Nonsteroidal antiinflammatory drugs in the nephrotic syndrome

Vriesendorp, Robert

NONSTEROIDAL ANTIINFLAMMATORY DRUGS IN THE NEPHROTIC SYNDROME

R. VRIESENDORP

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STELLINGEN

I.

Nonsteroidal antiinflammatory drugs - de zogenaamde antiflogistica - remmen de proteïnurie bij patiënten met een idiopatisch nefrotisch syndroom door verlaging van de glomerulaire waterdruk en/of het glomerulaire ultrafiltratie quotient.

II.

Nonsteroidal antiinflammatory drugs oefenen hun gunstige invloed op patiënten met een idiopatisch nefrotisch syndroom niet uit door in te grijpen in de noxe die aanleiding gaf tot het nefrotisch syndroom, maar door te interfereren met de aanpassing van de nier aan deze noxe.

III.

Nonsteroidal antiinflammatory drugs en angiotensin converting enzyme remmers dienen in een prospectief, met placebo gecontroleerd onderzoek bij patiënten met het nefrotisch syndroom getest te worden op hun te verwachten gunstig effect ten aanzien van behoud van nierfunctie.

IV.

De mate van proteïnurie is één van de belangrijkste prognostische factoren bij patiënten met een idiopatisch nefrotisch syndroom.

V.

Een verlaagde filtratiefractie is de meest gevoelige parameter van renale aktiviteit bij gesystematiseerde lupus erythomatodes.

VI.

Bij de onderhoudsbehandeling van angina pectoris is het ononderbroken gebruik van nitraat bevattende pleister zinloos.

VII.

Bij de behandeling van CARA-patiënten met theophylline-preparaten dient men te beseffen dat hiermee geen vermindering van de bronchiale hyperreactiviteit wordt bereikt.

VIII.

Allergie - een versterkte respons in de IgE klasse tegen gebruikelijke allergenen - is een autosomaal dominante aandoening.

IX.

De moleculaire biologie is de anatomie van de moderne geneeskunde.

X.

De belangrijkste richtlijn voor het verbeteren van de resultaten van de humane beenmergtransplantaties is het verkrijgen van een adequate balans in de spiegelreactie van host versus graft en graft versus host.

XI.

Vooralsnog mag de klinisch oncoloog lak hebben aan LAK-cellen.

XII.

De extreem hoge incidentie van borstkanker in Nederland rechtvaardigt een nationale aanpak van screening en vroege detectie met krachtige steun van de overheid.

XIII.

Wateroplosbare chemotherapeutische agentia zijn van beperkte waarde in de behandeling van maligne processen in het centraal zenuwstelsel door hun onvoorspelbare penetratie van de bloed-hersen barrière.

F. J. Vriesendorp et al
J. Neuro-Oncol 1984; 2: 301-314

XIV.

Bij de huidige stand van zaken is het voorschrijven van cytostatica gepingel op de vierkante meter.

H. M. Vriesendorp, R. Vriesendorp en F. J. Vriesendorp
Cancer Chemother Pharmacol 1987; 19: 273-276

XV.

Schaatsen is de enige sport die men met de handen op de rug wint.

XVI.

Het is niet onredelijk dat het algemeen bekende lied "Oh yes, we have no bananas" geen weerklank heeft gevonden op het Canarische eiland Tenerife.

J. J. Vriesendorp, 1984.

XVII.

De grootste vergissing van de jaren zeventig is de vertaling van het adagium van de zestiger jaren "de hel, dat zijn de anderen" in "de hemel, dat ben ik".

Stellingen
behorende bij het proefschrift van
Robert Vriesendorp

Nonsteroidal antiinflammatory drugs in the nefrotic syndrome

Groningen, 30 maart 1988

RIJKSUNIVERSITEIT GRONINGEN

NONSTEROIDAL ANTIINFLAMMATORY DRUGS IN THE NEPHROTIC SYNDROME

PROEFSCHRIFT

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit Groningen
op gezag van de Rector Magnificus Dr. S. K. Kuipers
in het openbaar te verdedigen op woensdag 30 maart 1988
des namiddags te 2.45 uur precies
door

ROBERT VRIESENDORP

geboren te 's Gravenhage

1988
DRUKKERIJ VAN DENDEREN B.V.
GRONINGEN

Promotores : Prof. Dr. G. K. van der Hem
Prof. Dr. A. J. M. Donker

Referenten : Dr. P. E. de Jong
Dr. D. de Zeeuw

Waarnemingen kosten tijd, gedachten niet; dat merk je het beste, als je droomt.
Belcampo

CONTENTS

VOORWOORD

Dit proefschrift is een bundeling van artikelen over de werking van antiflogistica bij patienten met een nefrotisch syndroom. De onderzoekingen, die aan de artikelen ten grondslag liggen, zijn van 1979 tot 1985 op de afdeling Nefrologie in de Interne Kliniek van het Academisch Ziekenhuis te Groningen (hoofd: Prof. Dr. G. K. van der Hem) verricht. Het onderwerp vormt al meer dan 15 jaar een lijn van onderzoek op deze afdeling. Veel mensen zijn bij het onderzoek betrokken geweest voordat ik er aan meewerkte. Hun werk en daarop gebaseerde gedachten ontwikkeling waren onontbeerlijk voor het tot stand komen van dit proefschrift.

Gjalt van der Hem, Ab Donker, Paul de Jong en Dick de Zeeuw hebben telkens gefungeerd als ideeënbron en prikkel voor dit proefschrift. Ze waren niet alleen klankbord, maar traden handelend op in de uitvoering van verschillende protocollen. Hun bijdragen, ook op het redactionele vlak, zijn groot geweest, waardoor deze artikelenserie het stempel van de hele groep draagt. Een groep, waarin ik met plezier en trots heb gewerkt. Ik ben hen voor hun inzet en intensieve persoonlijke contacten zeer dankbaar.

Hetzelfde geldt voor Wim Sluiter, wiens adviezen en uitleg van statistische technieken en relevantie significant voor dit onderzoek waren. John Pratt heeft de prostaglandine bepaling, vermeld in hoofdstuk 3, ontwikkeld en daarmee de weg naar één van de belangrijkste waarnemingen in dit onderzoek geplaveid.

Alie Bremer-Drent voerde gelukkig vaak luid lachend, efficient en accuraat de bepalingen van de glomerulaire filtratie snelheid en de effectieve renale plasma flow uit. Willy Bruins-van der Wey en Greet Smit verzorgden met grote precisie en geduld het secretariële deel van dit proefschrift.

De internisten van de Interne Oncologie te Groningen en van het Westeinde Ziekenhuis te Den Haag toonden mij hoe vanzelfsprekend de grenzen tussen maat- en vriendschap kunnen vervloeien. Zij gaven mij bovendien de mateloze ruimte, die ik nodig had voor het prepareren van dit proefschrift.

Mijn gezin stond als trouwe supporters telkens langs de lijn. Het wordt tijd voor de return, de thuiswedstrijd.

Rob Vriesendorp

Scheveningen, februari 1988.

CHAPTER 1

INTRODUCTION

Since many centuries abnormal urine is attributed to a renal disorder. Hippocrates was the first to describe the association of frothy urine with kidney disease (1). In more recent times Bright renewed the interest in this relationship and extended the existing knowledge by the observation of albuminous urine in renal patients (2). Since 1836 an ever increasing flow of reports regarding this subject has arisen trying to unravel the pathogenesis of proteinuria.

Proteinuria

The distinction between normal and pathological proteinuria is quantitative rather than qualitative, as healthy persons also excrete protein in their urine. The amount of protein excretred in healthy subjects seldom exceeds 150 mg per 24 hours (3,4), but in renal disease urinary protein loss can amount to many grams per day.

Different mechanisms can underly urinary protein loss. Firstly, when plasma protein levels are highly elevated and the protein is freely fittered in the urinary space the high load of filtered protein can exceed the reabsorptive capacity of the kidney (for example in case of the urinary excretion of immunoglobulin light chains). Secondly, defects in the glomerular capillary wall can result in leakage of proteins, particularly of albumin and immunoglobulins. Thirdly, tubular reabsorption (of low molecular weight proteins as beta-2-microglobulin and lysozyme) can be affected. Finally, increased tubular secretion (for example of Tamm Horsfall protein) can occur (5).

Excessive urinary protein loss is mostly of *glomerular* origin. Micropuncture studies in the rat report an albumin concentration of 1.0 mg per 100 ml of glomerular ultrafiltrate while the plasma concentration amounts to 4000 mg per 100 ml (6,7). Supposing such a tight glomerular barrier in man, urinary albumin loss should not exceed 2 gram per day.The observed elevation of the albumin concentration of the glomerular filtrate in rats with experimental glomerulonephritis suggests a defective filtration barrier in those diseased glomeruli (8-11). Not only in the rat, but also in other mammalian species the existence of a filtration barrier for plasma proteins has been demonstrated (12).

The selectivity of this filtration barrier has been tested with exogenous and endogenous proteins. By the use of exogenous neutral macromolecules of varying molecular size (like dextran or polyvinyl-pyrrolidone (PVP)) the *size-selectivity* of the glomerular capillary wall is well established. In rats and man fractional

clearances of neutral dextran with a molecular radius larger than 42 Å approach zero (13,14). Thus, uncharged large molecules do not pass the intact filtration barrier. The impressive difference of the Bowmans space to plasma ratio of albumin with that of neutral dextran of the same effective molecular radius (36 Å), suggests that also other mechanisms besides size influence the transport of macromolecules trough the glomerular capillary wall (6,13,15). Several investigators showed in rats that anionic dextran sulphate was cleared to a much lesser degree than neutral dextran with comparable molecular size (16,17). This restriction in filtration of anionic macromolecules is called the *charge selectivity* of the glomerular wall. It is believed to result from the electrostatic force between anionic parts of the glomerular capillary wall and the circulating charged macromolecules (18,19). This mechanism allows the rapid passage of water etc., while it minimizes the loss of negatively charged macromolecules like albumin (13,17). Characterization of the defect in the filtration barrier by endogenous proteins is only possible in subjects with substantial urinary protein loss, because larger plasma proteins can only be reliably detected when proteinuria reaches nephrotic proportions. Clearance ratios of proteins with different molecular weight can then be calculated. Usually the clearance ratio between IgG (molecular weight 170.000 daltons) and albumin or transferrin (molecular weight 69.000 and 90.000 daltons) is used as the *selectivity index of proteinuria* (20). One may argue that this selectivity index does not take into account the charge selectivity of the glomerular wall and the tubular reabsorption rate of proteins.

It has been speculated that failure of the tubular protein reabsorption accounts for the major part of renal proteinuria (21). However, some authors postulate the maximum reabsorptive capacity (Tm) of albumin close to the normal filtered amount (9,22). Furthermore, the tubular reabsorption of albumin as well as of low molecular weight proteins is supposed to be a low-affinity transport process (5,23). So, if excess albumin reaches the ultrafiltrate by defective filtration, it will not be reabsorbed to a high degree. As albumin is the major constituent of urinary proteins, significant proteinuria in the rat is most likely due to defective glomerular filtration. Likewise, in man alterations in glomerular filtration are accepted as the fundamental abnormality leading to heavy proteinuria.

The underlying renal disorders of heavy proteinuria range widely. They vary from conditions which present themselves mainly with substantial urinary protein loss (minimal change nephropathy) to entities with not only proteinuria but also with hypertension and renal function impairment (acute glomerulonephritis and systemic vasculitis) (see also chapter 4, Table I). As heavy proteinuria - more than 5 gram per day - is the principal characteristic of the nephrotic syndrome, one often is confronted with the other classical signs and symptoms of the nephrotic syndrome in these patients like hypoalbuminaemia, hyperlipidaemia and oedema.

Hypoalbuminaemia

Hypoalbuminaemia indeed is a common finding in patients with the nephrotic syndrome. Theoretically, it can result firstly, from decreased albumin synthesis (predominantly in the liver), secondly, from an increase in albumin catabolism and thirdly from an increased loss. Finally, changes in albumin distribution over the vascular and extravascular space can be involved. The first mechanism does not contribute to the hypoalbuminaemia in the nephrotic syndrome, since the hepatic synthesis of albumin is not reduced in proteinuric patients but most often enhanced (24,25). With respect to the second pathway, an increase in the fractional catabolic rate of albumin is observed in nephrotic patients (25,26). Animal studies suggest that this enhanced fractional catabolism of albumin takes place in the kidney and can be reversed by nephrectomy (15,27). Since, however, the total mass of albumin is reduced, the catabolic rate, expressed in grams per day, was normal or even subnormal (25). In patients with the nephrotic syndrome the loss of albumin from extrarenal sites has not been extensively investigated except for the gastrointestinal tract. Faecal loss of albumin appears not to be increased (26,28). Most likely therefore, renal loss of albumin plays the key role in the degree of hypoalbuminaemia. The final possible cause of hypoalbuminaemia, a change in albumin distribution does not seem to contribute significantly. Recent studies of Koomans et al showed that a fall in plasma albumin and plasma oncotic pressure coincides with a decrease in interstitial colloid oncotic pressure (29). In conclusion, enhanced renal catabolism of albumin and urinary loss of albumin are thought to be responsible for the observed hypoalbuminaemia in the nephrotic syndrome.

Hyperlipidaemia

Another feature of the nephrotic syndrome, hyperlipidaemia has been extensively documented (30-35). Although general agreement exists on the presence of hypercholesterolaemia, conflicting data are reported on the level of plasma triglycerides and plasma lipoproteins (31-35). As the severity of the nephrotic syndrome increases, the level of triglycerides rises. In one study, however, only patients with a serum albumin below 10 g/l showed hypertriglyceridaemia (31). The different results on estimations of plasma lipids and lipoproteins may not only be related to the severity of the nephrotic syndrome, but can also be influenced by (steroid) treatment, the nutritional state of the patient, sex ratio differences, complicating illnesses and the degree of renal insufficiency (31). Trying to avoid these interfering factors, Appel et al recently studied twenty consecutive nephrotic patients without diabetes mellitus, renal

insufficiency or corticosteroid treatment. In the majority of patients they noted elevated plasma cholesterol and low density lipoprotein levels, and a depressed high density lipoprotein level in comparison with the age- and sex-linked norms of a large scale study (the Lipid Research Clinics Prevalence Study) (35). Like others they established an inverse correlation between the total cholesterol level and both the albumin plasma concentration and the plasma oncotic pressure (30,35), but not with plasma viscosity (35). The mechanisms involved in hyperlipidaemia in nephrotic patients are complex and include increased hepatic synthesis of lipoproteins and impaired catabolism (32,34). As infusion of albumin as well of dextran ameliorates the hypercholesterolaemia in nephrotic and idiopathic hypoalbuminaemia (30,36), reduced plasma oncotic pressure is the most likely signal for enhancement of the hepatic (lipo-)protein synthesis.

It is questionable whether the risk of cardiovascular disease is elevated in patients with the nephrotic syndrome. Initially, an increased incidence of ischaemic heart disease was claimed in two reports of small number of patients (37,38). Later on conflicting results were published in larger groups of patients (39,40). In view of the observed depression of the high density lipoprotein level and the short follow up of some studies, the cardiovascular risk in patients with long-standing nephrotic syndrome may well be increased.

Oedema

The last feature of the nephrotic syndrome, oedema, is often the first symptom of patients with this condition. The pathophysiologic pathway of the phenomenon is still debated. However, the conventional theory in which oedema is the consequence of low colloid oncotic pressure and of salt- and water retention due to decreased plasma volume, is strongly opposed (41). Dorhout Mees et al noted that more than 60% of the reported nephrotic patients had a normal or even expanded plasma volume (42). Moreover, they demonstrated a decrease in plasma and blood volume in patients with minimal change nephrotic syndrome after successful remission induction, while plasma albumin and plasma renin activity rose and oedema disappeared (42). Thus, at least in some nephrotic patients salt- and water retention is induced by other pathways than hypovolaemia. As such, changes in renal haemodynamics, enhanced renal adrenergic activity and stimulation of aldosterone secretion are implicated (43-45), and apparently active in some, but not all patients with the nephrotic syndrome.

Metabolic derangements

The morbidity and the prognosis of patients with the nephrotic syndrome are

not only determined by the nature of the underlying renal disease, but also by the metabolic derangements secondary to urinary loss of trace metals, lipids, proteins and hormones. Trace metal deficiency (iron, copper, zinc) is reported in a substantial number of patients and can partly be explained by their tight binding to plasma proteins lost by proteinuria (46,47). Loss of vitamin D and its metabolites bound to an alpha-2-globulin can result in clinically manifest bone disease, osteomalacia as well as hyperparathyroidism, especially in children (48,49). Massive proteinuria and oedema of the bowel wall can lead to a negative nitrogen balance (50) and may cause serious retardation of development and growth in children (51). The protein malnutrition in patients with the nephrotic syndrome increases the susceptibility to (pneumococcal) infections. Urinary loss of IgG and of factors essential for bacterial opsonization are likely to contribute to this elevated susceptibility (52,53). The reported high incidence of renal vein thrombosis and other thrombo-embolic complications in nephrotic patients is mostly attributed to loss of clotting factors, to low plasma levels of anti-thrombin III and plasminogen and to hyperaggregation of platelets (54-56). Correction of hypoalbuminaemia will restore adequate colloid oncotic pressure and the transport capacity of plasma proteins.

Treatment

Ideally, treatment is directed to the cause of the disorder. Despite intensive research the cause and the precise pathogenesis of the nephrotic syndrome remain obscure in the majority of the patients. Although immune mechanisms appear to be involved in the initiation of the histological and functional lesions of most glomerulopathies, only in a very few the immunogen has been identified (57). The above described derangements urge for more than symptomatic treatment. Therefore, treatment of patients with the idiopathic nephrotic syndrome has been based on manipulation of the immune response and the inflammatory reaction.

Immunosuppressive treatment of glomerulopathies mostly consists of corticosteroids with or without azathioprine or alkylating antineoplastic drugs like cyclophosphamide and chlorambucil. From the early fifties immunosuppressive therapy has been administered to a great number of nephrotic patients. The usefulness of corticosteroids in patients with minimal change nephropathy is beyond question. In 85 to 90 per cent of the patients a remission can be obtained within weeks (58,59). In other glomerulopathies, however, the value of immunosuppressive therapy is less clear. Prospective trials with corticosteroids with or without chlorambucil revealed better preservation of glomerular filtration rate in the treated patients with membranous nephropathy

(60,61), while other investigators report no beneficial effect of immunosuppressive treatment (62). The results of corticosteroids or corticosteroid containing regimens in patients with membranoproliferative glomerulonephritis differ strongly (63-65). No effects of corticosteroids are seen in nephrotic patients with focal glomerulosclerosis (66,67). Although favourable responses to corticosteroids in some glomerulopathies have been noted, it is still doubtful whether this therapy influences the natural course or final outcome of these disorders. In most glomerulopathies convincing evidence of prospective and controlled long-term studies regarding this subject is lacking.

Based on the same intentions as with corticosteroids nonsteroidal antiinflammatory drugs have been used in the treatment of nephrotic patients. The first report on this treatment in the nephrotic syndrome is by Fieschi and Bianchi in 1955 (68). They treated 25 nephrotic patients with phenylbutazone and observed a decrease in proteinuria and an increase in serum albumin in 21 of them. Longterm administration was hampered by myelotoxicity. In 1960 de Vries et al reported similar effects of phenylbutazone and aminophenazone in proteinuric patients (69). Moreover, they noted an concomitant mild decrease in creatinine clearance during the treatment with both drugs. After withdrawal of the agents, creatinine clearance returned to baseline levels. In the mid-sixties Michielsen and co-workers drew attention to the antiproteinuric effect of indomethacin in nephrotic patients (70). Regardless the underlying glomerulopathy, indomethacin reduced proteinuria, but its effect was most impressive in patients with membranous glomerulopathy or with proliferative glomerulonephritis. Investigators from France, Italy and the Netherlands confirmed their findings (71-73). As with phenylbutazone the prompt decrease in urinary protein excretion was parallelled by a reduction in creatinine clearance.

Groningen, the nephrotic syndrome and indomethacin

At this stage regular research regarding nonsteroidal antiinflammatory drugs and the nephrotic syndrome was started in the department of Nephrology of the University Hospital in Groningen. Arisz et al and Donker et al showed that indomethacin had an antiproteinuric effect within one to two days and that it also decreased glomerular filtration rate (74,75). Besides the fall in proteinuria and glomerular filtration rate, filtration fraction decreased, plasma renin activity was suppressed and the residual proteinuria became more selective. These effects were greatly enhanced by sodium depletion. Moreover, the effects appeared rapidly reversible after withdrawal of indomethacin, even after longterm treatment with indomethacin (74,75). From these studies it was hypothesized that inhibition of the prostaglandin synthesis by nonsteroidal antiinflammatory drugs

was responsible for the observed changes in the urinary protein excretion and renal haemodynamic variables.

