases evokes hypersensitivity reactions within the first few minutes of dialysis. Symptoms range from mild reactions with flushing, edema and dizziness to life-threatening reactions with severe bronchospasm, hypotension and cardiorespiratory arrest. Hypersensitivity to ethylene oxide (Grammer et al., 1984; Dolovich et al., 1984), complement activation (Hakim et al., 1984) and passage of microbial products across the membrane (Man et al., 1988) have been considered to be causes of anaphylactoid reactions.

Recently, a new mechanism of severe anaphylactoid reactions occurring during hemodialysis with a polyacrylonitrile (PAN) membrane has been proposed (Tielemans et al., 1990; Verresen et al., 1990). Most of the reactions occurred in patients treated with angiotensin converting enzyme (ACE) inhibitor. Data from several hemodialysis centers on this type of reaction were reviewed in a survey of 591 patients dialyzed with a PAN membrane; 72 of them were under treatment with ACE inhibitor, 41 of these developed anaphylactoid reactions (Parnes and Shapiro, 1991). In contrast, among the remaining 519 patients not receiving ACE inhibitor treatment, only two reactions occurred. In comparison, 496 patients dialyzed with other membranes, mainly of the cellulose type, presented no reactions, even the 71 patients treated with ACE inhibitor.

The association of ACE inhibitors with the PAN-related anaphylactoid reactions suggested the participation of bradykinin (Tielemans et al., 1990; Verresen et al., 1990) and consequently the "bradykinin hypothesis" was suggested: i) plasma kallikrein released via contact activation by negative charges on the polyacrylonitrile membrane surface liberates bradykinin from high molecular weight kininogen; ii) as kininase II (=ACE) is inhibited by the ACE inhibitor the kinin released can accumulate in the circulation and cause the observed anaphylactoid reactions. In order to evaluate this hypothesis we performed the *in vitro* and *in vivo* studies described here.

## Material and Methods

### *In vitro studies*

Blood from healthy donors was used within 30 min after venipuncture. For studies on bradykinin release, citrated (0.38% citrate) or heparinized (5 U/ml)

plasma was used. Experiments on C5a generation or histamine release were carried out with heparinized whole blood (5 U/ml). The ACE inhibitor used was captopril, and the endotoxin *Salmonella minnesota* (Re595, List, Campbell, CA, USA).

Flat 32-cm<sup>2</sup> membrane sheets of either cellulose (CEL), or polyacrylonitrile (PAN) obtained from commercially available dialyzers were incubated in Petri dishes with 8.0 ml citrated human plasma or heparinized whole blood at 37°C for up to 1 h under gentle shaking. Control experiments without membranes were performed in the same way. For kinin measurements, 0.2 ml plasma was removed at specific times, mixed with 1 ml ethanol and heated to 70°C for 10 min. After centrifugation the supernatant was removed and dried under vacuum. The kinin content of the residues was determined by radioimmunoassay (Fink et al., 1985).

des-Arg-C5a was determined by an enzyme immunoassay (Behring, Marburg, Germany) having a sensitivity of 0.2 ng/ml. Histamine was determined by a radioimmunoassay (Immunotech/Dianova, Hamburg, Germany), with a sensitivity of 0.5 nM.

### *Statistical analysis*

Each experiment was done three times, each time with blood from a different donor. All samples were measured in triplicate. The data sets of nine values per time point were evaluated by the Duncan multiple range test using the SAS software package. Mean values were considered significantly different when  $P < 0.01$ .

### *In vivo studies*

*Study 1.* During the first few months of 1990 several patients of the dialysis center in Leuven who were under treatment with ACE inhibitor developed anaphylactoid reactions at the onset of dialysis with PAN dialyzers. During the anaphylactoid reactions blood samples were drawn into syringes containing potassium EDTA (1.5 mg/ml) from the lines entering the dialyzer and centrifuged within 10 min. Control blood samples were collected in the same way from patients without reactions on the PAN membrane and without ACE inhibitor treatment 5 min after the start of dialysis. All plasma samples were stored at -70°C. Kinin measurements were carried out in 1992. After thawing, plasma was mixed with an inhibitor cocktail (Shimamoto et al., 1988) (9:1 v/v) and 200  $\mu$ l of this mixture was added to 800  $\mu$ l ethanol at 0°C, kept 15 min at 4°C, and then heated

for 15 min at 70°C. After centrifugation the supernatant was removed and dried under a stream of air.

In order to determine the effect of the sampling procedure on kinin generation, blood samples from nine patients dialyzed with PAN membranes without reactions and not taking ACE inhibitors were collected into an inhibitor cocktail and processed according to Shimamoto et al. (1988), and in parallel, additional samples were drawn into EDTA and processed as described above. The kinin content of all samples was measured by radioimmunoassay (Fink et al., 1985).

