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In vivo clearance and elimination of nine marker substances during hemofiltration with different membranes

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ABSTRACT: The handling of low, middle and high molecular weight markers was examined in seven stable dialysis patients during hemofiltration with different membranes. Four membranes were examined in a randomized, crossover order (polysulfone, polyamide, AN69 polyacrylonitrile, Asahi polyacrylonitrile) by measuring plasma and dialysate concentrations of phosphate, creatinine, vitamin B_{12}, β_2-microglobulin, furanic acid, hippuric acid, retinol-binding protein, alpha-1-antitrypsin, and albumin. Sieving coefficients and plasma clearances of β_2-microglobulin or retinol-binding protein were markedly or slightly lower during hemofiltration with the Asahi polyacrylonitrile membrane than with the other membranes (highest removal with polysulfone/AN69 polyacrylonitrile membranes). No differences of obvious clinical relevance could be seen between the four membranes. A high β_2-microglobulin removal rate might be important to prevent dialysis-associated amyloidosis. (Int J Artif Organs 1992; 15: 408-12)

KEY WORDS: Hemofiltration, AN69 polyacrylonitrile, Asahi polyacrylonitrile, polysulfone, polyamide

INTRODUCTION

Controversies regarding the role of uremic toxins, e.g. middle molecules, in morbidity and mortality of patients with end-stage renal disease (ESRD) still persist (1, 2), but have stimulated the development of membranes that remove these middle molecules. Since the identification of β_2-microglobulin as the major component in dialysis-associated amyloidosis (3, 4), the removal of this protein during hemodialysis, hemofiltration and peritoneal dialysis has been widely studied (5-12).

β_2-microglobulin is removed better during hemodialysis with synthetic membranes (e.g. AN69 polyacrylonitrile, polysulfone), than with cellulosic membranes (12-14, 27, 28).

The present study compared characteristics of four different membranes *in vivo* with regard to parameters that serve to calculate clearances and elimination of markers mainly dependent on the size of the molecule (e.g. phosphate, creatinine, alpha-1-antitrypsin, albumin) and of markers more dependent on the protein binding and/or of potential influence as uremic toxins (e.g. β_2-microglobulin, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid = furanic acid, hippuric acid, vitamin B_{12}, retinol-binding protein).

PATIENTS AND METHODS

Seven stable patients with ESRD were studied after giving their informed consent. Their mean age was 54 ± 11 years (36-70) and they had been on chronic hemodialysis treatment for 51 ± 57 months (7-144); the etiology of the renal failure was diabetes mellitus (n=2), crescentic glomerulonephritis (n=1), mesangio-

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proliferative glomerulonephritis (n=1), interstitial nephritis (n=1), chronic glomerulonephritis (n=1), unknown (n=1). Post-dilution hemofiltration (3 h duration; 18 L of substitution fluid; 250 ml/min blood flow; 300 mm Hg transmembrane pressure; AFG 04 machine, DialyseTechnik, Rheinstetten-Forchheim, Germany) was performed using four different membranes [Filtral^{R} 16 (Hospal Medizintechnik GmBH, Nürnberg, Germany, AN69 polyacrylonitrile membrane, 1.7 m^2 surface), HF 80^{R}(Fresenius AG, Bad Homburg, Germany, polysulfone membrane, 1.8 m^2 surface), FH 88^{R} (Gambro AB, Lund, Sweden, polyamide membrane, 2.0 m^2 surface) PAN 250 NOVA^{R} (Diamed Medizintechnik GmbH, Köln, Germany, Asahi polyacrylonitrile membrane, 1.8 m^2)] in a randomized order in all patients. Three hemofiltration procedures were done with each membrane (six patients could be studied almost completely with all membranes, whereas one patient was studied incompletely).

Blood samples from the arterial and venous lines and samples of filtrate were obtained 15 min, 1h, 2h, and 3h after the start of the hemofiltration procedure. Hippuric acid and furanic acid were measured by HPLC (15), vitamin B_{12} by a radioreceptor test (Fa. Biermann, Bad Nauheim, Germany) modified to reach a detection limit of 10 pg/ml, and albumin, alpha-1-antitrypsin, and retinol-binding protein by nephelometry (Behring-Nephelometer-Analyzer, Behringwerke, Marburg, Germany) using antisera from Behring and modifications in the assay systems in order to reach detection limits of 0.2 mg/dl, 0.5 mg/dl, and 0.12 mg/dl, respectively. β_2-microglobulin was measured by a commercially available Elisa (Syn^{elisa}BETA-2-MIKROGLOBULIN^{R}, Elias Medizintechnik GmbH, Freiburg, Germany) with a detection limit of 0.05 mg/dl. Creatinine and phosphate were measured by routine laboratory methods.