In the renal unit of the University Hospital Groningen further studies were undertaken,that will be described in this thesis. First, the efficacy of other nonsteroidal antiinflammatory drugs than indomethacin was tested for their antiproteinuric effect (chapters 2 and 3). Chapter 2 describes a double-blind study comparing the renal haemodynamic and antiproteinuric effects of indomethacin with those of naproxen. In chapter 3 the antiproteinuric effects of different classes of nonsteroidal antiinflammatory drugs are studied. Since these different nonsteroidal antiinflammatory drugs were shown to possess in-vitro different prostaglandin synthesis inhibitory characteristics, the relationship between the alterations in proteinuria, glomerular filtration rate, plasma renin activity and urinary prostaglandin E_2 excretion were studied (chapter 3).

The influence of long term antiproteinuric treatment with indomethacin on progression of renal insufficiency was investigated in a retrospective study of 98 nephrotic patients with membranous glomerulopathy, focal glomerulosclerosis or membranoproliferative glomerulonephritis (chapter 4). In chapter 5 the Groningen studies are reviewed and finally, chapter 6 focusses on important aspects of intraglomerular pressure as a common determinant for urinary protein loss and the natural course of renal function loss in renal disease.

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CHAPTER 2

ANTIPROTEINURIC EFFECT OF NAPROXEN AND INDOMETHACIN

A double-blind crossover study.

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Abstract. In a double-blind crossover study in 10 salt-depleted nephrotic patients the reduction of proteinuria was significantly larger during indomethacin 50 mg three times daily than during naproxen 250 or 500 mg three times daily (72 vs. 44%, $p < 0.05$; 77 vs. 46%, $p < 0.05$, respectively). Both drugs induced similar reversible intrarenal hemodynamic changes, but indomethacin had more pronounced effects than naproxen. A common pathway, such as the reduction of the glomerular filtration rate and a reduction of the glomerular transcapillary hydraulic pressure, is likely to explain the observed phenomena and is most probably mediated by inhibition of intrarenal prostaglandin synthesis. If treatment with a nonsteroidal antiinflammatory drug is considered in patients with the idiopathic nephrotic syndrome, indomethacin appears up to now the most effective agent in reducing urinary protein loss.

Introduction

The first report on the antiproteinuric effect of a nonsteroidal anti-inflammatory drug (NSAID) was published in 1955 by Fieschi and Bianchi (1) who administered phenylbutazone to nephrotic patients. A decrease in urinary protein excretion and an increase in plasma albumin concentration were observed in 21 out of 25 patients. With aminophenazone, a similar effect was noted by de Vries et al. (2). These authors moreover observed a mild and reversible decrease in creatinine clearance after institution of phenylbutazone as well as aminophenazone. The antiproteinuric effect of indomethacin was described by Michielsen and Lambert (3) in 1967. Similar observations were reported by

Santoprade and Comellini (4), Conte et al. (5), and Wijdeveld (6).

Donker et al. (7) demonstrated an immediate decrease of proteinuria, plasma renin activity (PRA), glomerular filtration rate (GFR), effective renal plasma flow (ERPF), and filtration fraction (FF) after the administration of 50 mg indomethacin three times daily to sodium-restricted patients with the idiopathic nephrotic syndrome. Significant enhancement of these effects was noted when the nephrotic patients were sodium depleted by a low-sodium diet and 50 mg hydrochlorothiazide once a day resulting in stimulation of the renin-angiotensin axis. All the changes were reversible after withdrawal of indomethacin. These observations suggested an interference of indomethacin in the regulation of renal hemodynamics. Donker et al. (7) postulated that the antiproteinuric effect of indomethacin was mediated by an inhibition of the renal prostaglandin synthesis. If so, other NSAIDs may induce similar intrarenal hemodynamic changes.

Naproxen also inhibits prostaglandin synthesis and was selected for the present study because of its good tolerance (8). Therefore, we performed a double-blind crossover study with indomethacin and naproxen on the effects on proteinuria, GFR, ERPF, FF and PRA in patients with the nephrotic syndrome.

Patients and Methods

Ten consecutive patients presenting with a proteinuria of more than 3 g/day and with a GFR of at least 20 ml/min were studied (table I). Patients with an expected steroid-responsive nephrotic syndrome (such as minimal lesions or membranous glomerulopathy with selective proteinuria) were excluded. All patients were informed and gave verbal consent. During the whole study all patients received a

Table I. Patient characteristics at entry of the study.

Patient	Sex	Age years	Histological diagnosis	Proteinuria g/24 h	GFR ml/min	ERPF ml/min	FF	PRA nmol A_1/l/h
A	F	47	MGP	7.1	64	391	0.16	1.4
B	M	34	FG	15.0	24	151	0.16	4.3
C	F	55	amyloid	8.2	94	388	0.25	1.1
D	M	60	FG	8.3	50	194	0.25	3.7
E	F	71	MGP	3.9	36	225	0.15	2.0
F	M	29	MPGN	3.0	103	470	0.21	13.0
G	F	32	MGP	6.3	110	751	0.15	4.1
H	M	49	FG	9.5	25	201	0.12	5.2
I	M	57	MGP	17.0	23	225	0.10	0.4
K	M	54	MGP	12.0	40	372	0.10	4.2

MGP = Membranous glomerulopathy; FG = focal glomerulosclerosis; MPGN = membranoproliferative glomerulonephritis.

low-salt diet (30 mmol NaCl/day) and 50 mg hydrochlorothiazide once a day. All other medication was withdrawn except for atenolol in patient B. To secure the double-blind crossover design of the study, the hospital pharmacy produced identical capsules containing either 50 mg indomethacin or 250 or 500 mg naproxen and allocated the crossover order of NSAID treatment at random and unknown to the physicians and the patients. After sodium balance was reached, the patients were treated with indomethacin and naproxen, each during 1 week in the allocated order. Between both treatments NSAID was withheld for 1 week. All patients received 50 mg indomethacin and patients A-E 250 and patients F-K 500 mg naproxen three times a day.

Before the start, at the end, and 1 week after cessation of the treatment with either NSAID, GFR and ERPF were measured simultaneously with ^{125}I-sodium iothalamate and ^{131}I-hippuran, respectively, as described by Donker et al. (9). Both functions were corrected for 1.73 m^2 body surface area. Urine was collected daily, and protein was determined by biuret method in aliquots of 24-hour urine collections. At 8.00 h after overnight rest, fasting supine venous blood samples for estimation of PRA were drawn daily. PRA was measured with a commercially available radioimmunoassay kit (Becton Dickenson) for angiotensin I, generated under standard conditions (10). Proteinuria and PRA for each period are expressed as the mean of the values measured in the last 3 days of each period. All percentages or proportional changes are related to the value obtained in the no treatment period preceding the NSAID treatment involved.

For statistical analysis Student's t test (paired or unpaired when appropriate) and Spearman's rank test were used.

Results

Statistical analysis of all data prior to or 1 week after cessation of NSAID treatment did not show significant differences. This held true whether this treatment consisted of naproxen or of indomethacin.

The results in each patient were grouped into six separate periods: 1 week before the first NSAID, during the first NSAID, 1 week after the first NSAID (which equals that of 1 week before the second NSAID), during the second NSAID, and 1 week after the second NSAID. So a comparison could be made of naproxen 250 versus indomethacin 50 mg three times daily in 5 patients (patients A-E; table II, figure 1) and of naproxen 500 versus indomethacin 50 mg three times a day in another 5 subjects (patients F-K; table III, figure 2). As no statistically significant changes in proteinuria, renal function, and PRA were observed between patients treated with 250 and those with 500 mg naproxen three times daily, also a comparison could be made of the effects of naproxen,

irrespective of its dose, versus those of indomethacin 50 mg three times a day in all patients (patients A-K; table IV).

Patients treated with naproxen 250 and indomethacin 50 mg three times a day

In all patients (A-E) the decrease in proteinuria was significant during both naproxen and indomethacin treatment. The mean proteinuria during administration of naproxen fell from 8.5 ± 4.0 to 5.2 ± 3.7 g/24 h and during administration of indomethacin from 8.2 ± 6.1 to 2.5 ± 2.5 g/24 h (table II, figure 1). In every patient the antiproteinuric effect of indomethacin was better than that of naproxen ($p < 0.05$).

GFR during both NSAID administration fell significantly. The mean change in GFR during indomethacin treatment (34%) was twice as large as that during naproxen treatment (17%; $p < 0.05$). Decreases in ERPF and FF were observed during both NSAID administrations, but were not significantly different from each other.

On naproxen PRA did not change in 2 patients. On indomethacin all patients demonstrated a fall in PRA, although patient D still showed a high level (5.1 nmol A_I/l/h). The mean proportional fall in PRA during administration of naproxen was less than that during administration on indomethacin (26 and 64%, respectively; $p < 0.05$).

One week after cessation of the NSAID treatment all above-mentioned changes had been reversed to levels that did not significantly differ from the pretreatment levels.

Table II. Proteinuria, renal function, and PRA in nephrotic patients (A-E) treated with either naproxen 250 or indomethacin 50 mg three times a day during salt depletion (mean ± SD).

	Proteinuria g/24 h	GFR ml/min	ERPF ml/min	FF	Proteinuria per GFR, mg/ml	PRA nmol A_I/l/h
Naproxen						
Before treatment	8.5 ± 4.0	53 ± 25	268 ± 101	0.19 ± 0.05	0.16 ± 0.14	2.4 ± 1.5
During treatment	5.2 ± 3.7	43 ± 19	241 ± 92	0.17 ± 0.04	0.11 ± 0.11	1.7 ± 1.7
Proportional change, %	44 ± 13	17 ± 8	10 ± 5	10 ± 4	35 ± 12	26 ± 26
p value	< 0.005	< 0.02	< 0.02	< 0.025	< 0.005	n.s.
Indomethacin						
Before treatment	8.2 ± 6.1	52 ± 24	267 ± 100	0.19 ± 0.04	0.16 ± 0.17	2.7 ± 1.8
During treatment	2.5 ± 2.5	34 ± 13	221 ± 84	0.15 ± 0.03	0.08 ± 0.10	1.4 ± 2.1
Proportional change, %	72 ± 16[a]	34 ± 6[a]	17 ± 8	21 ± 7	57 ± 20[a]	64 ± 30[a]
p value	< 0.001	< 0.001	< 0.02	< 0.02	< 0.005	< 0.02

[a] The value during indomethacin administration differs significantly from that during naproxen administration.
n.s. = not significant.

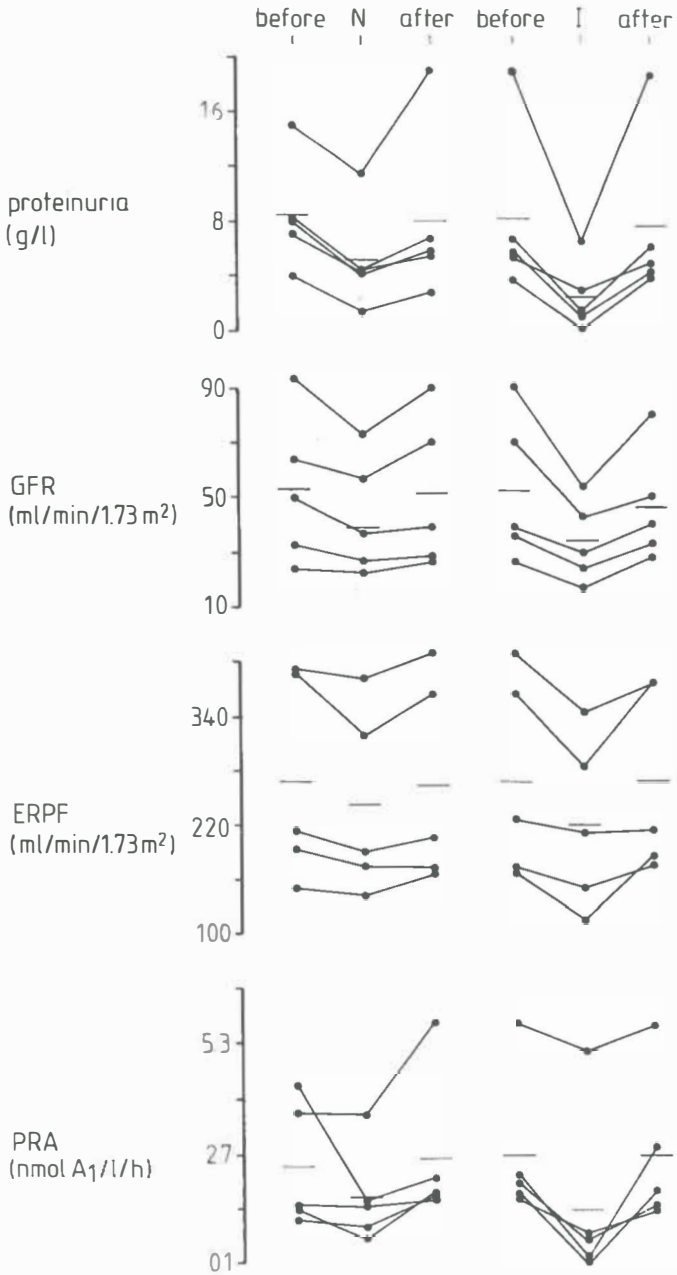

Figure 1. Proteinuria, GFR, ERPF, and PRA before, during, and after administration of naproxen (N) 250 and indomethacin (I) 50 mg three times a day in patients A-E.

Patients treated with naproxen 500 and indomethacin 50 mg three times daily

During treatment with both naproxen and indomethacin, proteinuria decreased significantly in all patients (F-K). When treated with naproxen the mean urinary loss of protein fell from 9.7 ± 5.1 to 5.3 ± 3.3 g/24 h. Indomethacin induced a mean decrease in proteinuria from 8.6 ± 4.4 to 2.5 ± 2.4 g/24 h (table III, figure 2). The fall in urinary protein excretion was larger during indomethacin administration than during that of naproxen ($p < 0.05$).

GFR always decreased except in patient G during naproxen treatment. At the average GFR decreased by 17% on naproxen and by 32% on indomethacin. No significant difference could be detected between these changes in GFR, although the GFR significantly decreased during administration of indomethacin but not during that of naproxen. ERPF and FF fell in most cases, but the changes were relatively small.

A significant decrease in PRA was noted both during naproxen (54%) and indomethacin (66%). The proportional changes in mean PRA during both NSAIDs did not differ significantly.

One week after cessation of the NSAID treatment all above-mentioned changes had reversed to levels not significantly different from their pretreatment levels.

Table III. Proteinuria, renal function, and PRA in nephrotic patients (F-K) treated with either naproxen 500 or indomethacin 50 mg three times a day during salt depletion (mean ± SD).

	Proteinuria g/24 h	GFR ml/min	ERPF ml/min	FF	Proteinuria per GFR, mg/ml	PRA nmol A_I/l/h
Naproxen						
Before treatment	9.7 ± 5.1	56 ± 34	382 ± 176	0.14 ± 0.03	0.21 ± 0.18	4.3 ± 2.7
During treatment	5.3 ± 3.3	50 ± 37	363 ± 195	0.12 ± 0.03	0.14 ± 0.12	2.2 ± 1.4
Proportional change, %	46 ± 16	17 ± 12	7 ± 7	9 ± 7	34 ± 19	54 ± 12
p value	< 0.005	n.s.	n.s.	< 0.05	< 0.025	< 0.001
Indomethacin						
Before treatment	8.6 ± 4.4	60 ± 39	398 ± 205	0.14 ± 0.04	0.18 ± 0.15	5.0 ± 4.8
During treatment	2.5 ± 2.4	38 ± 28	277 ± 123	0.12 ± 0.03	0.08 ± 0.08	1.5 ± 1.3
Proportional change, %	77 ± 14[a]	32 ± 20	25 ± 18	10 ± 19	70 ± 20[a]	66 ± 12
p value	< 0.001	< 0.05	n.s.	n.s.	< 0.005	< 0.001

[a] The value during indomethacin administration differs significantly from that during naproxen administration ($p < 0.05$).
n.s. = not significant.

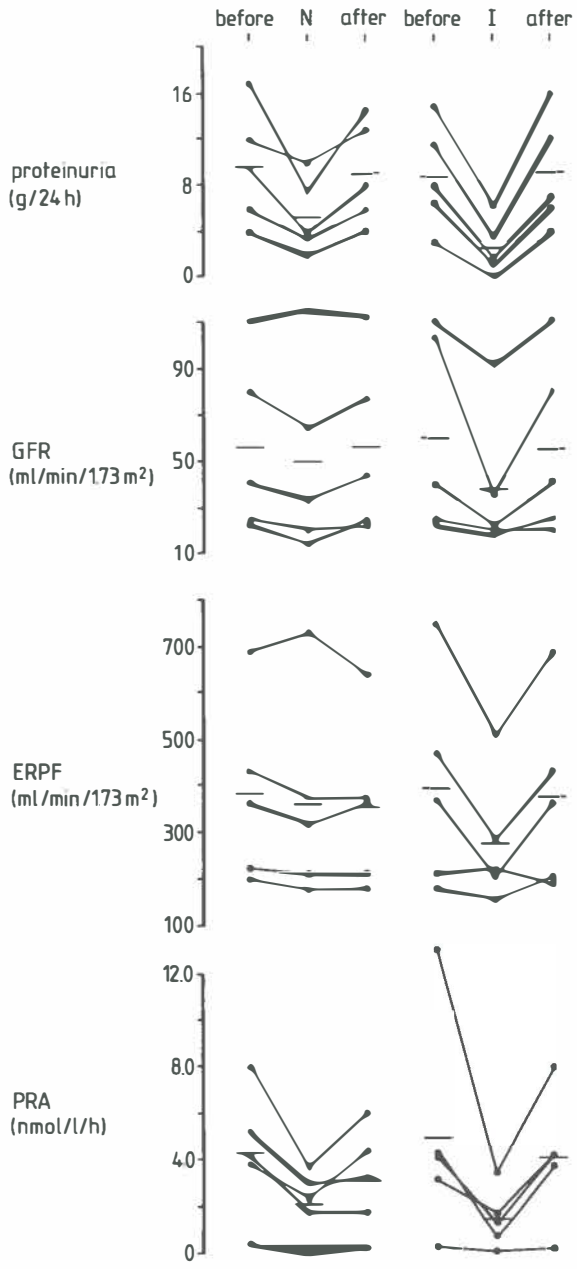

Figure 2. Proteinuria, GFR, ERPF, and PRA before, during, and after administration of naproxen (N) 500 and indomethacin (I) 50 mg three times a day in patients F-K.

Correlations

The proportional change in proteinuria correlated with the proportional change in GFR ($R = 0.57$, $p < 0.01$; figure 3). In 19 out of 20 NSAID treatments the proportional decrease in urinary protein loss was greater than the change in GFR (figure 3). In fact, the mean protein excretion per milliliter of GFR fell from 0.18 before, to 0.10 mg during NSAID treatment. Thus, assuming a linear relation, the change in GFR accounted for approximately 50% of the fall in proteinuria. As is shown in tables II-IV, the change in protein excretion per milliliter of GFR during indomethacin administration was larger than during naproxen treatment ($p < 0.05$). Like the proportional change in GFR, the proportional fall in ERPF correlated with the proportional change in urinary protein loss ($R = 0.53$, $p < 0.05$). The proportional change of PRA correlated with the absolute fall in proteinuria ($R = 0.49$, $p < 0.05$), but not with the proportional or absolute changes in the renal hemodynamics.

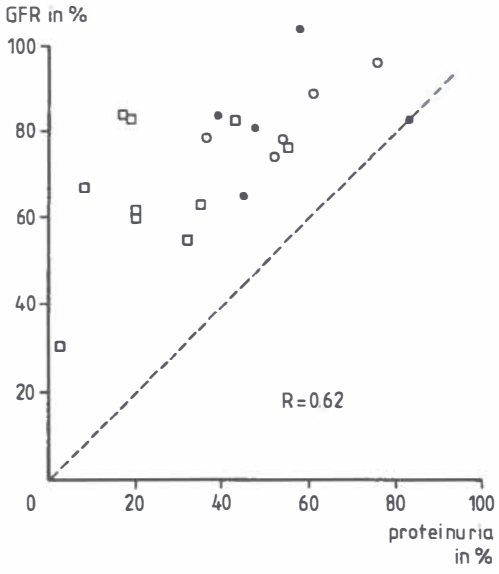

Figure 3. Relation between proteinuria and GFR during NSAID treatment, both expressed as the percentage of the preceding no treatment period. Squares and open and closed circles represent values obtained during treatment with indomethacin 50, naproxen 250, and naproxen 500 mg three times daily, respectively. $r = 0.62$.

Table IV. Proteinuria, renal function, and PRA in nephrotic patients (A-K) treated with either naproxen 250 or 500 or indomethacin 50 mg three times a day during salt depletion (mean ± SD).