*Study 2.* A 26-year old woman not treated with ACE inhibitor developed two episodes of anaphylactoid reaction 3 to 5 min after starting hemodialysis with a PAN dialyzer, but not when a CEL or a high-flux polysulfone dialyzer was used. Blood samples were collected 5 min after the start of hemodialysis and processed for kinin determination (Shimamoto et al., 1988).

## Results and Discussion

### *In vitro studies*

Extending our previous studies (Lemke and Fink, 1992a,b), we undertook a series of *in vitro* experiments to investigate the possibility that the combination of the PAN dialyzers and captopril therapy provokes anaphylactoid reactions. We investigated the effect of a PAN membrane on the release of kinin upon incubation with plasma in the presence of ACE inhibitor as well as the possibility that microbial lipopolysaccharides (endotoxin) can contribute to kinin generation and that PAN/captopril might enhance complement activation or histamine release and, thus, eventually lead to adverse reactions.

When plasma was incubated with a PAN hemodialyzer membrane in the presence of captopril (0.1 µg/ml), bradykinin levels increased from 27 to 2926 fmol/ml after 15 min, whereas the values at 15 min for the CEL membrane and for the control without membrane were 275 and 33 fmol/ml, respectively (means, N = 3 donors, P<0.01). The maximal kinin concentration and kinin stability in the incubation mixture were dependent on captopril concentration (Figure 1). Endotoxin, when added to the incubation mixtures, had no effect on the rate or extent of kinin generation.

A further possible cause of anaphylactoid reactions might be complement system activation. In the *in vitro* system, however, PAN/captopril did not cause activation of the complement system. In contrast, CEL did cause activation of the complement system, but these membranes are not involved in ACE inhibi-

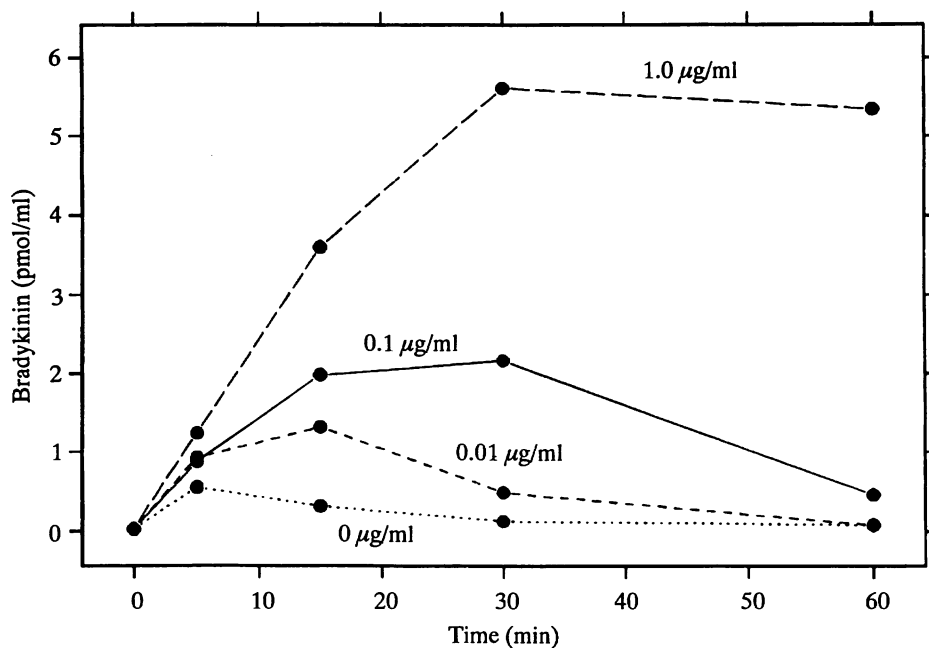


Figure 1 - The rate and extent of kinin accumulation in plasma during incubation with a PAN membrane is dependent on the concentration of captoril. Human plasma was incubated with a 32 cm<sup>2</sup> PAN membrane at 37°C in the presence of different concentrations of captoril. Aliquots of plasma were removed and processed for the measurement of kinin by radioimmunoassay.

tor-related patient reactions and have been used without complications for hemodialysis in patients who developed anaphylactoid reactions with the PAN membrane.

In order to determine whether histamine release from basophils might be involved in the PAN-related reactions we incubated heparinized whole blood in the presence of 0.1 µg/ml captoril. In the control and in the incubates with PAN or CEL membranes only a small histamine release of up to 5 pmol/ml was observed, whereas for a positive control, when anti-IgE was added the histamine concentration rose immediately to 80 pmol/ml.