Statistical analysis was done by Student's t-test and p values < 0.05 were considered statistically significant; data are given as mean $\pm$ SD.

The sieving coefficient of a marker was calculated as

$$\text{sieving coefficient} = \frac{2 \times C \text{ filtrate}}{C \text{ arterial} + C \text{ venous}}$$

where C stands for concentration of the marker in either the filtrate or arterial or venous plasma.

The plasma clearance of a marker was calculated as sieving coefficient x ultrafiltration rate. If the concentration of a marker in the filtrate was below the detection limit of the assay, the lowest measurable concentration was used for calculation of the sieving coefficient and consecutively of the plasma clearance. These sieving coefficients and plasma clearances are the maximum possible values and thus might cause some overestimation.

RESULTS

Mean sieving coefficients of phosphate, creatinine, β_2-microglobulin, hippuric acid, and retinol-binding protein are given in Table I. The β_2-microglobulin sieving coefficient was significantly lower ($p < 0.001$) during hemofiltration with the Asahi polyacrylonitrile membrane than with the other membranes and was highest with the AN69 and polysulfone membranes. A similar pattern was found for retinol-binding protein, removal being highest with the polysulfone membrane. Differences between membranes were only slight for creatinine and hippuric acid. Sieving coefficients were ≤ 0.02 for vitamin B_{12} and furanic acid, ≤ 0.004 for alpha-1-antitrypsin, and ≤ 0.001 for albumin.

Plasma clearances of phosphate, creatinine, β_2-microglobulin, hippuric acid, and retinol-binding protein are given in Table II. Plasma clearances of β_2-microglobulin and retinol-binding protein were significantly lower with the Asahi polyacrylonitrile membrane than with the other membranes. Plasma clearances for the AN69 membrane tended to be lower for the remaining markers. Plasma clearances were ≤ 1.5 ml/min for vitamin B_{12}, ≤ 1.0 ml/min for alpha-1-antitrypsin and furanic acid, and ≤ 0.2 ml/min for albumin.

DISCUSSION

Synthetic high-flux membranes used in a convective mode were studied with regard to marker substances with molecular masses ranging from 98 to 66500 Daltons. All studies were done *in vivo* because of the well-known effect of secondary membrane formation, due to contact of the membrane with blood, with the subsequent decrease of sieving coefficients not detectable *in vitro* (16).

TABLE I - MEAN SIEVING COEFFICIENTS (± SD) DURING HEMOFILTRATION WITH FOUR DIFFERENT MEMBRANES

	AN69 polyacrylonitrile n=72	Asahi n=60	polysulfone n=64	polyamide n=67
phosphate	0.77 ± 0.20	0.77 ± 0.15	0.78 ± 0.19	0.79 ± 0.18
creatinine	1.03 ± 0.22	1.07 ± 0.26	1.07 ± 0.14	1.09 ± 0.20*
hippuric acid	0.54 ± 0.10	0.61 ± 0.16**	0.58 ± 0.14*	0.57 ± 0.08*+
β_2-microglobulin	0.50 ± 0.18	0.08 ± 0.09***	0.54 ± 0.17+++	0.35 ± 0.20***+++ ###
retinol-binding protein	0.02 ± 0.02	0.01 ± 0.00	0.06 ± 0.07***+++	0.03 ± 0.02***+++ ###

*: in comparison with AN69 membrane; +: in comparison with Asahi membrane, #: in comparison with polysulfone membrane
$p < 0.05$, 1 symbol; $p < 0.01$, 2 symbols; $p < 0.001$, 3 symbols.