	Proteinuria g/24 h	GFR ml/min	ERPF ml/min	FF	Proteinuria per GFR, mg/ml	PRA nmol A_I/l/h
Naproxen						
Before treatment	9.1 ± 4.1	55 ± 30	325 ± 154	0.17 ± 0.05	0.18 ± 0.16	3.4 ± 2.2
During treatment	5.3 ± 3.1	47 ± 30	302 ± 165	0.15 ± 0.04	0.13 ± 0.12	1.9 ± 1.2
Proportional change, %	45 ± 14	17 ± 10	8 ± 6	10 ± 5	34 ± 16	40 ± 25
p value	< 0.001	< 0.01	< 0.05	< 0.005	< 0.02	< 0.02
Indomethacin						
Before treatment	8.4 ± 4.8	56 ± 32	332 ± 174	0.16 ± 0.05	0.17 ± 0.16	3.8 ± 3.4
During treatment	2.5 ± 2.2	36 ± 22	249 ± 109	0.13 ± 0.03	0.08 ± 0.09	1.4 ± 1.6
Proportional change, %	75 ± 15[a]	33 ± 15[a]	21 ± 14[a]	15 ± 15	63 ± 21[a]	65 ± 23[a]
p value	< 0.001	< 0.01	< 0.02	< 0.025	< 0.01	< 0.025

[a] The value during indomethacin administration is significantly different from that during naproxen administration ($p < 0.05$).

Discussion

As shown in this study, naproxen effectively decreases proteinuria in *salt-depleted* nephrotic patients. Naproxen 250 and 500 mg three times a day, however, appeared to be less effective as indomethacin 50 mg three times daily. The same held true for the decreases in PRA, GFR, ERPF, and FF induced by these agents (table IV). All these effects were reversible within one week after cessation of both agents in the dosages tested. Thus, it seems likely that naproxen and indomethacin have a common pathway in reducing proteinuria in *sodium-depleted* patients with the nephrotic syndrome.

Several pathways are possibly involved: change in configuration and electrical charge of the albumin molecule, reduction of the glomerular transcapillary hydraulic pressure, reduction of the GFR, and enhancement of the proximal albumin reabsorption.

Both naproxen and indomethacin are highly bound to albumin. This protein-bound fraction usually accounts for about 99% of the plasma concentration. This binding may well change the shape and the electrical charge of the albumin molecule. If this occurs, it can interfere with the passage of albumin through the glomerular basement membrane. This phenomenon, however, is not likely to play a major role in the observed antiproteinuric effect, because, for instance, indomethacin induces hardly any reduction in urinary protein in *sodium-repleted* patients with the nephrotic syndrome (7).

Assuming a linear relation between the proportional reduction in proteinuria and that in GFR, the mean fall in GFR accounts for only half the decrease in urinary protein. Therefore, another mechanism might be involved. In this respect, it is noteworthy that Arisz et al. (11) demonstrated an increase in the selectivity of the remaining proteinuria after indomethacin administration. Tiggeler et al. (12) confirmed this observation by using polyvinylpyrrolidone molecules. It is also worth mentioning that during intrarenal infusion of renin or angiotensin II in rats the permeability of the glomerular basement membrane for macromolecules, including polyvinylpyrrolidone, increased, while FF rose (13). Furthermore, in Munich-Wistar rats nonvasodepressor doses of PGE_2 and PGI_2 did raise the mean glomerular transcapillary hydraulic pressure difference ($\bar{\Delta}$ P) significantly, an effect that could be abolished by preceding salarasin infusion (14). In sodium-depleted healthy volunteers and nephrotic patients it has been shown that indomethacin decreases PRA and FF (15), the latter in accordance with a decrease in $\bar{\Delta}$ P. Thus, it may well be that administration of indomethacin during sodium depletion decreases $\bar{\Delta}$ P and thereby the GFR as well as the size permeability of the glomerular basement membrane for macromolecules. Both angiotensin II and norepinephrine appear to increase the synthesis of renal prostaglandins which, in turn, counteract with the vasoconstrictive actions of angiotensin II and norepinephrine on the renal vasculature (16). As the hemodynamic and antiproteinuric effects of indomethacin are strongly augmented by salt depletion and the resulting relative stimulation of the renin-angiotensin axis (7), the known renal prostaglandin synthesis' inhibition of naproxen and indomethacin can explain the hemodynamic and proteinuric changes observed in this study.

The fourth mechanism by which indomethacin may reduce the urinary protein excretion is an interference with the albumin-reabsorptive capacity of the proximal tubule. Although debated, it has been stated that quantitatively the proteinuria in the nephrotic syndrome can solely be attributed to a failure of tubular albumin absorption without assuming altered permeability of the glomerular membrane (17). Indomethacin as well as naproxen are thought to be excreted in the urine via the anion carrier in the basolateral membrane of the proximal tubule (18). Usberti et al. (19) reported a reversion of proximal tubular dysfunction during indomethacin treatment. So, one cannot exclude the possibility that the antiproteinuric effect of NSAID is at least partially mediated by an enhancement of the proximal tubular reabsorptive capacity. If a fall in GFR would increase this function of the proximal tubule, it is hard to understand why the indirect stimulation of the proximal tubular reabsorption by salt restriction and hydrochlorothiazide does not reduce proteinuria in patients with the nephrotic syndrome (7). Therefore, in our opinion, enhanced absorption of albumin in the proximal tubule does not seem to be of major importance in the

above-described NSAID-induced changes in renal function and proteinuria.

With the exception of minimal change disease, glomerular pathology accompanied by substantial urinary protein loss is associated with a poor prognosis for the kidney (20). Although it has not been proven whether glomerular protein loss itself is harmful for the kidney, several investigators (21, 22) have suggested that long-standing increased permeability of the glomerular basement membrane for macromolecules might facilitate the development of focal glomerulosclerosis and might precipitate renal function deterioration. Thus, the antiproteinuric effect of any drug per se might be beneficial to the nephrotic patient. In this respect the recent observations of Zimmerman et al. (23) are worth mentioning. In a prospective trial of warfarin and dipyramidole in patients with membranoproliferative glomerulonephritis the anticoagulant therapy resulted not only in a significant decrease in urinary protein but also in stabilization of the kidney function. Similarly, the antiproteinuric effect of indomethacin might ultimately preserve renal function despite a reversible fall in GFR after institution of the drug. In view of the strong antiproteinuric effect of indomethacin, controlled prospective studies are warranted to disclose whether this drug is not only useful in symptomatic treatment leading to a significant rise in serum albumin (1), but also prohibits progressive kidney function loss in salt-depleted patients with the nephrotic syndrome.

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CHAPTER 3

REDUCTION OF URINARY PROTEIN AND PROSTAGLANDIN E_2 EXCRETION IN THE NEPHROTIC SYNDROME BY NONSTEROIDAL ANTIINFLAMMATORY DRUGS.

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Key Words: Nephrotic syndrome - Prostaglandin synthesis' inhibition - Nonsteroidal antiinflammatory drugs - Prostaglandin E_2 excretion.

Abstract. Seven salt-depleted patients with the idiopathic nephrotic syndrome were treated with various nonsteroidal antiinflammatory drugs. Indomethacin, diclofenac-sodium and flurbiprofen decreased proteinuria, glomerular filtration rate, plasma renin activity and renal prostaglandin E_2 excretion by 59%, 19%, 55% and 68% respectively. Sulindac induced no major changes in proteinuria, glomerular filtration rate, plasma renin activity and renal prostaglandin E_2 excretion. The relative change in proteinuria and glomerular filtration rate during nonsteroidal antiinflammatory drug treatment correlated strongly with that of the renal prostaglandin E_2 excretion ($R = 0.89$ and $R = 0.70$, respectively $p < 0.05$). It is likely that the antiproteinuric effect of nonsteroidal antiinflammatory drugs is dependent on their potency to inhibit renal prostaglandin synthesis and it is suggested that this effect is mediated by lowering transcapillary glomerular hydraulic pressure.

Introduction

Several nonsteroidal antiinflammatory drugs (NSAID) are shown to be effective antiproteinuric agents in salt depleted patients with the nephrotic syndrome of different origin (1-4).

The mechanism by which these drugs affect urinary protein loss in these

patients is not yet established. Initially, the antiproteinuric effect of indomethacin is ascribed to its antiinflammatory property, directly interfering with the glomerular pathologic process itself (2). Also other mechanisms can be involved such as an interference of NSAID with the regulation of renal hemodynamics (5). Indeed, during administration of indomethacin, glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) are generally reduced in salt depleted normal subjects as well as in salt depleted nephrotic patients (6). Furthermore, the proteinuria that persists during indomethacin treatment is more selective than before (7, 8), and the NSAID-induced changes in GFR, proteinuria and its selectivity appear to be readily reversible after withdrawal of these agents (4-7). Finally, only an activated renin-angiotensin-aldosterone system as achieved by dietary salt-restriction and diuretic therapy, ensures an optimal reduction of proteinuria by indomethacin (5). These observations suggest that not the glomerular disease itself, but the renal adaptation to that process is influenced by NSAID.

Renal prostaglandins modulate the pressor effects of substances like angiotensin II, norepinephrine and vasopressin (9). Most NSAID are well known for their prostaglandin synthesis' inhibition. In contrast to other NSAID, sulindac affects GFR and renal prostaglandin E_2 (PGE_2) excretion only minimally (10). As it is likely that urinary prostaglandin excretion reflects renal prostaglandin synthesis (11), sulindac probably does not inhibit renal prostaglandin synthesis, although it markedly inhibits platelet cyclo-oxygenase (12). To elucidate further the antiproteinuric mechanism of NSAID, we studied the effect of various NSAID with different in vitro prostaglandin synthetase inhibitory potencies (13) on PGE_2 excretion, on proteinuria and on renal hemodynamics.

Patients and Methods

Patients.

Seven patients with the nephrotic syndrome were studied in an out-patient setting. Kidney biopsies revealed membranous glomerulopathy in three subjects, membranoproliferative glomerulonephritis in three, and focal glomerulosclerosis in one. All patients gave informed consent. The initial values shown in Table I were obtained when the patients adhered to one to three gram salt intake and 50 mg hydrochlorothiazide per day. This regimen was continued throughout the whole study period. Indeed, most patients adhered adequately to the diet, except patients 2 and 6 who - according to the urinary sodium excretion - used about six to eight grams of salt a day. Concomitant antihypertensive medication was used by patients 1, 2 and 6. Patients 1 through 4 had been

instituted on indomethacin from three months to two years prior to the study. In these subjects indomethacin had been withdrawn at least two weeks before the initial values were obtained.

Table I. Patient characteristics and initial values obtained during sodium restriction and hydrochlorothiazide 50 mg a day.

Patient No.	Age/Sex	Histological diagnosis	Blood pressure mm Hg	GFR-ERPF ml/min	Proteinuria g/24 h	Serum albumin g/l	Antihypertensive medication	NSAID tested
1	37/m	MPGN	150/100	51-488	12.4	34	atenolol 100 mg furosemide 80 mg	D, F, I, S
2	40/m	MPGN	135/100	41-247	4.6	39	atenolol 100 mg	F, I, S
3	61/m	FG	110/80	54-264	3.1	38	–	F, S
4	48/m	MGP	135/80	96-556	9.8	26	–	D
5	48/m	MGP	135/80	70-378	7.0	33	–	D, I
6	37/m	MPGN	145/105	81-369	11.1	26	clonidine 450 μg	F
7	49/m	MGP	130/90	40-424	10.3	25	–	I

MPGN = membranoproliferative glomerulonephritis; FG = focal glomerulosclerosis; MGP = membranousglomerulopathy; D = diclofenacsodium; F = flurbiprofen; I = indomethacin; S = sulindac.

NSAID were selected by their in vitro potency to inhibit prostaglandin synthesis. Four NSAID were used: indomethacin (Indocid®, 150 mg daily), diclofenac sodium (Voltaren®, 200 mg daily), flurbiprofen (Froben®, 200 mg daily), and sulindac (Clinoril®, 400 mg daily).

Data of fourteen NSAID treatments, preceded by a one to two week period without antiphlogistic drugs were available for statistical analysis. And the effects of NSAID's dose modification were tested in patients 1, 2 and 5 (indomethacin once, diclofenac sodium once and flurbiprofen twice). During the last three days of a NSAID treatment or of a wash-out period, urine was collected daily for determination of protein and PGE_2. The patients were asked to store the urine at 4°C immediately after voiding. The next day GFR and ERPF were measured in supine position using continuous infusion of ^{125}I-iothalamate and ^{131}I-hippuran, respectively (14). That day blood was drawn for plasma renin activity (PRA) after three hours rest.

Laboratory methods.

Urinary protein was measured by the biuret method.

Prostaglandins were measured by radioimmunoassay. A survey of the literature and our own experience indicated that immunogens prepared by coupling prostaglandin to an immunogenic protein using carbod-

imides resulted in antisera of poor specificity. The mixed anhydride method using isobutyl chloroformate gave solubility problems: in low concentrations of organic solvent in the reaction mixture, the prostaglandins precipitated; at higher concentrations of organic solvent, the protein denatured. We found that the protein thyroglobulin is exceedingly soluble in high concentrations of dimethyl formamide (DMF) and could be recovered in immunogenic form upon removal of the dimethyl formamide by dialysis. This observation formed the basis of the following synthetic method.

Dimethyl formamide was rendered amine- and water-free by storage over P_2O_5 under vacuum for about ten days. It was then cooled in an ice-water bath. Bovine thyroglobulin was dissolved in water to a concentration of 10 mg per ml and cooled to 0°C. To one volume of the thyroglobulin solution, four volumes of the ice-cold DMF were added with vigorous stirring. This was solution A. It was prepared 15 minutes before use.

Two mg of prostaglandin (5.6 μmole) plus a trace amount of the corresponding ^{3}H-labelled prostaglandin were dissolved in 0.5 ml of ice-cold DMF. Three microliters of tri-n-butylamine in 0.1 ml DMF and 0.75 microliter of isobutyl chloroformate (6.75 μmole) in 0.1 ml DMF were added. This was solution B. It was incubated at 0°C for 30 min.

Solution B was then added to 10 ml of solution A with vigorous stirring. Eight hundred and ten g of NaOH (20.3 μmole) in 0.1 ml of water was added. The mixture was maintained at 0°C for two hours and then dialysed against 3 x 5 liters of the following solution: NaCl:0.15 M, $NaCO_3$:0.03 M. This solution must be pre-cooled to 4°C before dialysis begins. The immunogen was stored at –80°C until use. It contained between 30 and 50 prostaglandin residues per molecule of thyroglobulin. Rabbits were repeatedly immunized with a suspension of the immunogen in Freund's complete adjuvant (100 μg thyroglobulin per rabbit per immunization).

Each urine sample was extracted using the Sepak®-C_{18} system (Waters Associates Inc.) after adding a tritium-labelled recovery standard. The samples were subjected to purification on LH-Sephadex® used in reversed-phase mode. The eluent was 1.5 M ammonium sulphate in water. Prostaglandins $F_{2\alpha}$ and E_2 were co-eluted. The specificities of the antisera were sufficient to assay both prostaglandins in this fraction without further purification. Using this 5 chromatography system, PGE_2 and $PGF_{2\alpha}$ were separated from PGE_1 and $PGF_{1\alpha}$ (which was also co-eluted) and from all other readily available analogues and metabolites. The intra-assay and inter-assay variation coefficients were 9% and 15%, respectively. The results of radioimmunoassays for PGE_2 and $PGF_{2\alpha}$ using the reversed-phase LH-Sephadex purification fulfilled the usual criteria for analytical accuracy and correlated well with the results of assays using high performance liquid chromatography.

PRA was determined by a radioimmunoassay (15).

For protein and PGE_2 excretions the means of three 24-hour urine collections are given.

For determination of correlation the Spearman Rank test was used.

Results

Proteinuria, PGE_2 excretion, PRA and GFR during the various wash-out periods did not vary by more than 10% except during the fourth wash-out in patient 1 (Figure 1).

The effect of seven to ten days of treatment with the various NSAID on proteinuria in all subjects is shown in Figure 2. Indomethacin, diclofenac-sodium and flurbiprofen induced a marked decrease in proteinuria. During treatment with sulindac, however, no significant change in proteinuria was noted.

In Table II the percentage changes in proteinuria, GFR, PRA and urinary PGE_2 excretion during the treatment with sulindac and with the three other NSAID are given. The median fall of proteinuria, GFR, PRA and urinary PGE_2 excretion observed during treatment with the antiproteinuric NSAID is 59, 16, 55

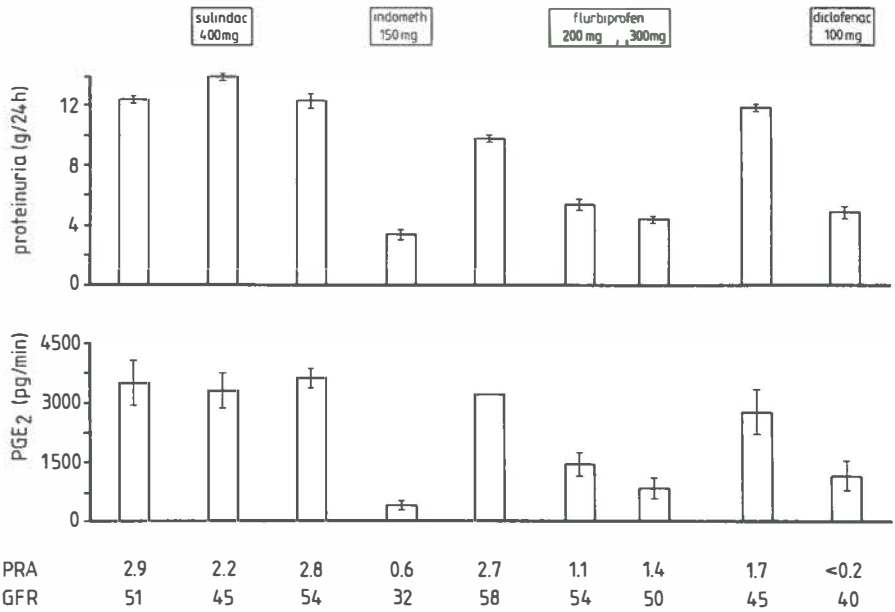

Figure 1. The effect of 4 different NSAID on proteinuria, PGE_2 excretion, PRA and GFR in patient 1.

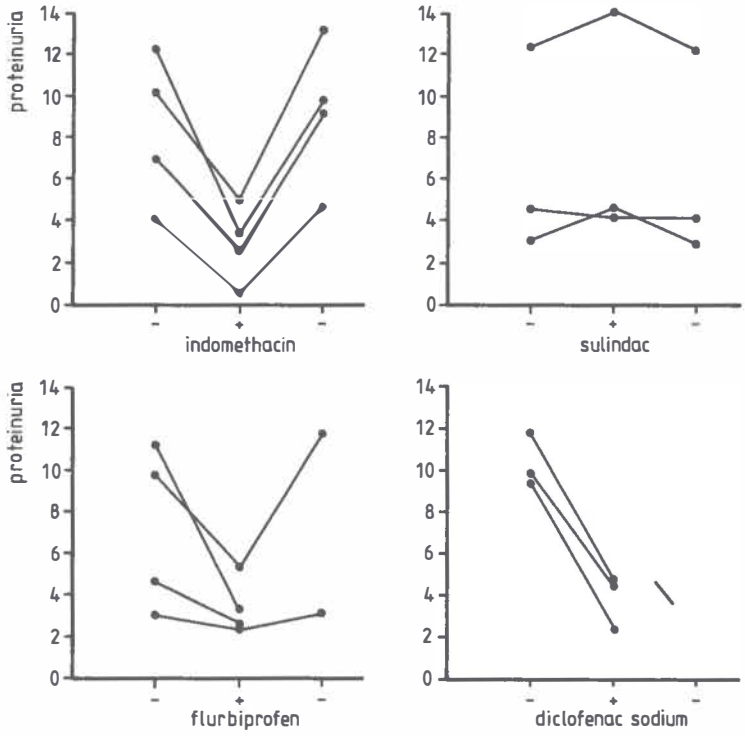

Figure 2. Proteinuria before, during, and after treatment with indomethacin, sulindac, flurbiprofen and diclofenac-sodium.

and 68%, respectively (Table II). During treatment with sulindac no major change in GFR and PGE_2 excretion is found. An exact order of the various NSAID to their antiproteinuric effect cannot be given due to the small numbers in this study. The changes during sulindac, however, are the smallest in every parameter tested.

The results in an individual patient are given in Figure 1 which demonstrates the

Table II. Median change in proteinuria, GFR, PRA and urinary PGE_2 excretion after seven to ten days NSAID treatment, expressed as the percentage of the previous wash-out period. (Between brackets the range is indicated)

	Proteinuria	GFR	PRA	Urinary PGE_2 excretion
Sulindac (n = 3)	13 (+45 to −12)	−13 (0 to −19)	−24 (6 to −38)	+1 (+9 to −7)
Other NSAID (n = 11)	−59 (−18 to −88)	−16 (0 to −40)	−55 (10 to −88)	−68 (−37 to −89)

acute and parallel changes in proteinuria, GFR, PRA and PGE_2 excretion. In this patient the dose of flurbiprofen was increased without a preceding NSAID wash-out. As is shown proteinuria, urinary PGE_2 excretion, PRA and GFR still further declined. Similar results were obtained in the other patients in whom different doses of indomethacin, diclofenac-sodium and flurbiprofen were tested. The higher dose elicited a more profound decrease in proteinuria, in renal PGE_2, in PRA and in GFR.