Taken together, the results of our *in vitro* studies demonstrate that PAN causes a release of kinin, and in the presence of a sufficiently high concentration of ACE inhibitor kinin accumulates in the incubation medium because its inactivation is inhibited. Our studies indirectly exclude the possibility that other factors such as histamine or complement, considered to be causes of anaphylactoid reactions, play a part in provoking the PAN/ACE inhibitor-related reactions. The

results of these and our previous studies (Lemke and Fink, 1992a,b) do not fully agree with results reported by Schulman et al. (1993) who used a somewhat different experimental approach. These authors incubated pieces of hollow fiber membranes with whole citrated blood and found a large increase of kinin concentration within 5 min for PAN even when ACE inhibitors were omitted, whereas with CEL a slow, but finally also large increase was observed during the 1-h observation time. To explain the reason for the difference in relation to our results (i.e., an increase of kinin concentration in the absence of ACE inhibitor and a slow but significant kinin generation by CEL), we can only speculate that the properties of the surface hollow fiber membrane, though of the same material, may differ from that of flat sheet membranes. Another possibility is that the surface area of the 5-g pieces of hollow fiber membrane is much larger than that used by us, and/or the antibody used by Schulman et al. (1993) in the radioimmunoassay also detected degradation products of kinin. However, their results and ours are consistent in that both groups demonstrated that the PAN membrane causes a very rapid release of an amount of kinin *in vitro*, which should be high enough to provoke anaphylactoid reactions in the *in vivo* situation.

### *In vivo studies*

For ethical reasons it is impossible to perform a prospective clinical study on the use of PAN hemodialyzers in patients treated with ACE inhibitors. However, we could perform a retrospective study with plasma samples collected in 1990 from several patients who were treated with ACE inhibitor and developed anaphylactoid reactions at the onset of dialysis with a PAN membrane (Verresen et al., 1994) (Table 1, group I). The kinin levels ( $2392 \pm 53$  pmol/l) were about seven-fold higher than those of the control group II ( $350 \pm 16$  pmol/l). Since the samples were not obtained in a way that would optimally suppress kinin generation during blood collection and handling (kinin determination had not been planned at that time), the data were validated further by comparing kinin levels of samples prepared by the incorrect (group III,  $250 \pm 15$  pmol/l) and the correct technique (Shimamoto et al., 1988) (group IV,  $63 \pm 8$  pmol/l). Comparison of groups III and IV clearly demonstrates that an incorrect sampling technique causes elevated kinin plasma levels which, however, are within the same range as those of the control group II, whereas the kinin levels of patients with anaphylactoid reactions, group I, are by far too high to be explained by this artifactual kinin release during inappropriate sample handling. Comparison with the control groups clearly demonstrates that the high levels in group I indeed reflect generation of bradykinin during hemodialysis with PAN dialyzers, which accumulates in the

Table 1 - Plasma kinin levels in patients with and without anaphylactoid reactions.

All samples were obtained from the line entering the dialyzer either after the onset of the reaction or, for groups II-IV, 5 min after starting dialysis. Group I: patients under treatment with ACE inhibitor who developed an anaphylactoid reaction (AR) at the onset of hemodialysis with a PAN membrane. Group II: control patients without ACE inhibitor treatment and without AR but dialyzed with PAN. The blood samples of groups I and II were not drawn directly into an inhibitor cocktail (Shimamoto et al., 1988) and artefactually elevated kinin levels were expected. The effect of this incorrect sampling procedure on plasma kinin levels was studied in a control experiment in which blood samples from groups III and IV were drawn with and without using an inhibitor cocktail, respectively.

	Groups			
	I Patients with AR	II Control	III Control	IV Control
Number	9	9	9	9
Sampling with inhibitor cocktail	no	no	no	yes
Kinin concentration				
Mean $\pm$ SEM (pmol/l)	2392 $\pm$ 53	350 $\pm$ 16	250 $\pm$ 15	63 $\pm$ 8

circulation because its breakdown is diminished by the ACE inhibitor.

Very high plasma kinin levels during hemodialysis with PAN membranes were observed in a 26-year old patient who developed anaphylactoid reactions although not being treated with an ACE inhibitor (Table 2) (Verresen et al., 1993, 1994). She remained symptom-free and had normal plasma levels of kinin during dialysis with CEL and polysulfone membranes. Obviously, some special properties of the PAN membrane

Table 2 - Plasma kinin levels (fmol/ml) before and during hemodialysis of a patient not treated with ACE inhibitor who developed anaphylactoid reactions with PAN but not with other dialyzer membranes.

Blood samples were collected from the lines entering, A, and leaving, V, the dialyzer into an inhibitor cocktail and processed (Shimamoto et al., 1988) for kinin determination by radioimmunoassay.