TABLE II - PLASMA CLEARANCE (ml/min) OF DIFFERENT MARKERS DURING HEMOFILTRATION WITH FOUR DIFFERENT MEMBRANES (mean ± SD)

		AN69 polyacrylonitrile n=72	Asahi n=60	polysulfone n=64	polyamide n=67
phosphate	15 min	74	82	82	86
	1 h	69	77	80	78
	2 h	70	72	74	71
	3 h	52	60	69	73
	mean	66 ± 23	73 ± 20*	76 ± 24**	77 ± 22**
creatinine	15 min	100	109	112	117
	1 h	93	103	109	107
	2 h	94	93	102	98
	3 h	71	96	90	92
	mean	90 ± 30	101 ± 30*	105 ± 25**	105 ± 23**
hippuric acid	15 min	54	67	59	66
	1 h	50	63	58	59
	2 h	47	57	56	52
	3 h	37	48	51	45
	mean	47 ± 15	59 ± 22***	56 ± 16***	56 ± 15***
β_2-microglobulin	15 min	34	7	63	27
	1 h	49	9	60	31
	2 h	55	8	52	36
	3 h	37	7	41	39
	mean	44 ± 20	8 ± 8***	54 ± 22 ++	33 ± 21 ++###
retinol-binding protein	15 min	3	1	11	3
	1 h	2	1	4	3
	2 h	2	1	4	3
	3 h	1	1	3	2
	mean	2 ± 2	1 ± 1***	6 ± 8 +++	2 ± 3 ++##

*: in comparison with AN69 membrane; +: in comparison with Asahi membrane, #: in comparison with polysulfone membrane
$p < 0.05$, 1 symbol; $p < 0.01$, 2 symbols; $p < 0.001$, 3 symbols.

Sieving coefficients and plasma clearances of low molecular mass markers (98-240 Daltons) do not markedly differ with the various membranes, provided that the molecules are sufficiently soluble as free substances in plasma. This is demonstrated by the high values for phosphate and creatinine, no noteworthy removal of furanic acid due to > 99% protein binding and an intermediate response of hippuric acid partly due to the 40-50% protein binding (Tabs. I and II).

The removal of low molecular markers seemed to be somewhat lower with the AN69 membrane. Phosphate, creatinine and hippuric acid plasma clearances decreased by about 10-20% during three hours of hemofiltration with no obvious difference between membranes. These results are in accordance with the literature, but most authors either investigated only one or two membranes or a limited number of marker substances (only creatinine was included in the majority of reports) (12, 17-19). The role of hippuric acid and furanic acid as uremic toxins has not yet been established, but markedly elevated plasma levels have been reported (15, 20, 21). Schoots et al. (21) reported that hippuric acid is an independent parameter that correlates closely with residual renal function.

With the exception of the markedly lower removal of β_2-microglobulin and retinol-binding protein by the polyacrylonitrile Asahi membrane (and the better results with the polysulfone and AN69 membranes), the sieving coefficients and plasma clearances of middle molecular mass markers (1355-21000 Daltons) were not different with the membranes tested. Low removal rates of vitamin B_{12} and retinol-binding protein are due to protein binding (e.g. transcobalamin, prealbumin), and are in accordance with previous reports (16, 22, 23). No appreciable removal of vitamin B_{12} was seen despite its low molecular mass (1355 Daltons), and this makes vitamin B_{12} a useful marker only *in vitro*. Sieving coefficients of β_2-microglobulin range from about 0.4-0.8 (12, 16, 17, 19, 22-24) and tend to decrease moderately to markedly (16, 22, 23) or remain constant during hemofiltration (24). Interestingly, Asahi polyacrylonitrile (PAN series) seems to be almost impermeable to β_2-microglobulin, in contrast to AN69 polyacrylonitrile (16, 22, 26), which seems to have a high initial absorption of β_2-microglobulin (24, 25, 27, 28). As reported elsewhere the high molecular mass markers albumin and alpha-1-antitrypsin were not removed in substantial amounts in the present study (16, 19, 22, 23); minor removal of alpha-1-antitrypsin and albumin has been demonstrated during the very first few minutes (23) before secondary membranes formed, but in the present study the first measurements were made after 15 minutes.

In conclusion, with the exception of the very low removal of β_2-microglobulin and to a minor extent of retinol-binding protein with the Asahi polyacrylonitrile membrane, the other differences between the synthetic membranes examined with regard to removal of marker substances with molecular masses from about 100 to about 67000 Daltons seem to be of little clinical relevance.

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