In Figure 3 the proportional fall in proteinuria is plotted versus the percentage decrease in GFR. During all NSAID treatments in which a reduction of proteinuria was noticed, the relative fall in proteinuria exceeded that of GFR.

In Figure 4 the relationship between the proportional decrease in proteinuria and that of the renal PGE_2 is shown. There existed a strong positive correlation ($R = 0.89$, $p < 0.001$). The larger the decrease in PGE_2 excretion, the more marked was the change in proteinuria. Also a positive significant correlation was found between the proportional decrease in PGE_2 excretion and that in GFR ($R = 0.70$, $p < 0.05$).

Finally, the absolute change in PGE_2 excretion was significantly related with that in log PRA ($R = 0.62$, $p < 0.05$) demonstrating the interplay of the prostaglandin system with the renin-angiotensin axis in these patients with the nephrotic syndrome.

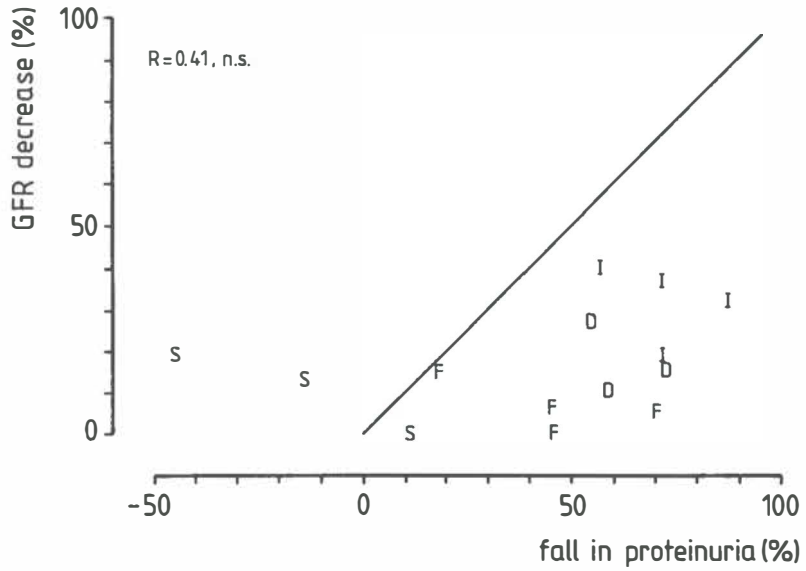

Figure 3. The relation between the percentage decrease in GFR and the percentage fall in proteinuria induced by 4 differents NSAID's. The line of identity is drawn. See Table I for explanation of symbols.

Discussion

In this study a reduction of proteinuria was induced by indomethacin by diclofenac-sodium and by flurbiprofen but not by sulindac. A fall in proteinuria was accompanied by a decrease in renal PGE_2 excretion, GFR and PRA. Our observations confirm and extend earlier reports of Lianos et al (16) and Gutierrez et al (17). Both demonstrated a concordant fall in proteinuria, PGE_2 excretion and GFR in nephrotic patients during treatment with indomethacin. Our findings are also in accordance with the data of Ciabattoni et al (12). They showed that sulindac did not influence PGE_2 excretion and GFR in patients with chronic glomerular disease. On the other hand some studies found that sulindac inhibits PGE_2 excretion after furosemide stimulated prostaglandin synthesis (18). Brater's suggestion that different pathologies and models have different susceptibilities to prostaglandin synthesis' inhibition by different NSAID can well explain these contradictory findings. Other arguments for this assumption are the discrepancy between the platelet and renal prostaglandin synthesis' inhibition of sulindac (12, 18) and the dose-related phenomena of NSAID in salt depleted nephrotic patients noted by Wijdeveld (19) and also suggested in this study.

It is generally accepted that in patients with reduced renal mass or low volume states renal blood flow and GFR are at least partially dependent on the integrity of the prostaglandin system (20). This is probably the case in salt depleted patients with the nephrotic syndrome. With this assumption the observed relationships between the changes of the renal PGE_2 excretion with those of GFR and PRA during NSAID treatment fit well.

In a previous study we reported that in salt depleted nephrotic patients the fall of GFR significantly correlated with the reduction in urinary protein loss during either indomethacin or naproxen treatment (4). In the present study the relationship between the decrease of GFR and proteinuria did not reach significant levels. However, like in the previous study, NSAID, except sulindac, solicited a larger reduction in proteinuria than in GFR (Figure 3). This, the fall in GFR may not fully account for the reduction in urinary protein loss.

The most important observation in the study is the significant correlation between the falls in proteinuria and renal PGE_2 excretion ($R = 0.89$, Figure 4). This correlation supports the hypothesis that the antiproteinuric effect of NSAID is related to their property to inhibit renal prostaglandin synthesis.

As the inhibition of renal prostaglandin synthesis plays a major role in the reduction of proteinuria by NSAID in salt-depleted nephrotic patients several pathophysiologic explanations should be considered. Indomethacin is known to promote tubular reabsorption (21). So, enhancement of the tubular reabsorption capacity for albumen might at least partially explain the antiproteinuric effect of NSAID. If this mechanism plays an important role, some phenomena are not

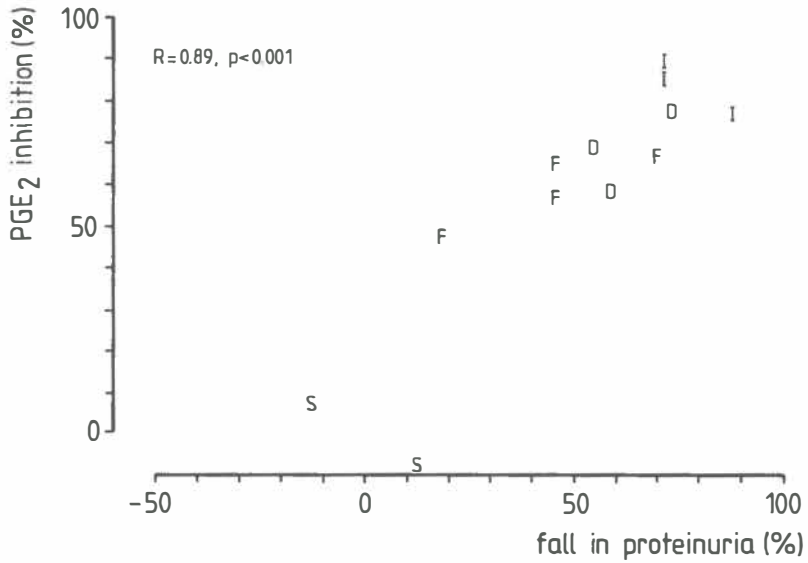

Figure 4. The relation between the percentage decrease in PGE_2 excretion and the percentage fall in proteinuria by 4 different NSAID's. For unknown reasons the urine collections for the PGE_2 excretions during two periods of NSAID treatment were lost. See Table I for explanation of symbols.

accounted for: Why does indirect stimulation of the proximal reabsorption by salt restriction and/or hydrochlorothiazide not reduce urinary protein loss (5), and why is the proteinuria during NSAID treatment more selective than before treatment (7, 8)? The latter observation focuses to NSAID-induced changes in the glomerular basement membrane (GBM) level. Renal prostaglandins and renal vasopressors govern glomerular transcapillary hydraulic pressure. An elevated pressure over a diseased GBM enhanced the glomerular protein loss by facilitation of the macromolecular diffusion. Reduction of this pressure can then explain the fall in GFR, the decrease in proteinuria, the relationship between the changes in GFR and proteinuria, as well as the increased in selectivity of the residual urinary protein loss.

NSAID, however, might not only interfere with the size selectivity, but also with the charge selectivity of the GBM. Suzuki et al (22) recently reported a decrement of polyamin content of the GBM in nephrotic rats after treatment with azapropazone, a pyrazolone derivative, chemically related to phenylbutazone.

In conclusion, in this study the antiproteinuric effect of NSAID was related to their potency to inhibit renal prostglandin synthesis. It is suggested that this effect is mainly mediated by lowering transcapillary hydraulic pressure.

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CHAPTER 4

THE INFLUENCE OF INDOMETHACIN ON DECLINE AND FINAL OUTCOME OF RENAL FUNCTION IN NEPHROTIC PATIENTS

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Submitted.

Running head: Indomethacin and renal function in nephrotic patients.

Key words: Indomethacin - Nephrotic syndrome - Renal function - Proteinuria - Renal failure

Abstract. In this retrospective study of 98 nephrotic patients treatment with indomethacin (n = 58) significantly delayed the development of terminal renal failure compared to a non-treated group (n = 40). After ten years follow-up only 34 per cent of the treated patients needed renal function replacement therapy in contrast to 69 per cent of the untreated patients ($p < 0.05$). Testing for differences between the two groups with respect to blood pressure control and urinary urea excretion did not reveal any significant differences. Besides treatment with indomethacin the amount of proteinuria and the histological diagnosis did significantly influence the rate of renal function decline. Heavy proteinuria and membranoproliferative glomerulonephritis were associated with a rapid doubling of serum creatinine concentration.

These retrospective findings suggest that nonsteroidal antiinflammatory drugs ultimately might preserve renal function in patients with non-steroid sensitive nephrotic syndrome.

Introduction

Nonsteroidal antiinflammatory drugs (NSAID) - in particular indomethacin - are effective antiproteinuric agents in salt-depleted nephrotic patients (1-5). The reduction of the proteinuria is accompanied by a decrease in glomerular filtration rate (GFR) (3, 4). It is not known whether this early decrement in renal function ultimately results in irreversible renal function loss. However, when indomethacin is withheld after one to three years of administration, the proteinuria and GFR reverse to pretreatment levels (3). Furthermore, several reports include nephrotic patients with adequate renal function after NSAID treatment for years (2, 5).

Proteinuria appears to be an important determinant of the rate of renal function decline as the degree of urinary protein loss is reported to have prognostic significance in patients with membranous glomerulopathy (MGP), focal glomerulosclerosis (FG) and membrano-proliferative glomerulonephritis (MPGN) (2, 5-8).

Single nephron hyperfiltration and altered glomerular permeability enhance the development of proteinuria and glomerulosclerosis. They also accelerate the decline of renal function (9). A decrease of the proteinuria by NSAID then might reflect a reduction of single nephron hyperfiltration and a decrease in glomerular permeability for macromolecules. This might ultimately lead to preservation of renal function in patients with the nephrotic syndrome. Therefore, we retrospectively compared the final renal outcome of indomethacin-treated and untreated nephrotic patients with MGP, FG and MPGN, and analysed the factors influencing the rate of kidney function decline.

Patients and methods

Since 1968, we treated many nephrotic patients, who were expected not to benefit from corticosteroid treatment, with indomethacin in order to reduce proteinuria and to raise serum albumin. We now retrospectively evaluated the course of renal function in these indomethacin-treated patients in comparison with a group of patients with the nephrotic syndrome who did not receive any NSAID.

The records of all patients who had undergone renal biopsy in our department from January 1st, 1968, to December 31th, 1982, were reviewed. Subsequently, 354 patients with proteinuria of more than 3 g per day were identified (table I). The distribution of histological diagnoses given in table I does not represent a normal distribution of causes of the nephrotic syndrome in a general nephrology unit. As we only evaluated patients in whom a renal biopsy was performed (thus

Table 1. Distribution of diagnosis in 354 patients with proteinuria of more than 3 g per day, who underwent renal biopsy from January 1, 1968, to December 31, 1982, at the University Hospital Groningen, The Netherlands.

Idiopathic membranous glomerulopathy	49
Membrano-proliferative glomerulonephritis	34
Focal glomerulosclerosis	31
"Minimal lesions" glomerulopathy	47
Systemic vasculitis	32
Renal amyloidosis	28
Local and focal glomerulonephritis	20
Chronic glomerulonephritis	20
IgA-nephropathy	19
Acute glomerulonephritis	16
Hypertensive sclerosis	10
Drug-related membranous glomerulopathy	9
Familial renal disease	7
Diabetic glomerulosclerosis	7
Other diagnosis	6
No diagnosis	19

excluding many diabetics) and as our unit is a referral centre, particularly for patients with amyloidosis and systemic vasculitis, these diseases are probably under- and overpresented, respectively. For the present study we only included patients with a clinical presentation and a histological diagnosis compatible with idiopathic MGP, FG and MPGN (n = 114). Patients in these 3 diagnosis groups were frequently treated with indomethacin in our unit in an attempt to lower urinary protein excretion. Although debate exists about the potential benefit of corticosteroids and/or immunosuppressive drugs in membraneous glomerulopathy (10), we did not treat those patients with these agents.

During the study period from 1968 to 1982, criteria for indomethacin treatment were: 1. signs of severe nephrotic syndrome, either clinically (severe oedema) or biochemically (proteinuria of more than 5 g per day and/or a low serum albumin), 2. glomerular filtration rate of more than 30 ml/min, and 3. no signs of active or recent peptic ulcer. After 1974, these indications for treatment with indomethacin tended to broaden somewhat: proteinuric patients without severe oedema or without a low serum albumin were sometimes treated with indomethacin. Also patients with a more impaired renal function were given indomethacin when a severe nephrotic condition was present, and sometimes patients with a gastrointestinal history were treated with indomethacin when gastric acid secretion had been suppressed adequately.

Arbitrarily, indomethacin treatment of at least half a year was chosen as adequate exposition to this agent for evaluation of its long-term effect on renal function. Thus, another 16 patients were excluded from the study because indomethacin treatment (n = 13) or follow-up (n = 3) did not extend over six months. Of the 98 remaining patients, 58 were treated with indomethacin for

more than six months. Seven patients received indomethacin from 6 to 12 months, 17 for 1 to 2 years and the other 34 patients for more than 2 years. The control group consisted of 40 patients.

The records of the 98 evaluated patients (86 per cent of the eligible patients) were reviewed with regard to age, sex, serum creatinine, serum albumin and cholesterol, mean arterial pressure (MAP), proteinuria, creatinine clearance, and urea excretion. The date of entry was set as the date of biopsy (controls, n = 40) or the last day before indomethacin treatment started (n = 58). All patients had been asked to adhere to a 80-100 g protein, low salt diet. In addition, they were treated with diuretics, mostly hydrochlorothiazide 25-50 mg a day. Antihypertensive treatment was prescribed to both groups of patients if needed, with a goal diastolic blood pressure of less than 95 mm Hg. Angiotensin converting enzyme inhibitors were given to only two patients in the control group. In the indomethacin-treated group, the NSAID was generally given in a dose of 150 mg per day (dose range 75-225 mg per day). The levels of MAP, serum creatinine, 24 hour protein and urea excretion, and creatinine clearance were recorded each half year in the first five years of follow-up and each year thereafter till either the end of indomethacin treatment, renal insufficiency requiring hemodialysis treatment, or death. Follow-up extended to January 1st, 1984.

Indomethacin treatment was tested for its effect on final renal outcome by the renal survival time, defined as the time between the date of entry into the study and the date of start of hemodialysis or death related to end-stage renal failure.

Indomethacin treatment, serum creatinine, MAP, proteinuria, serum albumin, age, sex and diagnosis were separately assessed in all 98 patients for their effect on renal function decline. For all these variables, except the diagnosis, two classes were made. For serum creatinine the classes were split at 110 μmol per litre, for MAP at 107 mm Hg, for proteinuria at 5 g per day, and for serum albumin at 30 g per litre. As measure of renal function decline the creatinine doubling time was chosen, defined as the permanent occurrence of a serum creatinine concentration twice the value of the reference level. The reference value for the controls was the serum creatinine level at entry into the study, the reference of the indomethacin-treated patients was the serum creatinine concentration after approximately one month of indomethacin administration in order to allow for the initial increase in serum creatinine after the institution of indomethacin (3). Thus, in the treated patients only serum creatinine values during indomethacin therapy were taken into account. Life-tables of renal survival and creatinine doubling time were analysed by the log-rank test, as described by Peto et al (11). All endpoints or "events" in the renal survival curves were defined as above. However, in the serum creatinine doubling times' curves also another endpoint than the above defined was included. If indomethacin was withheld for renal toxicity before doubling of serum creatinine occurred the patient was scored as

"event". In case of indomethacin withdrawal for extrarenal toxicity (in five patients because of gastrointestinal side effects - see also result section - and in one patient because of dizziness and headache) or for spontaneous remission (n = 8) a patient was scored as "lost" in the serum creatinine doubling time life-table.

The groups of patients to be tested by log-rank analysis were evaluated for differences in patient characteristics by the two-tailed Wilcoxon test. If significant differences in patient characteristics between the groups of patients occurred, each group of patients was divided into the classes of every significantly different patient characteristic. The extent of exposure was calculated in all classes of each group of patients. The ultimate extent of exposure in a group of patients was then determined by the addition of the extents of exposure in the classes which were part of that group of patients.

In any test a level of $p < 0.05$ was considered statistically significant.

Serum and urinary creatinine concentration, urinary urea concentration, serum albumin and serum cholesterol were routinely measured by a computerized multichannel autoanalyser. Urinary protein excretion was measured by biuret method in 24 hour urine collections.

Results

From table II it is evident that the patients characteristics at entry are not randomly distributed over the control group and the indomethacine-treated group in this retrospective study. Serum creatinine was lower and proteinuria was more severe in the indomethacin-treated patients at entry (thus, before indomethacin was given). This reflects our policy of treating particularly those patients with a more pronounced nephrotic syndrome and with a higher initial glomerular filtration rate. The range of these two parameters, however, is very wide, reflecting that these criteria were not taken too strictly. MAP at entry was higher in the untreated patients. Urinary urea excretion was lower in the control group. No statistical significant differences existed in creatinine clearance, serum albumin, serum cholesterol, age, sex ratio or diagnosis ratio between both groups.

The data given in table II moreover show the well known *short-term* effects of indomethacin. Median proteinuria had decreased from 8.2 (range 3.0 to 26.2) before indomethacin treatment to 3.8 (0.0 to 10.7) g/24 h ($p < 0.05$) after one mouth of treatment, while serum albumin had increased from 27 (12 to 48) to 30 (15 to 46) g/l ($p < 0.05$) and serum creatinine from 92 (35-327) to 120 (53 to 409) μmol/l ($p < 0.05$). As a consequence, after one month of treatment (at reference time) renal function was not different between both groups and only MAP and proteinuria were significantly lower in the indomethacin-treated group.

Table II. Characteristics of the indomethacin-treated and untreated patients at entry into the study and at the reference time after one month of indomethacin treatment (median and range).

	untreated patients	indomethacin-treated patients			
	at entry	at entry	p**	at reference time	p**
number of patients	40	58		58	
Serum creatinine (μmol/l)	115 (59-566)	92 (35-327)	<0.05	120 (53-409)	n.s.
Creatinine clearance (ml/min)	77 (10-183)	90 (16-236)	n.s.	81 (17-158)	n.s.
MAP (mm Hg)	107.5 (90-160)	105.8 (80-132)	<0.05	103.3 (77-143)	<0.05
Proteinuria (g/24 h)	5.7 (3.0-15.5)	8.2 (3.0-26.2)	<0.05	3.8 (0.0-10.7)	<0.05
Urinary urea excretion (mmol/24 h)	283 (67-550)	333 (167-767)	<0.05		
Serum albumin (g/l)	28 (12-46)	27 (12-48)	n.s.	30 (15-46)	n.s.
Serum cholesterol (mmol/l)	7.83 (2.79-20.90)	8.25 (3.63-14.76)	n.s.		
Age (years)	36 (14-79)	35 (14-70)	n.s.	35 (14-70)	n.s.
Sex ratio (M/F)	27/13	32/26	n.s.	32/26	n.s.
Diagnosis ratio*	14/10/16	28/17/13	n.s.	28/17/13	n.s.

* diagnosis ratio shows the number of patients with MGP, FG and MPGN, respectively.
** value of comparison between indomethacin-treated and untreated patients.
n.s. = not significant, M = male, F = female.

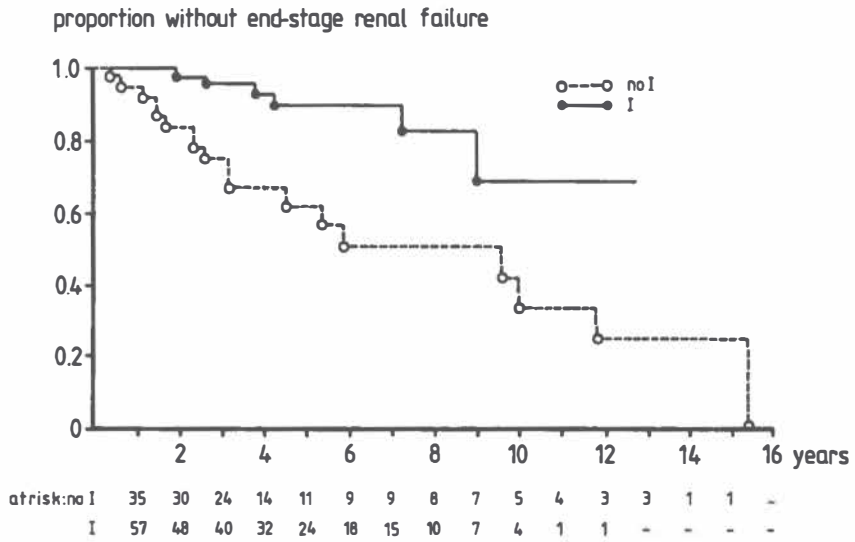

Figure 1. Renal survival of indomethacin-treated and untreated nephrotic patients with MGP, FG or MPGN. I and no I denote patients with and without indomethacin therapy, respectively.