	0 min	5 min A	5 min V
PAN (1st episode)	212	2112	5280
PAN (2nd episode)	76	2107	10468
CEL (one treatment, no reaction)	0	36	0
Polysulfone (one treatment, no reaction)	29	0	123

must be responsible for the bradykinin release, but in this case, the development of anaphylactoid reactions must be due to a hitherto unrecognized patient inability to degrade kinin efficiently. A similar case was reported recently by Schaefer et al. (1993).

In conclusion, the results of the *in vitro* and *in vivo* studies described here support the "bradykinin hypothesis" (cf. Introduction), demonstrating that contact of blood with the PAN hemodialyzer membrane provokes a significant generation of kinin and that the presence of an ACE inhibitor can effectively block kinin degradation in plasma. *In vivo*, the accumulation of kinin in the circulation finally causes anaphylactoid reactions. Not all patients exposed to the combination of PAN dialyzer and ACE inhibitor developed anaphylactoid reactions and, on the other hand, some patients who are treated with PAN but not with ACE inhibitor are occasionally affected. Therefore, one has to assume that factors other than blocking kinin degradation by ACE inhibition also play a role in this phenomenon.

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## References

- Dolovich J, Marshall CP, Smith EKM, Shizmizu A, Pearson FC, Sugona MA & Lee W (1984). Allergy to ethylene oxide in chronic hemodialysis patients. *Artificial Organs*, 8: 334-337.
- Fink E, Schill W-B, Fiedler F, Krassnigg F, Geiger R & Shimamoto K (1985). Tissue kallikrein of human seminal plasma is secreted by the prostate gland. *Biological Chemistry Hoppe-Seyler*, 366: 917-924.
- Grammer LC, Roberts M, Nicholls AJ, Platts MM & Patterson R (1984). IgE against ethylene oxide-altered human serum albumin in patients who have acute dialysis reactions. *Journal of Allergy and Clinical Immunology*, 74: 544-546.
- Hakim RM, Breillat J, Lazarus JM & Port FK (1984). Complement activation and hypersensitivity reactions to hemodialysis membranes. *New England Journal of Medicine*, 311: 878-882.
- Lemke H-D & Fink E (1992a). Generation of bradykinin in human plasma using AN69 and PAN 17DX membranes in the presence of an ACE inhibitor *in vitro*. *Nephrology, Dialysis and Transplantation*, 7: 727-728.
- Lemke H-D & Fink E (1992b). Accumulation of bradykinin formed by the AN69- or PAN 17DX-membrane is due to the presence of an ACE inhibitor *in vitro*. *Journal of the American Society of Nephrology*, 3: 376.
- Man NK, Cianconi C, Faivre JM, Diab N, London G, Maret J & Wambergue FP (1988). Dialysis-associated adverse reactions with high flux membranes and microbial contamination of liquid bicarbonate concentrate. *Contributions to Nephrology*, 62: 24-34.



- Parnes EL & Shapiro WB (1991). Anaphylactoid reactions in hemodialysis patients treated with the AN69 dialyzer. *Kidney International*, 40: 1148-1152.
- Schaefer RM, Fink E, Schaefer L, Barkhausen R, Kulzer P & Heidland A (1993). Role of bradykinin in anaphylactoid reactions during hemodialysis with AN69 dialyzers. *American Journal of Nephrology*, 13: 473-477.
- Schulman G, Hakim R, Arias R, Silverberg M, Kaplan AP & Arbeit L (1993). Bradykinin generation by dialysis membranes: possible role in anaphylactic reaction. *Journal of the American Society of Nephrology*, 3: 1563-1569.
- Shimamoto K, Ando T, Tanaka S & Iimura O (1988). The determination of kinin and glandular kallikrein in human biological fluids. *Atemwegs-und Lungenkrankheiten*, 14 (Suppl 1): S29-S36.
- Tielemans C, Madhoun P, Lenaers M, Schandene L, Goldman M & Vanherweghem J-L (1990). Anaphylactoid reactions during hemodialysis on AN69 membranes in patients receiving ACE inhibitors. *Kidney International*, 38: 982-984.
- Verresen L, Waer M, Vanrenterghem Y & Michielsens P (1990). Angiotensin-converting-enzyme inhibitors and anaphylactoid reactions to high-flux membrane dialysis. *Lancet*, 336: 1360-1362.
- Verresen L, Fink E, Dekens M, Leenaerts P & Vanrenterghem Y (1993). Bradykinin - mediator of anaphylactoid reactions during dialysis with AN69. *4th Benelux Dialysis Symposium*, 2-3 April 1993, Amsterdam. *Nephrology, Dialysis and Transplantation* (in press).
- Verresen L, Fink E, Lemke H-D & Vanrenterghem Y (1994). Bradykinin - mediator of anaphylactoid reactions during hemodialysis with AN69 membranes. *Kidney International*, 45: 1497-1503.

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