During the long-term follow-up of the effects of indomethacin on renal function the frequency of visits to the out-patient clinic were similar in both groups. The patients who received indomethacin were seen 4.6 (2-13) and the patients from the control group 4.5 (1.5-12) times a year. With respect to the *long-term* effects of indomethacin on renal function, figure 1 shows the renal survival curves of the indomethacin-treated and untreated patients. At five years follow-up, 10 per cent of the treated patients and 38 per cent of the untreated group had entered terminal renal failure. At ten years follow-up the renal survival difference between both groups of patients had further increased: twice as many untreated patients required renal replacement therapy (69 vs 34%). Allowing for different classes of serum creatinine, MAP, and proteinuria at entry (table II), the difference between both renal survival rates was significant ($p < 0.05$).

When the patient variables were tested for their influence on renal function decline, only proteinuria and diagnosis could divide the 98 patients into groups with significantly different creatinine doubling times. In figure 2 the curves of the patients with proteinuria of less or more than 5 g per day are drawn. The high proteinuric patients doubled their serum creatinine earlier than the patients with a low urinary protein loss ($p < 0.05$). Also the decline in renal function between the three diagnostic groups differed significantly ($p < 0.05$) (figure 3). Patients with MPGN doubled their serum creatinine significantly faster than patients with

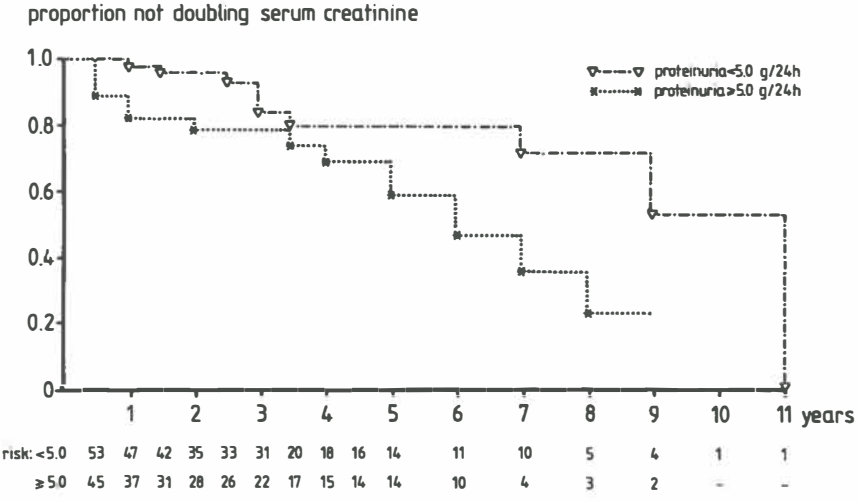

Figure 2. Serum creatinine doubling time in patients with more or less than 5 g urinary protein loss per day. The selection of the indomethacin-treated patients for both groups was made according to the amount of proteinuria after approximately one month of treatment.

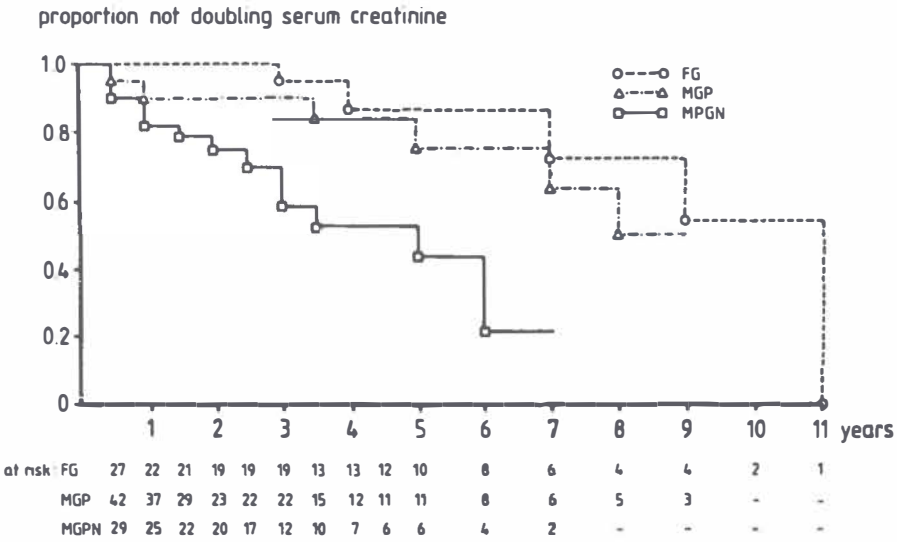

Figure 3. Serum creatinine doubling in nephrotic patients with MGP. FG and MPGN.

MGP or FG. No difference between the creatinine doubling curves of the patients with MGP and with FG was observed. Because we made use of classes for the patient characteristics that are maldistributed over the tested groups of patients, proteinuria and MPGN are probably independent prognostic factors for the decline of renal function in the studied nephrotic patients. This supposition is strengthened by the observations that no significant maldistribution of the

Table III. Characteristics of the patients with more and with less proteinuria than 5 g per day (median and range). For the indomethacin-treated patients the values after one month of treatment are included.

		proteinuria < 5.0 g/24 h	proteinuria ≥ 5.0 g/24 h	p
number of patients		53	45	
serum creatinine	(μmol/l)	115 (53-292)	109 (62-566)	n.s.
MAP	(mm Hg)	103.3 (83.3-153.3)	106.7 (76.7-160,0)	n.s.
proteinuria	(g/24 h)	2.9 (0.0-4.8)	7.4 (5.0-15.5)	-
albumin	(g/l)	32 (23-46)	26 (12-43)	<0.05
age	(years)	32 (14-70)	38 (14-79)	n.s.
sex ratio	(M/F)	33/20	26/19	n.s.
diagnosis ratio*		21/16/16	21/11/13	n.s.
indomethacin ratio**		18/35	22/23	n.s.

* see table II.

** indomethacin ratio denotes the number of patients in the untreated and in the indomethacin-treated group of patients.

Table IV. Patients characteristics in the three diagnosis group (median and range). For the indomethacin-treated patients the values after one month of treatment are included.

		MGP	FG	MPGN
number of patients		42	27	29
serum creatinine	(μmol/l)	107 (56-409)	126 (53-230)	124 (71-566)
MAP	(mm Hg)	105.0 (76.7-143.3)	105.0 (90.0-153.3)	110.0 (83.3-160.0)
proteinuria	(g/24 h)	4.9 (0.4-11.3)	4.1 (0.1-10.8)	4.3 (0.0-15.5)
serum albumin	(g/l)	30 (14-38)	31 (20-45)	30 (12-46)
age	(years)	40 (14-70)	32 (15-79)	29 (14-78)*
sex ratio	(M/F)	26/16	16/11	17/12
indomethacin ratio**		14/28	10/17	16/13

* differs significantly from MGP ($p < 0.05$)
** see table III.

MPGN patients appeared in the proteinuric classes (table III) and that the median proteinuria was approximately equal in the MGP, FG and MPGN patients (table IV).

The indomethacin-treated patients doubled their serum creatinine at a lower rate than their untreated counterparts (figure 4). After ten years 62 per cent of the treated versus 22 per cent of the untreated patients had not doubled their serum creatinine concentration. However, taking the maldistribution of MAP- and proteinuria over both groups into account (see table II), this difference was not statistically significant ($0.2 < p < 0.3$, figure 4). When evaluating the effect of indomethacin on the course of creatinine clearance a similar result was observed.

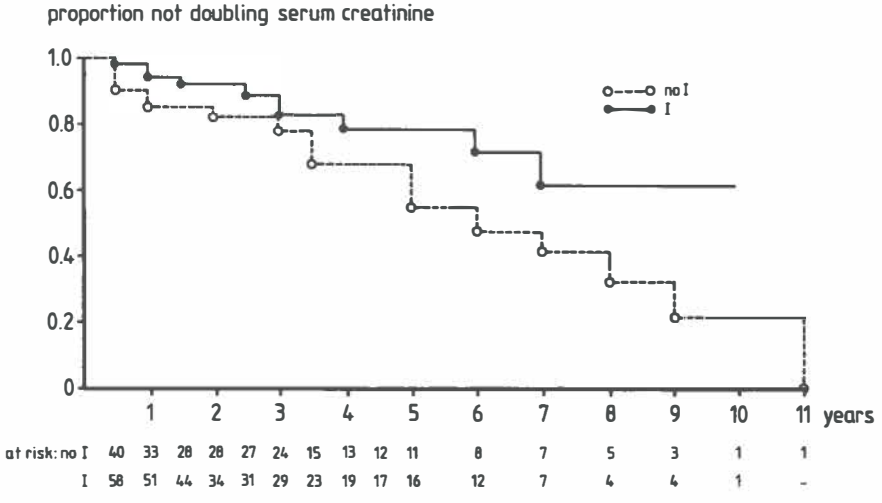

Figure 4. Serum creatinine doubling in nephrotic patients with and without indomethacin treatment.

After 10 years, creatinine clearance had not fallen by more than 50 per cent in 44 per cent of the indomethacin-treated patients versus 14 per cent of the untreated patients. Allowing for MAP and proteinuria classes the difference again, was not statistically significant.

Next we investigated whether indomethacin therapy delayed serum creatinine doubling in certain subsets of patients. All classes of the various patient characteristics at entry were studied, but only in patients with a serum creatinine level below 110 μmol per litre the indomethacin-treated patients fared significantly better than their untreated controls (figure 5). Some improvement of creatinine doubling time was noted in several other subsets of patients, e.g. those with proteinuria in excess of 5 g per day, those with a serum albumin below 30 g per litre, those younger than 35 years, in women and in MPGN patients. However, in all these subsets the difference between the treated and untreated patients did not reach statistically significant levels. As only proteinuria and diagnosis had prognostic significance for renal function decline in this study, one may wonder whether the distribution of the MPGN patients over the treated and control patients influenced the renal survival curves (figure 1) and the creatinine doubling time curves of patients with a serum creatinine less than 110 μmol (figure 5). Allowing for proteinuria and diagnosis classes at entry, the renal survival of the treated and untreated patients remained statistically different ($p < 0.01$). However, the difference of the creatinine doubling time curves of both groups lost its significant level ($0.1 < p < 0.2$), when both prognostic factors were

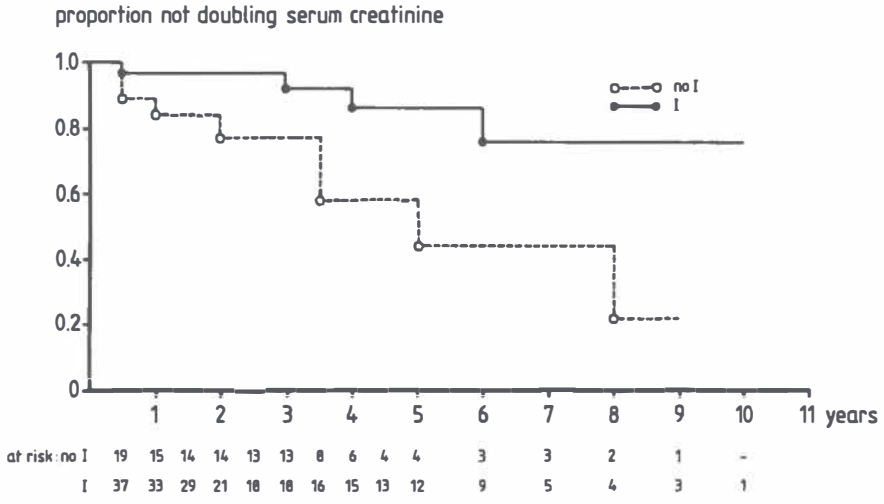

Figure 5. Serum creatinine doubling in the subset of patients with a serum creatinine concentration less than 110 μmol/l at entry into the study.

taken into account in the statistical analysis. So, it is likely that the poor distribution of MPGN-patients has no impact on the renal survival curves (figure 1), but is partially responsible for the difference of the creatinine doubling time in the treated and control patients with a serum creatinine less than 110 μmol/l at entry (figure 5).

It can be questioned whether the proteinuria in the treated patients was effectively suppressed by long-term treatment with indomethacin. The reduction of urinary protein loss after one month of treatment (table II) persisted in the treated patients during follow-up (figure 6). Their median fall in proteinuria was significantly different from that in the untreated patients at nearly all intervals of follow-up (figure 6, $p < 0.05$). However, at any interval a wide overlapping range was noted in both treatment groups.

Data on other confounding factors, which could influence the final renal outcome in patients with renal disease are given in table V. Although at entry into the study MAP was higher in the untreated controls as compared to the indomethacin-treated patients, this difference was no longer present at 1, 3 and 5 years of follow-up. During the follow-up, MAP also was never significantly different from the entry value, neither in the controls nor in the indomethacin-treated patients. Urinary urea excretion, which can be used as an estimate for protein intake, never was lower in the indomethacin-treated patients. In fact,

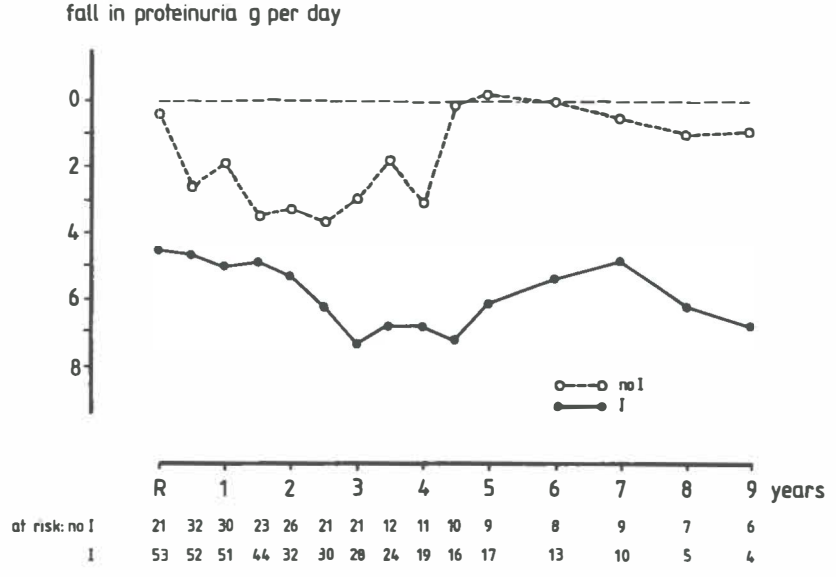

Figure 6. The median fall of proteinuria in indomethacin-treated and untreated patients during the study. R denotes reference time e.g. after approximately one month of treatment.

Table V. Characteristics of the two patients groups during the observation period (median and range). The statistical significance of the difference between the two groups at any time is given.

	Entry	1 year	3 year	5 year
MAP (mm Hg)				
controls	107.5 (90-160)	108.3 (83-147)	103.3 (83-140)	113.3 (103-123)
indomethacin	105.8 (80-132)	103.3 (75-133)	106.7 (67-147)	106.7 (87-147)
p	<0.05	n.s.	n.s.	n.s.
urinary urea excretion (mmol/24 hr)				
controls	283 (67-550)	233 (67-533)	300 (200-550)	289 (90-333)
indomethacin	333 (167-767)	330 (167-533)	340 (120-633)	350 (230-733)
p	<0.05	<0.01	n.s.	<0.02

urea excretion was significantly higher in the indomethacin group at entry and at 1 and 5 years of follow-up. In both groups urea excretion was never significantly different compared to the value at entry.

NSAID treatment is frequently associated with gastrointestinal side effects (12, 13). As mentioned in the patients and methods section, we were reluctant to prescribe indomethacin to patients with a history of active ulcer disease. As a consequence, only 2 out of the 58 patients in the indomethacin group had experienced peptic ulcer prior to entry into the study, in contrast to 9 out of the 40 patients in the control group. During the study period 6 patients in the indomethacin group developed a peptic ulcer and 2 had biopsy proven gastritis. Thus, 8 out of the 58 patients (= 14 per cent) had documented gastrointestinal side effects. Four additional patients had gastrointestinal complaints with normal gastroscopy findings. In 5 out of these 12 patients indomethacin was withdrawn because of these gastrointestinal side-effects. During the study period 3 patients from the control group experienced peptic ulcer and one had documented gastritis, together 10 per cent of the control population.

NSAID have also been reported to cause acute renal toxicity (14, 15). We observed acute deterioration of renal function (defined as a fall in creatinine clearance to less than 50 per cent of the pre-treatment value within one week of treatment) in 4 patients. In these 4 subjects withdrawal of indomethacin resulted in all cases in a prompt reversal of that renal functional deterioration. Indomethacin was restarted either at lower dosis (n = 2), or after correction of a pre-existing volume depletion (n = 2), and indomethacin was then well-tolerated with respect to renal function.

Discussion

In this study two groups of patients were retrospectively evaluated with respect

to the long-term effects of indomethacin treatment on renal function outcome. The patients in the indomethacin group had a lower pre-treatment serum creatinine and mean arterial pressure than the control group. This could indicate that renal disease in this group was less severe than in the control group. However, 24 hour urinary protein excretion before treatment was significantly higher in the indomethacin group, which may imply a more worse prognosis. We showed in the present study that patients with a higher protein excretion showed an enhanced rate of decline in renal function independent whether indomethacin was given or not. In contrast, neither serum creatinine level, nor mean arterial pressure had any influence on renal function decline. Thus, we argue that the patient group that was going to receive indomethacin was, based upon entry criteria, not expected to fare a better course than the control group.

At ten years follow-up twice as many treated as untreated patients required no renal replacement therapy (69% versus 34%, see figure 1). Additionally, a similar proportion of both groups, 62 and 22 per cent, respectively, had not doubled their serum creatinine after ten years follow-up. However, the difference in the latter criterion did not reach statistical significance. One could question whether serum creatinine represents an adequate measure for renal function in patients with the nephrotic syndrome. However, when evaluating changes in creatinine clearance, the effects of indomethacin were nearly identical. Alternatively, the discrepancy between the renal survival and the creatinine doubling could also be due to the fact that in the creatinine doubling time life-tables the indomethacin-treated patients were taken into account only during their treatment and not thereafter as in the renal survival time curves. Moreover, the criteria used for "event" and "lost" in the creatinine doubling time life-table play also a role as some of the criteria account only for the treated patients. Drug withdrawal for renal toxicity before serum creatinine doubling was scored as "event" and spontaneous remissions as "lost" in the treated patients, but not in the controls. Both criteria lead to a proportionally high rate of events in the treated patients. So, this use of event and loss criteria definitely underestimates the beneficial effect of indomethacin on renal function preservation in the studied patients.

As reported in the literature the rate of renal failure loss in the diagnostic groups ranges widely, undoubtedly due to a variance in diagnostic criteria, patient characteristics and forms of therapy. Still the renal survival data in our groups of patients correspond remarkably well with some of comparable groups in the literature. The clinical course of 64 untreated patients with idiopathic membranous glomerulopathy was recently reported by Davidson et al (8). They observed a significant deterioration of kidney function in 43 per cent of their patients, fitting well in the quoted range of renal failure rates from 19 to 63 per cent. The renal failure rate in our MGP patients is 11 per cent for the

indomethacin-treated and 36 per cent for the untreated patients. We used nearly the same entry criteria and end points as the Collaborative Study of the Adult Idiopathic Nephrotic Syndrome, e.g. proteinuria more than 3.5 g per day and doubling of serum creatinine (16). Based on the data of the latter study, the cumulative proportion of treated (short-term prednisolone) and untreated patients with membranous glomerulopathy that not doubled their serum creatinine, could be calculated and compared with our results at five years. The similarity is striking: 55 and 56 per cent in the untreat and 92 and 88 per cent in the treated patients. Our patients with focal glomerulosclerosis had a relatively benign clinical course. At five and ten years follow-up 85 and 60 per cent of them were alive without terminal renal failure. In general these findings parallel the five and ten years actuarial survival of patients reported by Velosa et al (5), Beaufils et al (6) and Cameron et al (17). In our study, 77 per cent of the indomethacin-treated and 10 per cent of the untreated MPGN patients did not require renal function replacement therapy at ten years follow-up. Lagrue et al reported a ten years renal survival rate of 82 per cent for their NSAID-treated patients and 27-46 per cent for three series of untreated MPGN patients (18).

In the present study the degree of proteinuria was, besides the diagnosis, the only single factor that influenced the rate of decline in renal function significantly (figure 2). For the various diagnoses the prognostic significance of proteinuria on renal function outcome has been reported by several authors (2, 5-8). These investigations mainly concerned analysis by univariate techniques. In the only study using multivariate analysis, Tu et al found that proteinuria in patients with idiopathic membranous glomerulopathy was of significant importance in their univariate but not in their multivariate analysis (7). They accredited the hypoalbuminemia as the most important prognostic factor. In the present study serum albumin had no statistical significance as single prognostic factor. This apparent disagreement may be due to the different proportion of patients with a low level of serum albumin (less than 15 g/l): 25 per cent in Tu's series and 3 per cent in this study. Based on renin profiling it has been speculated that in nephrotic patients with low serum albumin and relatively high degrees of oedema other mechanisms of the regulation and preservation of the glomerular filtration rate are involved than in nephrotic patients with higher levels of serum albumin and lesser degrees of oedema (19).

Indomethacin can alleviate proteinuria and raise hypoalbuminemia in salt-depleted nephrotic patients (1-5). In this study proteinuria had declined after approximately one month of indomethacin therapy (table II). The observed reduction of proteinuria persisted in the treated patients during nine years of observation, while urinary protein loss hardly changed in the control patients (figure 6). So indomethacin apparently maintains its antiproteinuric effect during many years. Since urinary protein excretion immediately increases to pre-treat-

ment levels after withdrawal of indomethacin (3), this argues for a good compliance of the patients with the prescribed indomethacin. As proteinuria influences the rate of kidney decline unfavourably, it is not surprising that an antiproteinuric agent as indomethacin delays the doubling of serum creatinine considerably (figure 4). What other factors could have influenced our results? It has been suggested that a more strict follow-up of the patient by itself could result in a better outcome of renal survival (20). In our patient groups, however, no difference existed in the frequency of out-patient visits. Secondly, better blood pressure control has been found to improve the course of renal function impairment, at least in some studies (21). Although blood pressure at entry indeed was lower in the indomethacin-treated group, during the follow-up blood pressure regulation was similar in both groups. A more optimal blood pressure control in the indomethacin-treated patients was therefore not likely to contribute to the better renal survival in the indomethacin-treated patients. Thirdly, also the use of angiotensin converting enzyme inhibitors has been found to improve renal survival, at least in animal experiments (22). However, only two of our patients - in the control group - used captopril. Finally, restriction of protein intake has been advocated as a measure to delay renal function deterioration in patients with renal disease (23). In the indomethacin-treated group urinary urea excretion - a measure for protein intake - never was lower compared to the control group. Therefore, the better renal survival in the indomethacin-treated patients cannot be due to a lower protein intake in that group.

The observed beneficial effects of indomethacin on renal survival seem contradictory to the well known renal functional disturbances induced by NSAID (14, 15). Indeed in our study, 4 patients experienced a rapid fall in creatinine clearance during the first week of treatment. In all cases this was reversible after withdrawal and was not observed after correction of a coexisting volume depletion or during lower doses of the drug. One should, however, be aware of this potential risk. Also gastrointestinal discomfort is a common side effect of NSAID (12, 13). In fact in our study group the presence of previously active ulcer disease often was reason not to prescribe indomethacin. Taking into account this selection, gastrointestinal side-effects were documented in 14 per cent (and suspected in another 7 per cent) of the patients who received indomethacin in contrast to 10 per cent of the patients who were not treated with NSAID. No serious bleeding occurred in these patients. We, therefore, feel that the documented beneficial effects of indomethacin, such as the inhibition of urinary protein excretion and the better renal survival in patients with the nephrotic syndrome outweigh the potential risks of that treatment.

What are the mechanisms, whereby indomethacin delays renal failure in nephrotic patients? Indomethacin may influence the course of disease by

interrupting the pathophysiologic pathways of the disease. Like dipyramidole, indomethacin interferes with platelet aggregation and thus may modify the manifestations of glomerulonephritis (24, 25). Indomethacin also suppresses renal prostaglandin synthesis and likewise interferes with the renal adaptation to the pathologic process. Renal vasodilating substances such as prostaglandins, and renal vasoconstrictive stimuli like angiotensin-II and the adrenergic system, govern glomerular transcapillary hydraulic pressure and Kf (26-28). During activated renal vasoconstriction, as in salt-depleted nephrotic patients, renal blood flow and Kf are dependent on an adequate counteraction of renal vasodilators (9, 26-28). Renal prostaglandin synthesis' inhibition will then reduce glomerular transcapillary hydraulic pressure and Kf, resulting in a decrease of GFR and a change in glomerular permeability. The concommitant decrease in urinary PGE_2 excretion, GFR, proteinuria and selectivity of the residual urinary protein loss observed during indomethacin treatment of salt-depleted nephrotic patients, and the immediate reversal of these changes after withdrawal of the antiphlogistic agent (1-5, 29, 30), support the latter hypothesis. In view of the hyperfiltration concept it is noteworthy that only in the subset of patients with a low serum creatinine concentration indomethacin improved serum creatinine doubling time significantly (figure 5).

Thus, in this retrospective study of nephrotic patients with well-defined clinical and histological entities from one centre, indomethacin improved renal survival, most likely because of its antiproteinuric effect. An effect, that is thought to be mediated by renal prostaglandin synthesis' inhibition resulting in reduction of the mean glomerular transcapillary hydraulic pressure and Kf.

We realize that the retrospective character of our study urges for careful conclusions. Indeed, the patients were not randomly allocated to one of the two studied groups. As a result some differences existed between both groups at entry. For calculation of statistical significance of our data such differences between the two groups always were taken into account. With our data, however, it seems justified to perform a prospective study, to further document the beneficial effects of indomethacin in patients with a non-steroid sensitive nephrotic syndrome.

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CHAPTER 5

EFFECTS OF NONSTEROIDAL ANTIINFLAMMATORY DRUGS ON PROTEINURIA

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Abstract. Most nonsteroidal antiinflammatory drugs are antiproteinuric agents, especially if the patient is sodium-depleted. The decline in urinary protein excretion induced by these agents always markedly exceeds the decrease in glomerular filtration rate. Moreover, the remaining proteinuria appears to be more selective. Together, these findings suggest that the antiproteinuric effect of nonsteroidal antiinflammatory drugs is hemodynamically mediated. Nonsteroidal antiinflammatory agents that reduce renal prostaglandin E_2 excretion also decrease proteinuria, whereas sulindac decreases neither prostaglandin E_2 nor protein excretion. In a retrospective study, it appeared that administration of indomethacin improved renal survival of nephrotic patients with an initial serum creatinine concentration of less than 110 μmol/liter. The antiproteinuric effect of indomethacin itself or indomethacin- induced hemodynamic changes might explain this observation.

Introduction

Proteinuria is usually a sign of increased permeability of the glomerular capillary wall to macromolecules. In the majority of glomerulopathies, humoral immune mechanisms and inflammation are involved in the initiation of the histologic and functional glomerular lesions.This concept has prompted the introduction of corticosteroids and nonsteroidal antiinflammatory drugs in the treatment of patients with nephrosis since the early 1950s.

The usefulness of corticosteroids in the treatment of minimal-lesion glomerulopathy or "lipoid nephrosis" is beyond question, as most patients achieve a rapid and complete remission with these drugs (1). However, whether corticosteroids are indicated in the treatment of patients with other glomerulopathies is still unclear. In a collaborative study on adult idiopathic nephrotic syndrome due to membranous nephropathy, a favorable influence of a short-term alternate-day prednisone regimen on the glomerular filtration rate has been described (2). Another prospective, controlled study demonstrated an even better preservation of kidney function (3); improvement of proteinuria was noted in patients with idiopathic membranous glomerulopathy treated with prednisone and chlorambucil. In contrast to these two studies, however, other investigators have not obtained beneficial effects with corticosteroids in membranous nephropathy (4).

Although adult patients with several other idiopathic glomerulopathies associated with the nephrotic syndrome may have an initially favorable response to corticosteroids (5), such a response has not been shown to influence the final outcome of these diseases. Thus, definite conclusions about the role of corticosteroids in adult patients with the nephrotic syndrome, other than that caused by "lipoid nephrosis", still await the results of controlled, long-term trials.

Nonsteroidal antiinflammatory drugs (NSAIDs) have been used in the treatment of patients with nephrosis with the same purpose as corticosteroids. In 1955, Fieschi and Bianchi (6) were the first to report the antiproteinuric effect of a NSAID: phenylbutazone induced a reduction in proteinuria and an increase in plasma albumin concentration in 21 of 25 nephrotic patients. Long-term administration, however, was hampered by myelotoxicity. De Vries et al (7) extended those observations by demonstrating the antiproteinuric effect of phenylbutazone as well as aminophenazone. Moreover, they drew attention to a mild, though reversible, decrease in creatinine clearance during administration of these drugs. The most extensively studied NSAID in the treatment of the nephrotic syndrome is indomethacin. In 1967, Michielsen et al (8) reported the antiproteinuric properties of indomethacin, which were confirmed by several other investigators (9-11). Indomethacin reduced proteinuria regardless of the underlying disease, although the effect was most impressive in patients with membranous glomerulopathy or proliferative glomerulonephritis (8, 9).

Although immune mechanisms and inflammation appear to be involved in the initiation of glomerular lesions, it is not clear whether they are always responsible for the progression of glomerular damage (5). Since the primary, initiating antigen is often identified in only small amounts in patients with chronic nephritis, if at all, secondary immune mechanisms, such as induction of autoimmunity against DNA (12, 13) and IgG (14), might play a role in the progressive deterioration of renal function in those diseases. Recently, however, non-immu-

nologic mechanisms have also been implicated (15, 16). In animal studies, a major reduction in renal mass results in hypertension, advanced focal glomerulosclerosis, and progressive renal failure (17-19). Hostetter et al (20) have demonstrated a considerable increase in single nephron glomerular filtration rate, glomerular plasma flow, and transcapillary hydraulic pressure difference in the remnant glomeruli. Similar observations have been made in animal models of hypertension, i.e., hyperfiltrating glomeruli and the development of glomerulosclerosis (21, 22). An elevation of the transcapillary hydraulic pressure difference has also been noted in experimentally induced glomerulonephritis in rats (23, 24). Together, these findings raise the possibility that changes in glomerular hemodynamics also contribute to the development of chronic renal failure in nephrotic patients. In the anticipation of the latter idea, we will review our investigations regarding the effects of indomethacin and other NSAIDs on kidney function, plasma renin activity, and urinary prostaglandin E_2 excretion in normal volunteers, non-proteinuric patients with reduced renal function, and patients with the nephrotic syndrome.

Indomethacin in healthy volunteers and in non-proteinuric patients with reduced renal mass

In healthy subjects, glomerular filtration rate and effective renal plasma flow decrease only slightly during indomethacin administration (4 and 3 percent, respectively; Table I) (25). Roughly similar results have been obtained when dietary sodium is restricted to a maximum of 50 mmol per day (Table II) (25). In uninephrectomized but otherwise healthy persons and in non-proteinuric

Table I. Data of patients given a sodium constant diet before and after three days of indomethacin treatment.

	GFR (ml per minute)		ERPF (ml per minute)		PRA (ng A_I/10 ml per three hours)	
Patients*	—†	+‡	—	+	—	+
Normal (n = 8)	113	108 (96)§	420	408 (97)	171	71 (42)
Uninephrectomized, healthy (n = 5)	99	80 (81)	385	308 (80)	ND	ND
Nonproteinuric, with reduced renal function (n = 3)	73	58 (79)	288	232 (81)	ND	ND

GFR = mean glomerular filtration rate; ERPF = effective renal plasma flow; PRA = plasma renin activity; ND = not determined.

* Data were gathered during sodium constant (more than 80 mmol per 24 hours) diet.

† Before three days of indomethacin treatment.

‡ After three days of indomethacin treatment (50 mg three times daily) (25,26).

§ Numbers in parentheses refer to percentage of change.

Table II. Data of patients given a sodium-depleted diet before and after three days of indomethacin treatment.

Patients*	GFR (ml per minute)		ERPF (ml per minute)		PRA (ng A_I/10 ml per three hours)	
	—†	+‡	—	+	—	+
Normal (n = 6)	100	91 (91)§	396	385 (97)	942	341 (36)
Uninephrectomized, healthy (n = 5)	78	63 (81)	289	247 (86)	ND	ND
Nonproteinuric, with reduced renal function (n = 8)	63	48 (76)	230	187 (81)	ND	ND

GFR = mean glomerular filtration rate; ERPF = effective renal plasma flow; PRA = plasma renin activity; ND = not determined.

* Data were gathered during sodium depletion (50 mmol per day).

† Before three days of indomethacin treatment.

‡ After three days of indomethacin treatment (50 mg three times daily) (25,26).

§ Numbers in parentheses refer to percentage of change.

patients with reduced renal function, indomethacin reduces the glomerular filtration rate and the effective renal plasma flow to a greater extent than in healthy volunteers with two normal kidneys, both during normal sodium intake (Table I) and during sodium restriction (Table II) (25, 26). In healthy volunteers and in patients with a renal disease with or without reduced renal function, sodium excretion and plasma renin activity decline at the first day on indomethacin administration both during a normal sodium intake and during sodium restriction (25). After a single dose of 50 mg of indomethacin to healthy volunteers, fractional excretion of sodium, chloride, and potassium decreases, whereas fractional phosphate excretion rises substantially (27). Therefore, the antinatriuretic effect of indomethacin might be ascribed to an enhanced sodium chloride reabsorption in the ascending limb of Henle's loop (27). The decline in plasma renin activity, however, cannot be explained by sodium retention only, since it is observed to occur within two hours after administration of a single dose of indomethacin and since it also occurs in severely sodium-depleted subjects (25).

Indomethacin and other NSAIDs in patients with the nephrotic syndrome

As in uninephectomized healthy individuals and in non-proteinuric patients with impaired renal function, indomethacin reduces glomerular filtration rate, effective renal plasma flow, and the filtration fraction in nephrotic patients, irrespective of the sodium balance (Table III) (28). In contrast to the effect of indomethacin on glomerular filtraltion rate, the antiproteinuric effect of the drug is enhanced by sodium depletion (Table III) (28).

Table III. Data of seven nephrotic patients receiving sodium constant diet without and with hydrochlorothiazide before and after three days of indomethacin treatment.

	GFR (ml per minute)		ERPF (ml per minute)		FF		Proteinuria (g per 24 hours)	
Diet	—*	+†	—	+	—	+	—	+
Sodium constant‡	106	87 (82)§	501	449 (90)	0.21	0.19	10.9	6.6 (61)
Sodium depletion**	91	69 (76)	478	375 (79)	0.19	0.18	10.4	2.1 (20)

GFR = mean glomerular filtration rate; ERPF = effective renal plasma flow; FF = filtration fraction.

* Before three days of indomethacin treatment.

† After three days of indomethacin therapy (50 mg three times daily) (28).

‡ Diet with 50 mmol of sodium chloride per day.

§ Numbers in parentheses are the values of the variables given as percentages of the values before indomethacin treatment.

** Diet with 20 mmol of sodium chloride per day and 50 mg of hydrochlorothiazide per day.

In a double-blind, crossover study with naproxen, 10 consecutive, salt-depleted patients with a proteinuria of more than 3.0 g per day were treated with 50 mg of indomethacin, three times per day (29). As shown in Figure 1, proteinuria, plasma renin activity, urinary sodium excretion, and diuresis decreased from the first day of indothacin treatment. The level of these variables plateaued after three days of therapy and returned to pretreatment values within one week after discontinuation of the drug. Glomerular filtration rate, effective renal plasma flow, and filtration fraction during indomethacin administration decreased by 36, 25 and 12 percent, respectively (Figure 1; Table IV) (29). After withdrawal of indomethacin, these changes appeared to be completely reversible (Figure 1) (28-30).

Table IV. Data of 10 salt-depleted nephrotic patients before and at the end of one week of treatment with indomethacin or naproxen.

		Proteinuria (g per 24 hours)	GFR (ml per minute)	ERPF (ml per minute)	FF	PRA (nmol A_I/liter per hour)	Proteinuria per GFR (mg/ml)
Indomethacin*	:–	8.4	56	332	0.17	3.8	0.17
	+	2.5 (30)†	36 (64)	249 (75)	0.15	1.4 (37)	0.08 (47)
Naproxen‡	:–	9.1	55	325	0.17	3.4	0.18
	+	5.3 (58)†	47 (86)	302 (93)	0.16	1.9 (56)	0.13 (72)

GFR = mean glomerular filtration rate; ERPF = effective renal plasma flow; FF = filtration fraction; PRA = plasma renin activity.

* Treatment with 150 mg of indomethacin daily.

† Numbers in parentheses refer to values of the variables during treatment given as percentages of the values before treatment.

‡ Treatment with 750 or 1,500 mg of naproxen daily (29).

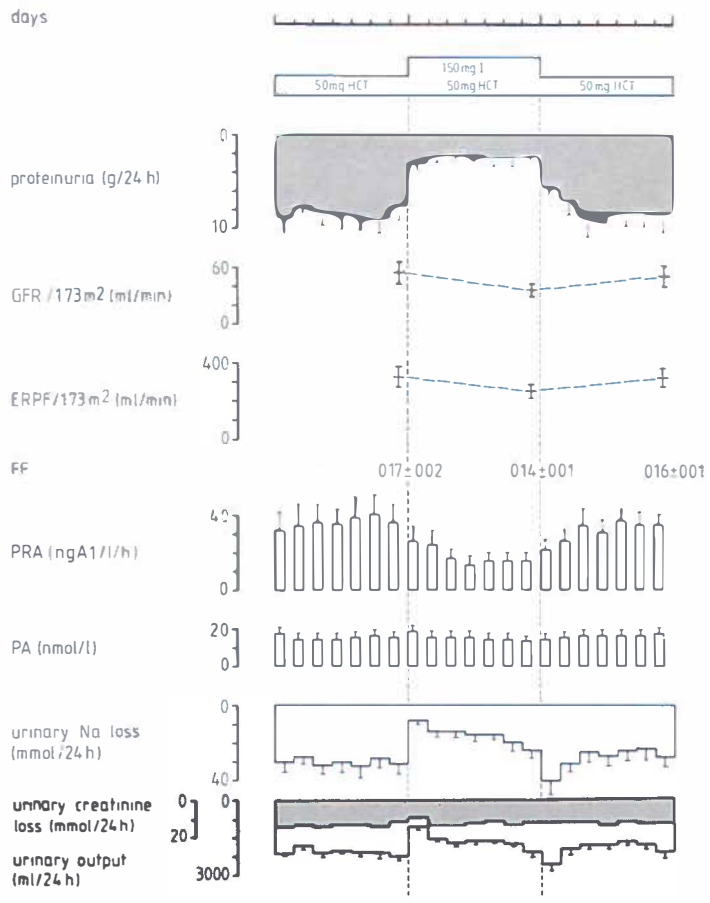

Figure 1. Proteinuria, glomerular filtration rate (GFR), effective renal plasma flow (ERPF), filtration fraction (FF), plasma renin activity (PRA), plasma aldosterone (PA), urinary sodium (Na) loss, creatinine excretion, and urinary output in 10 sodium-depleted patients before, during, and after one week of treatment with 150 mg of indomethacin (I) daily (mean ± SD). HCT = hydrochlorothiazide.

Even after long-term treatment, the effects of indomethacin are reversible. In 14 patients with nephrosis, indomethacin was withdrawn after one to three years of administration. Five patients showed no recurrence of proteinuria and their glomerular filtration rate roughly equaled that before therapy (Table V) (28). In the other nine patients, proteinuria recurred at pretreatment levels. Their mean glomerular filtration rate was 80 percent of the value before treatment. However, a comparison of glomerular filtration rates just before and after discontinuation of indomethacin revealed a mean increase of 29 percent (28). The amount as well as the selectivity of proteinuria changed during indomethacin administration.

Table V. Mean proteinuria and glomerular filtration rate in 14 patients before and after long-term treatment with indomethacin (28).

	Proteinuria (g per 24 hours)		GFR (ml per minute)	
	Before	After	Before	After
No recurring proteinuria (n = 5)	10	*0.3 (3%)*	111	103 (93%)
Recurring proteinuria (n = 9)	12	12 (100%)	79	63 (80%)

GFR = glomerular filtration rate.
* Numbers in parentheses refer to values of the variables during treatment given as percentages of the values before treatment.

The selectivity of proteinuria, as estimated by the ratio of IgG and transferrin clearances, increased in all tested patients (Figure 2) (30). These results have been confirmed and extended by Tiggeler et al (31), who demonstrated a relatively decreased renal clearance of polydisperse polyvinylpyrolidone molecules larger than 40 angstroms during indomethacin administration (31). Moreover, they documented a higher incidence of indomethacin-induced rises in

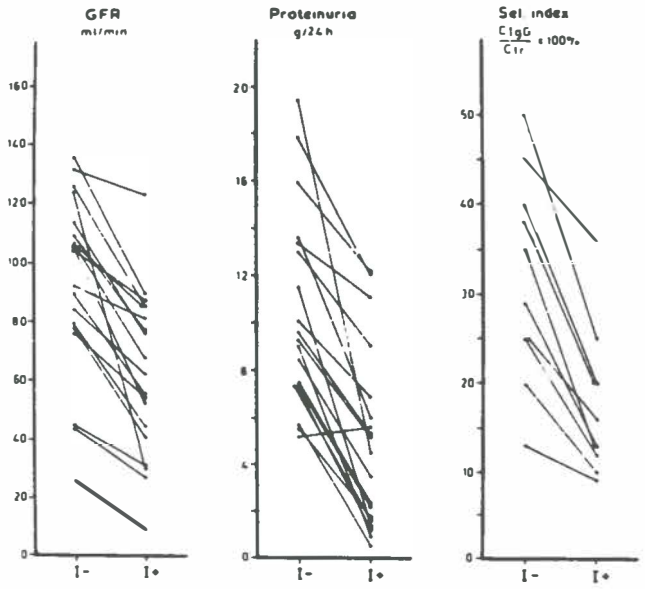

Figure 2. Glomerular filtration rate (GFR), proteinuria (mean of three consecutive days), and selectivity index before (I–) and during (I+) indomethacin administration. Reproduced with permission from (30).

selectivity in salt-depleted patients compared with salt-repleted ones (16 of 23 versus three of 10) (31).

In the already mentioned crossover study, we treated 10 sodium-depleted nephrotic patients for one week with indomethacin 50 mg three times daily or naproxen 250 (n = 5) to 500 (n = 5) mg three times daily in a blind order (29). Between both treatment periods, no NSAID was given for one week in order to study the reversibility of the NSAID-induced effects. The effects of both NSAIDs on proteinuria, renal function, and plasma renin activity are shown in Table IV. The reduction in proteinuria appeared to be significantly larger during indomethacin than during naproxen administration. The reduction in proteinuria was nearly always accompanied by a decline in glomerular filtration rate, suggesting that this decline and the decrease in proteinuria during NSAID treatment are related (Figure 3). However, the decline in the glomerular filtration rate cannot be solely responsible for the decrease in urinary protein loss

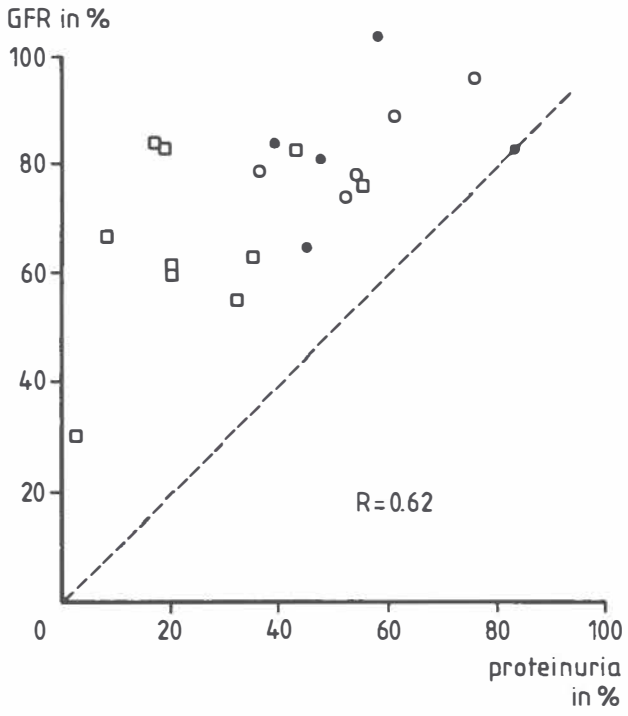

Figure 3. Relationship between the change in glomerular filtration rate (GFR) and proteinuria in 10 sodium-depleted nephrotic patients during treatment with indomethacin (50 mg three times daily, □) or naproxen (250 mg three times daily, ○, n = 5, or 500 mg three times daily, ●, n = 5). Reproduced with permission from (29).

since, in 19 of 20 studied periods of NSAID administration, the proportional decrease in proteinuria exceeded that in glomerular filtration rate (Figure 3) (29). Thus, the proteinuria per ml glomerular filtration rate fell during indomethacin treatment as well as during naproxen treatment at rates of 53 and 28 percent, respectively (Table IV). Both drugs induced similar, reversible intrarenal hemodynamic changes, but indomethacin had more profound effects than did naproxen (Table IV) (29).

In another study of seven sodium-depleted patients with the idiopathic nephrotic syndrome, indomethacin, diclofenac sodium, flurbiprofen, and sulindac were tested for their effects on proteinuria, renal function, and plasma renin activity (32). Due to the small numbers of treatments with each drug, an exact ranking order of the antiproteinuric effect could not be made. Sulindac, however, induced no major changes in proteinuria, glomerular filtration rate, or plasma renin activity, whereas during treatment with indomethacin, diclofenac sodium, and flurbiprofen, proteinuria, glomerular filtration rate, and plasma renin activity declined by 59, 16 and 55 percent, respectively (Table VI) (32). At this time, we were able to measure urinary prostaglandin E_2 (PGE_2) excretion.

Table VI. Data of seven salt-depleted nephrotic patients after seven to 10 days of NSAID treatment.

	Percentage of previous wash-out period*			
	Proteinuria	GFR	PRA	PGE_2 excretion
Sulindac† (n = 3)	+13	—13	—24	+1
	(+45 to —12)‡	(0 to —19)	(6 to —38)	(+9 to —7)
Other NSAIDs§ (n + 11)	—59	—16	—55	—68
	(—18 to —88)	(0 to —40)	(+10 to —88)	(—37 to —89)

GFR = glomerular filtration rate; PRA = plasma renin activity; PGE_2 + prostaglandin E_2.

* For further information, see (32).

† Sulindac was dosed 200 mg twice daily.

‡ Numbers in parentheses refer to range.

§ The NSAIDs were indomethacin (150 mg three times daily), diclofenac sodium (100 mg twice daily), and flurbiprofen (100 mg twice daily).

Assuming that urinary prostaglandin excretion reflects renal prostaglandin synthesis (33), it is noteworthy that only during treatment with NSAIDs that induced a reduction in proteinuria a concomitant decrease of the urinary PGE_2 excretion was noted (Table VI; Figure 4) (33). Similar findings have been reported by Lianos et al (34) and Gutierrez Millet et al (35). The relative change in proteinuria during NSAID therapy correlated strongly with that of the renal PGE_2 excretion ($R = 0.89$, $p < 0.001$; Figure 4). As can be expected, the reduction in glomerular filtration rate appeared to be related to the change in urinary PGE_2 excretion ($R = 0.70$, $p < 0.05$). Finally, the absolute change in

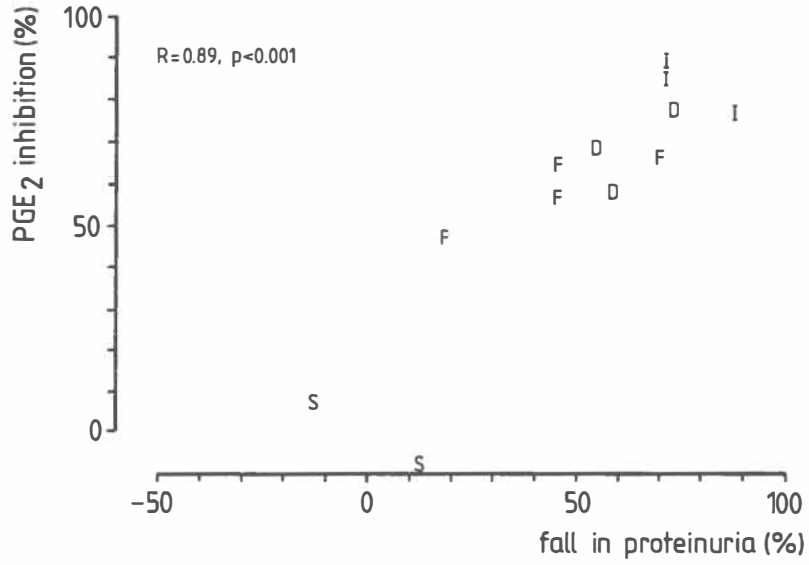

Figure 4. Relationship between the percentage decrease in PGE_2 excretion and decline in proteinuria by indomethacin (I), diclofenac-sodium (D), flurbiprofen (F), and sulindac (S). Reproduced with permission from (32).

PGE_2 excretion correlated with the change in log plasma renin activity during NSAID treatment ($R = 0.62$, $p < 0.05$; Figure 5), demonstrating the interplay of the prostaglandin system with the renin-angiotensin axis in sodium-depleted patients with the nephrotic syndrome (32).

Thus, in comparative trials (29, 32), indomethacin appears to be the strongest antiproteinuric agents, probably due to its marked inhibition of the renal prostaglandin synthesis. On the other hand, sulindac has not shown strong antiproteinuric properties and hardly decreases urinary PGE_2 excretion. The latter had already been noted by Ciabattoni et al (36) in patients with chronic glomerulonephritis. However, sulindac can inhibit renal PGE_2 excretion after stimulation of prostaglandin synthesis by furosemide (37). These contradictory findings may well be explained by the suggestion of Brater and co-workers (37) that different pathophysiologic conditions have different susceptibilities to prostaglandin synthesis inhibition. According to this concept, the discrepancy between platelet- and renal prostaglandin synthesis inhibition by sulindac (36) and the dose-related effects of NSAIDs in sodium-depleted nephrotic patients (11, 32) fit well. Thus, under particular conditions or in high doses sulindac may not be as renal-sparing as was hoped (38-40).

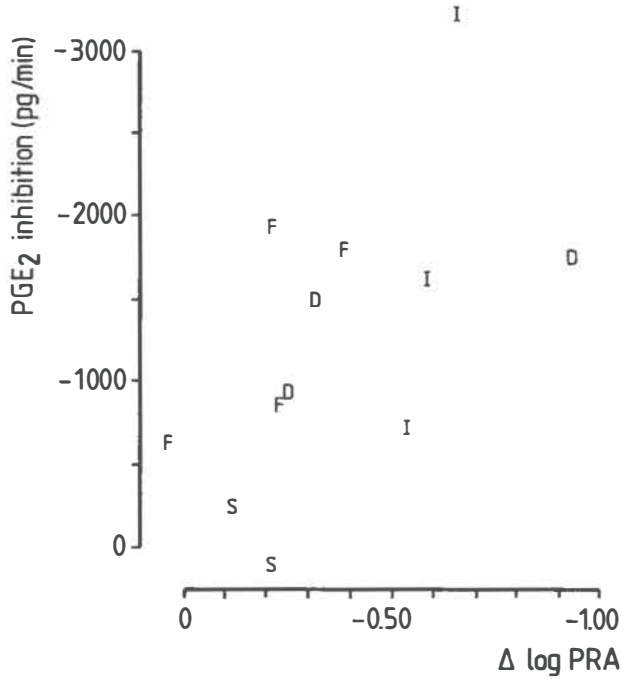

Figure 5. Relationship between the decrease in PGE_2 excretion and the change in log plasma renin activity (PRA) by indomethacin (I), diclofenac-sodium (D), flurbiprofen (F), and sulimdac (S).

How do NSAIDs influence proteinuria?

Several explanations have been brought forward to explain the effect of NSAIDs on proteinuria in patients with the nephrotic syndrome. At first, some investigators suggested a beneficial effect on the underlying disease itself, as expressed by an improvement of the histologic changes in consecutive renal biopsies (41). The phenomenon was attributed to the antiphlogistic or antiplatelet activity of NSAIDs. Another explanation might be a change in the configuration and charge of the albumin molecule because of its binding to NSAIDs. Most NSAIDs are highly and tightly bound to albumin. The protein-bound fraction usually accounts for about 99 percent of the plasma concentration. If this binding changes the shape and charge of the albumin molecule, it can influence the permeability of the glomerular basement membrane for this molecule. However, it is unlikely that this plays an important role in the antiproteinuric effect of NSAIDs because those drugs induce less reduction in proteinuria in salt-replete patients than in salt-deplete patients (28), and because

they raise the selectivity of the proteinuria (30) (Table III; Figure 2). A third mechanism that should be considered is the interference of NSAIDs with the albumin reabsorptive capacity of proximal tubules. It has been speculated that the urinary protein loss in the nephrotic syndrome can be completely attributed to a failure of tubular albumin reabsorption (42). Several NSAIDs, including indomethacin and naproxen, are excreted by the basolateral membrane of the proximal tubule (43). Since indomethacin has been shown to reverse proximal tubular dysfunction (44), one cannot exclude the possibility that NSAIDs improve a failing tubular reabsorption of albumin. It is, however, difficult to understand why an indirect stimulation of proximal tubular reabsorption by salt restriction and/or administration of hydrochlorothiazide does not results in a significant reduction of proteinuria (Table III). Therefore, enhanced tubular reabsorption of proteins is not a major pathway in the NSAID-induced reduction of proteinuria. In the mid-1970s, it already had been commented that interference of NSAIDs with prostaglandin synthesis and the renal hemodynamic control of the filtration process might be the most important contributor to the reduction of proteinuria by these agents (28). Since indomethacin inhibits the renal synthesis of prostaglandins and renal prostaglandins modulate the pressor effects of angiotensin II (45), norepinephrine (46), and antidiuretic hormone (47), it is now generally accepted that certain types of NSAID-induced renal reactions occur only if this function is dependent on the vasodilatory action of those prostaglandins (48). Thus, the modulating effect of prostaglandins plays little role in the control of renal function in healthy, euvolemic individuals. However, renal prostaglandins are probably involved in the maintenance of adequate renal function after sodium depletion, after uninephrectomy, in the elderly, in patients with reduced glomerular filtration rate, in patients with cirrhosis of the liver and ascites, and in patients with congestive heart failure (49-51). This also seems true in sodium depleted patients with nephrosis, as the reduction in glomerular filtration rate and renal blood flow by NSAIDs is markedly enhanced by sodium depletion (Table III), and the NSAID-induced reversible decline in glomerular filtration rate correlates with the decrease in PGE_2 excretion (32). In rats, intrarenal infusion of renin and angiotensin II leads to a rise in the filtration fraction and to a greater permeability of the glomerular capillary wall to macromolecules (52). These effects can be explained by an elevation of the glomerular transcapillary hydraulic pressure difference (by efferent vasoconstriction), a phenomenon documented in rats during infusion of exogenous angiotensin II (53, 54), and during stimulation of the renin-angiotensin axis with chronic low-salt diets (55). Brenner and Schor (56) have shown that low doses of PGE_2 and prostaglandin I_2 (PGI_2) also induce an increase in the glomerular transcapillary hydraulic pressure difference in anesthetized euvolemic rats (56). Concomitant infusion of saralasin inhibited this action of

PGE_2 and PGI_2 (56). Since prostaglandins stimulate renin release (57), it seems likely that in the rat a prostaglandin-induced maintenance or elevation of the glomerular transcapillary hydraulic pressure difference is (partly) mediated by angiotensin II. An alternative explanation is based on the work of Dunn et al (58). Determining the planar surface area of glutaraldehyde-fixed glomeruli by a millipore particle counter, Scharschmidt et al (59) measured glomerular contraction as an indirect measurement of mesangial contraction and the filtration surface area, one of the determinants of the glomerular ultrafiltration coefficient. They showed that arachidonic acid and PGE_2 attenuate the angiotensin II-induced glomerular contraction (59). Furthermore, they demonstrated an increased sensitivity of the glomeruli to the constrictive effects of angiotensin II after preincubation with indomethacin. Consequently, it might be that angiotensin II-induced prostaglandin synthesis prevents the angiotensin II-mediated decrement in the glomerular ultrafiltration coefficient. In further studies, Dunn et al (58) found thromboxane to be an effective constrictor of mesangial cells. In pathologic conditions, e.g., nephrotoxic serum nephritis, they showed a concomitant increase of thromboxane A_2 and a decrease of the filtration fraction that could be inhibited by pretreatment with a thromboxane synthetase inhibitor (58).

Thus, from the just mentioned studies, one may assume that in the rat, the prostaglandin system, the renin-angiotensin system, and their feedback systems are involved in the regulation of the glomerular transcapillary hydraulic pressure difference and the glomerular ultrafiltration coefficient. Whether this applies to humans remains to be determined. However, the reversible reduction of the glomerular filtration rate and filtration fraction, the enhancement of the antiproteinuric effects of NSAIDs by stimulation of the renin-angiotensin axis, the increase in the selectivity of residual proteinuria, and the correlation of the changes in filtration, proteinuria, and plasma renin activity with that of the urinary PGE_2 excretion during NSAID therapy may all be related to a decrease of the glomerular transcapillary hydraulic pressure difference and/or ultrafiltration coefficient. Both changes in the main determinants of glomerular filtration can explain the observed effects of NSAIDs in salt-depleted patients with nephrosis. In this respect, it is worth mentioning that angiotensin-converting enzyme inhibition in nephrotic patients can decrease urinary protein loss (60), as angiotensin-converting enzyme inhibition has been shown to decrease intracapillary glomerular pressure (61).

Effect of indomethacin on final renal outcome in patients with nephrosis.

It is well established that proteinuria is an important prognostic factor in the

final renal outcome in several glomerulopathies (62-64). Glomerular hyperfiltration enhances proteinuria and the development of glomerulosclerosis, and accelerates the decline in renal function. Since NSAIDs may counteract hyperfiltration in nephrotic patients by reducing the glomerular transcapillary hydraulic pressure difference and/or the ultrafiltration coefficient (see earlier), one expects a better preservation of renal function in NSAID-treated nephrotic patients despite the initial reduction of the glomerular filtration rate. Therefore, we retrospectively studied the influence of indomethacin as an antiproteinuric agent on renal function decline and final renal outcome in patients with nephrosis (65).

One hundred fourteen patients with proteinuria of more than 3 g per day and with a diagnosis of membranous glomerulopathy, focal glomerulosclerosis, or membranoproliferative glomerulonephritis were identified from our renal biopsy records covering the period of 1968 to 1983. Sixteen patients were excluded because follow-up was less than six months. Fifty-eight patients had been treated with indomethacin. The median dose was 150 mg (range: 75 to 225 mg) and the median duration of treatment was three years (range: six months to nine years). The 40 untreated patients were used as controls. Five of them received indomethacin therapy for less than one months. All patients were routinely treated with a low-salt diet and diuretics to control edema. None of the 98 patients had received corticosteroids or similar drugs. Some patient characteristics of both groups at entry are given in Table VII. The treated and untreated patients differed significantly in serum creatinine concentration, mean arterial pressure, and proteinuria. Through statistical means, these differences were taken into account in the final results.

As assessed by log rank analysis, renal survival was significantly better in the

Table VII. Median and range of the entry characteristics of indomethacin-treated and untreated nephrotic patients*.

	Untreated patients	Treated patients	p value†
Number of patients	40	58	
Serum creatinine (μmol/liter)	115 (59-166)	92 (35-327)	<0.05
Mean arterial pressure (mm Hg)	107.5 (90.0-160.0)	105.2 (80.0-131.7)	<0.05
Proteinuria (g per 24 hours)	5.7 (3.0-15.5)	8.2 (3.0-26.2)	<0.05
Serum albumin (g/liter)	28 (12-46)	27 (12-48)	NS
Age (years)	36 (14-79)	35 (14-70)	NS
Sex (male/female)	27/13	32/26	NS
Membraneous glomerulopathy/focal glomerulosclerosis/membranoproliferative glomerulonephritis	14/10/16	28/17/13	NS

NS = not significant.

* For further information, see (65).

† The p values were determined by two-tailed Wilcoxon tests.

indomethacin-treated patients (Figure 6). At 10 years' follow-up, 31 percent of the treated and 66 percent of the untreated patients needed dialysis ($p < 0.05$) (65). Similar observations have been reported by Lagrue et al (66). Using various NSAIDs as antiproteinuric agents in patients with membranoproliferative glomerulonephritis, these investigators demonstrated (also in a retrospective study) a significant improvement in renal survival in treated patients compared with untreated control subjects. Furthermore, we investigated the influence of indomethacin on renal function decline as measured by serum creatinine doubling time. Log-rank analysis showed some, but not significant, difference in creatinine doubling time (65). The discrepancy between the results in renal survival and creatinine doubling time might be due to patients who had spontaneous remissions. Spontaneous remissions were taken into account in the renal survival curves of both the untreated and treated patients from the start of the study to the last follow-up, which often extended beyond the occurrence of the remission. In the creatinine doubling time curve of the treated patients, the patients with spontaneous remissions were not involved after the determination of the remission, because indomethacin therapy was withdrawn at that event. Since such patients were included in the control group during the entire follow-up,

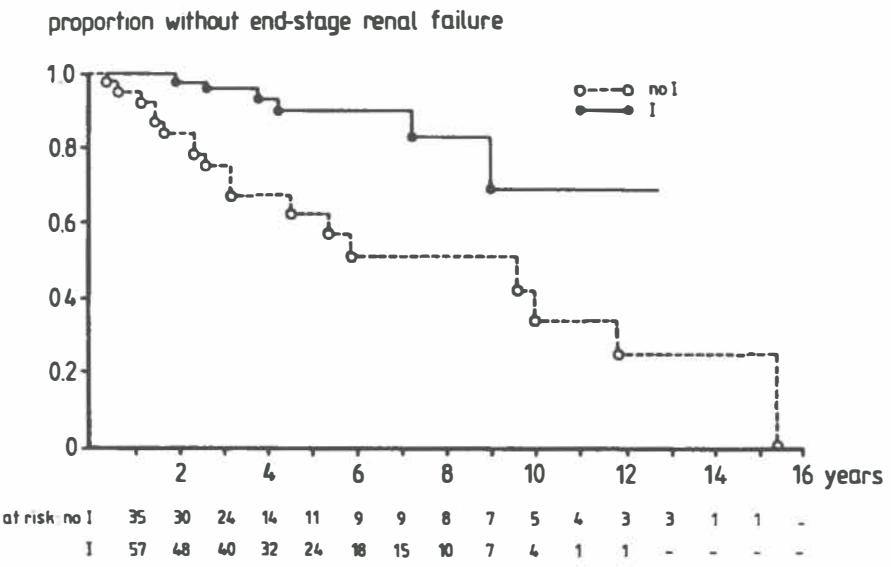

Figure 6. Renal survival curves for nephrotic patients with membraneous glomerulopathy, focal glomerulosclerosis, or membranoproliferative glomerulonephritis treated with indomethacin (I), compared with those of patients who did not receive this treatment (no I).

there was a smaller difference between the serum creatinine curves of the treated and untreated patients than would be expected.

The initial serum creatinine concentration, mean arterial pressure, degree of proteinuria, serum albumin concentration, age, sex, and diagnosis were also evaluated for their prognostic significance by log-rank analysis of the creatinine doubling time. In this univariate analysis, proteinuria and diagnosis significantly influenced the rate at which renal function declined. Severe proteinuria and membranoproliferative glomerulonephritis were associated with a rapid doubling of serum creatinine concentration (65). Thus, although the initial serum creatinine concentration and mean arterial pressure were higher in the patients not receiving treatment, neither factor influenced the decline in renal function. Although large urinary protein loss was a poor prognostic sign for renal function deterioration, the patients receiving treatment had, despite their initial proteinuria, a better renal survival than did the patients without treatment.

Finally, we searched for a subset of patients who fared better with indomethacin therapy. We could not establish a significant benefit in the patients with severe proteinuria or with membranoproliferative glomerulonephritis. Even in the subset with both unfavorable prognostic factors, indomethacin did not significantly delay creatinine doubling time. Only patients with an initial serum creatinine concentration less than 110 μmol/liter benefited significantly from treatment with indomethacin (65).

Comments

To date, only symptomatic treatment can be offered to most patients with idiopathic glomerulonephritis associated with the nephrotic syndrome. Corticosteroids are likely to induce a complete remission in patients with minimal-change nephropathy and membranous glomerulopathy with highly selective proteinuria. Whether corticosteroid therapy favorably influences the long-term prognosis of membranous nephropathy is still a matter of debate.

NSAIDs, in particular indomethacin, are often effective antiproteinuric agents in salt-depleted nephrotic patients. This action probably depends on the ability of NSAIDs to inhibit renal prostaglandin synthesis. Although these drugs induce a (reversible) reduction in renal function, it can be speculated that the antiproteinuric effect per se ultimately preserves glomerular filtration rate. Prospective, placebo controlled studies of the influence of NSAIDs on long-term renal function in salt depleted patients with the nephrotic syndrome are warranted.

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CHAPTER 6

DISCUSSION

In this thesis some aspects of the antiproteinuric properties of indomethacin and other nonsteroidal antiinflammatory drugs (NSAID) have been investigated. It extends the earlier work of Arisz and Donker in our Department, showing that indomethacin lowers urinary protein loss, glomerular filtration rate, filtration fraction, selectivity of the residual proteinuria and plasma renin activity (1-4).

From these and other studies several hypotheses have been brought forward regarding the mechanism by which indomethacin induces its antiproteinuric effect. The most prominent one involves the renal haemodynamic effect of NSAID. Indeed the reduction of proteinuria occurs within the first 24 hours of treatment and reversal of its effect is observed within the first days after withdrawing medication. The fast onset of the antiproteinuric effect favours the hypothesis of renal haemodynamic changes being of relevance rather than explaining the changes in protein excretion by changes in the course of the underlying disease. In this context it is of importance to note that the major known biological effect of NSAID is inhibition of the prostaglandin synthesis by an interaction with prostaglandin synthetase (5). This effect, combined with the indomethacin-induced changes in plasma renin activity (1,6),led to the interesting idea that indomethacin may reduce renal protein excretion through an intervention in the hormonal (angiotensin-II and prostaglandins) balance governing renal filtration (2-4). However, no clinical studies were available to date with regard to any relation between renal prostaglandins and proteinuria during NSAID treatment.

In chapter 2 we showed that the effects of indomethacin are also obtained by naproxen, although naproxen (750-1500 mg a day) was less effective than indomethacin (150 mg a day). Both drug regimens were given in the highest recommended dose. Moreover, no different response was found between the 750 mg naproxen and the 1500 mg naproxen treatment. Although no proper dose response curves were generated, this result suggests that different NSAID possess a different antiproteinuric potency. To test whether this difference is related to the capacity of these drugs to inhibit renal prostaglandin synthesis, we studied the antiproteinuric effect of different NSAID in relation to their effects on urinary prostaglandin E_2 excretion. Indeed, in chapter 3 it is shown that besides indomethacin also flurbiprofen and diclofenac are able to lower urinary protein excretion, whereas sulindac has no effect. Interestingly, these different NSAID also showed distinct differences in their effects on urinary prostaglandin E_2 excretion. Sulindac showed no significant inhibition, whereas indomethacin did reduce prostaglandin E_2 excretion by 70-90 per cent. Moreover, the

percentage fall in urinary protein loss showed a significant correlation with the percentage fall in urinary PGE_2 excretion. These data were somewhat different as was expected from the reported in-vitro capacity of these NSAID to inhibit prostaglandin synthesis. Flurbiprofen for example was expected to lower urinary prostaglandin excretion (and thus probably renal prostaglandin synthesis) more markedly than indomethacin, since flurbiprofen is more potent compared to indomethacin in in-vitro prostaglandin synthesis inhibition (7). Thus, apparently not only in-vitro capacities are of importance, but also other characteristics such as for instance (intrarenal) pharmacokinetics play a role. It remains, however, that the positive correlation between the anti-proteinuric effect of these drugs and their in-vivo inhibition of renal prostaglandin synthesis strongly suggests that the drug-induced prostaglandin synthesis inhibition plays a role in the anti-proteinuric effect.

How may inhibition of renal prostaglandin synthesis lead to changes in protein excretion? To answer this question it is of importance to review the alleged renal effects of prostaglandins. Brenner and Schor have shown that low doses of PGE_2 and PGI_2 induce a rise in postglomerular resistance without affecting preglomerular resistance. As a result the glomerular transcapillary hydraulic pressure difference will rise (8). In fact, these results mimic the well-known effects of angiotensin-II on glomerular haemodynamics. Indeed, concomitant infusion of saralasin inhibited the action of PGE_2 and PGI_2 (8). Based upon these studies one thus could argue that indomethacin treatment would result in a fall in the glomerular transcapillary hydraulic pressure difference as a consequence of a decrease in postglomerular resistance, without an effect on preglomerular vessels. Jensen et al studied the effects of indomethacin on glomerular haemodynamics in streptozotocin diabetic rats (9). They showed that indomethacin indeed caused a fall in intraglomerular capillary pressure. However, this fall was found to be due to an increase in preglomerular resistance without a fall in postglomerular resistance; the latter was even slightly increased. Animal studies therefore, seem to support the hypothesis that indomethacin induces a fall in intraglomerular capillary pressure.

Do the human experiments support this hypothesis? The decrease in proteinuria obtained with the different NSAID was positively correlated with the change in glomerular filration rate. Moreover, we have shown that the fall in urinary protein excretion with the different NSAID exceeds the fall in glomerular filtration rate. This indicates that the filtration of the smaller molecule of creatinine (or inulin and iothalamate) is less inhibited by the NSAID compared to the filtration of the greater molecule of albumin. This interpretation can be extrapolated to explain the fall in selectivity of the residual proteinuria: the fall in filtration of albumin is less inhibited as the fall in filtration of the much larger IgG. Lastly, the effects of indomethacin on renal haemodynamics and renal protein

excretion are more pronounced in the situation of a stimulated renin angiotensin system (for instance after pretreatment with a diuretic).

Taken together, these animal and human data suggest that the effects of the NSAID could well be due to interference with the delicate balance between angiotensin-II and prostaglandins which governs the glomerular filtration rate. Thus, indomethacin may lower the intraglomerular capillary pressure and thereby exert its antiproteinuric effect.

The above mentioned hypothesis is based upon the assumption that urinary prostaglandin excretion is a good reflection of intrarenal prostaglandin synthesis (10), and furthermore, that inhibition of prostaglandin excretion is correlated with haemodynamic changes at the glomerular level. However, prostaglandin synthesis and stores mainly prevail in the renal medulla (11), and to date no clear evidence has been presented that these medullary prostaglandins actually reach the glomerulus or its afferent and efferent arteriole. Moreover, it has been demonstrated in the experimental model of nephrotoxic serum nephritis (12) and in adriamycin nephrosis (13) that the proteinuria coincides with an increased thromboxane B_2 excretion, the stable breakdown product of thromboxane A_2 synthesis. Zoja et al showed that the antiproteinuric effect of indomethacin was paralleled by a fall in this thromboxane B_2 excretion (14). Since thromboxane is an effective constrictor of mesangial cells (15),an effect of indomethacin on the glomerular ultrafiltration coefficient resulting in a decrease of glomerular protein leakage could well explain the antiproteinuric effect of indomethacin. The clinical studies presented in this thesis, however, showed a good correlation between the effects of indomethacin on prostaglandin excretion and protein excretion. This does not preclude that the NSAID in fact also inhibit intraglomerular thromboxane synthesis in parallel with medullary prostaglandin synthesis. We should, however, keep in mind that the rat may not be a good model for the human renal physiology.

In interpreting the antiproteinuric mechanism of the NSAID, one should also consider some other therapeutic interventions that result in a lower urinary protein loss. We will discuss the angiotensin-I converting enzyme (ACE) - inhibitors, dipyridamole and the low protein diet.

After the documentation of manifest effects of *ACE-inhibitors* on renal haemodynamics, first reports on the effects of these drugs on proteinuria appeared in 1985. Taguma et al found that captopril lowered urinary protein loss in a group of diabetic subjects without an effect on blood pressure in those patients (16). Heeg et al recently showed in a group of patients with urinary protein loss of different aetiology a decrease in proteinuria during ACE-inhibition. The fall in protein excretion (of 60%) was more pronounced than the fall in glomerular filtration rate (of 20%) and was positively correlated with the fall in

overall renal vascular resistance and particularly in postglomerular capillary resistance (17). The findings in these clinical studies are supported by the well known animal studies: most authors agree that angiotensin II induces a preferential efferent vasoconstriction (18) with a rise in intraglomerular pressure whereas ACE-inhibitors induce the reverse, a preferential efferent vasodilatation with a fall in intraglomerular pressure (19,20).

Data on the antiproteinuric effect of *dipyridamole* were first reported in 1974 (21). Since then different case reports mentioned an effect of this platelet aggregation inhibitor on proteinuria in diabetic nephropathy (22) and lupus nephritis (23). The two thus far known trials with dipyridamole show a significant fall in urinary protein loss in approximately 50% of the patients (24,25). The beneficial effect was suggested to be due to an inhibition of the platelet aggregation in diseases with enhanced platelet aggregation (26). It has recently been shown that the fall in urinary protein excretion after intravenous administration of dipyridamole is positively correlated with a fall in filtration fraction (27). Although the overall effects were quite small, this again could be compatible with a decrease of intraglomerular capillary pressure as the cause of the antiproteinuric effect of the drug. We are not aware of any animal studies on the effect of dipyridamole that would corroborate this hypothesis.

Finally, also *low protein diets* have been found to lower urinary protein loss both in animals (28) and in humans (29). Animal studies have given ample evidence that a low protein intake results in a decrease in intraglomerular pressure (30).

Interestingly, the different therapeutic interventions described above (ACE-inhibitors, dipyridamole and the low protein diet) have been advocated as a strategy to retard the progression of renal disease to end stage renal failure. Particularly low protein diet has proven its efficacy in animal (30) and also in human (29) studies. Such data have also been provided for the ACE-inhibitors in animal experiments (31,32), and are under way for human studies (33). With respect to dipyridamole the data are more difficult to interpret since generally combinations of dipyridamole with warfarin, with cyclofosfamide and an anticoagulant, or with aspirin were used (34-36). It has, however, been argued that these combinations slowed the deterioration of renal function in patients with type I membranoproliferative glomerulonephritis (34,36).

In this context it is of importance to evaluate data on the long-term outcome of renal function in patients who received NSAID as an antiproteinuric therapy. Such data, albeit in a retrospective analysis, are given in chapter 4. From this analysis the beneficial effect of indomethacin to retard the progression to end stage renal failure does emerge.

In summary, the parallelism between the different therapeutic modalities is striking. Does it indeed imply that these different strategies lower proteinuria via a final common pathway in lowering the intraglomerular capillary pressure? And will this thereby also have a beneficial effect on the long-term course of renal function in those patients? These questions can only be answered more precisely in prospective studies in which the different therapeutic regimens are evaluated both in their effects on proteinuria and renal haemodynamics. Such prospective trials are also warranted to evaluate the suggested beneficial effects for the preservation of renal function with either NSAID or ACE-inhibitors.

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SAMENVATTING

In dit proefschrift is een aantal onderzoekingen over de werking van indometacine en soortgelijke antiflogistica - de zogenaamde nonsteroidal antiinflammatory drugs (NSAID's) - op de nierfunktie en eiwituitscheiding in de urine van patienten met een nefrotisch syndroom gebundeld. In eerder onderzoek van onze afdeling was aangetoond dat indometacine binnen 24 uur de glomerulaire filtratiesnelheid, de filtratiefractie, het eiwitverlies in de urine, de selectiviteitsindex van de proteinurie en de plasma-renine-activiteit verlaagt. Tevens is gebleken dat de remmende werking van indometacine vooral optreedt, indien de nefrotische patienten in sterke mate ontzout zijn door een strenge dietaire zoutbeperking en een diureticum. Tenslotte is uit eerder onderzoek duidelijk geworden dat alle door indometacine veroorzaakte veranderingen in nierfunktie en eiwituitscheiding binnen enkele dagen na het staken van dit middel verdwijnen. De effecten van indometacine werden toegeschreven aan de meest bekende werking van de NSAID's: de remming van de prostaglandine-synthese door blokkade van het enzymcomplex prostaglandine synthetase. Uit dierexperimenten is bekend geworden, dat angiotensine en prostaglandine-synthese-blokkers de renale doorbloeding veranderen. Op basis van deze gegevens is vanuit de Groningse Kliniek de veronderstelling geuit, dat indometacine het eiwitverlies in de urine doet afnemen door zijn interferentie in het hormonale evenwicht van het renine-angiotensine systeem en de prostaglandines, hetgeen leidt tot een verandering van de glomerulaire doorbloeding.

Ter verdere beoordeling van deze hypothese is een aantal klinische onderzoeken verricht en in dit proefschrift beschreven. In de eerste instantie is nagegaan of een ander NSAID dan indometacine soortgelijke effecten op nierfunktie en het eiwitverlies in zout-gedepleerde nefrotische patienten heeft als indometacine. In hoofdstuk 2 beschrijven we, dat naproxen vergelijkbare veranderingen in nierfunktie, eiwitverlies en plasma-renine-activiteit teweeg brengt als indometacine, maar in maximale dosering minder effectief is dan indometacine. Tevens blijkt in dit onderzoek dat naarmate de daling van de glomerulaire filtratiesnelheid tijdens de behandeling met de NSAID's groter is, het eiwitverlies meer afneemt, maar dat de procentuele daling van de glomerulaire filtratiesnelheid telkens kleiner is dan die van de proteinurie. Er lijken dus meer, of andere factoren dan de achteruitgang van de glomerulaire filtratiesnelheid verantwoordelijk voor de daling van de proteinurie tijdens de behandeling met indometacine en naproxen.

Eén van deze factoren kan de mate van prostaglandine-synthese remming zijn. Zoals uit dierexperimenteel en in vitro onderzoek is gebleken remmen NSAID's op molaire basis de prostaglandine-synthese niet in gelijke mate. Of dit ook geldt

voor de remming van de prostaglandine-synthese in de nier, is het onderwerp van onderzoek, beschreven in hoofdstuk 3. Bij dit onderzoek is ervan uitgegaan dat de uitscheiding van prostaglandine E_2(PGE_2) in de urine een betrouwbare afspiegeling van zijn renale synthese is, indien voldoende voorzorgsmaatregelen bij het verzamelen van de urine in acht zijn genomen. Op basis van in vitro activiteit op de prostaglandine-synthese is een aantal NSAID's voor dit onderzoek geselecteerd. Het blijkt dat flurbiprofen en diclofenac natrium evenals indometacine de proteinurie in zoutgedepleerde nefrotische patienten verlagen. Het valt echter op, dat sulindac geen effect heeft op eiwitverlies. Eenzelfde fenomeen wordt bij beoordeling van de PGE_2-uitscheiding opgemerkt: tijdens de behandeling van flurbiprofen, diclofenac natrium en indometacine neemt de PGE_2-uitscheiding wel af, maar tijdens de behandeling met sulindac niet. De afname van de PGE_2-uitscheiding komt niet alleen met de daling van de proteinurie overeen, maar ook met die van de glomerulaire filtratiesnelheid. Het verband tussen de veranderingen in PGE_2-uitscheiding en eiwitverlies is opvallend groot ($R = 0.89$). Deze bevinding is een krachtige ondersteuning van de hypothese dat NSAID's het eiwitverlies in de urine doen afnemen door blokkade van de prostaglandine-synthese in de nier.

Op welke wijze veroorzaakt remming van de renale prostaglandine-synthese afname van de proteinurie?
Om deze vraag te kunnen beantwoorden is het nodig een aantal dierexperimenten te beschouwen. De groep van Brenner uit New York heeft zich op basis van een natuurkundig rekenmodel vooral beziggehouden met druk- en flowveranderingen in de glomerulus. In de rat gaan intrarenale infusies van angiotensine, PGE_2 en PGI_2 gepaard met een toename van de postglomerulaire weerstand en van de glomerulaire intracapillaire waterdruk. Het effect van de PGE_2 en PGI_2 kan voorkomen worden door een gelijktijdige infusie met saralasin. In diabetische ratten leidt behandeling met indometacine inderdaad tot een verlaging van de glomerulaire intracapillaire waterdruk, echter niet door afname van de postglomerulaire weerstand, maar door stijging van de preglomerulaire weerstand.

Een andere hemodynamische uitleg van de werking van indometacine steunt op het werk van Dunn en zijn medewerkers. Zij tonen in vitro aan dat angiotensine II een glomerulaire contractie veroorzaakt en dat deze door arachidonzuur en PGE_2 kan worden afgezwakt. Indomethacine blijkt in dit model de gevoeligheid van glomeruli voor angiotensine II te vergroten. Glomerulaire contractie is te beschouwen als een maat voor mesangiale contractie en voor filtratie-oppervlak, één van de determinanten van de glomerulaire ultrafiltratie-coefficient. Op grond van bovenstaande dierexperimenten lijkt het zeer waarschijnlijk dat tijdens de behandeling van ratten met indometacine er intrarenale hemodynamische veranderingen (daling van de glomerulaire transcapillaire druk en/of ultrafil-

tratie-coëfficient) ontstaan, die afname van glomerulaire filtratiesnelheid en eiwitverlies in de urine verklaren.

Treden soortgelijke veranderingen ook op bij de mens?
Gedurende de behandeling van zoutgedepleerde nefrotische patienten met NSAID's neemt de proteinurie sterker af dan de glomerulaire filtratiesnelheid en daalt de selectiviteitsindex van de proteinurie. Met andere woorden, de passage van het kleinere molecuul (de glomerulaire tracer ^{125}I-iothalamaat) over de glomerulaire wand wordt door NSAID's in mindere mate belemmerd dan de passage van het grotere molecuul albumine, en dit laatste weer minder dan het nog grotere eiwitmolecuul IgG. De lekken in de glomerulaire wand lijken dus tijdens de behandeling met NSAID's in grootte af te nemen. Bovendien bestaat er een verband tussen de veranderingen in eiwituitscheiding en filtratiefractie, een indicator voor glomerulaire transcapillaire druk. Naarmate de proteinurie tijdens de behandeling met NSAID's meer daalt, neemt de filtratiefractie sterker af. De grootte-afname van de glomerulaire lekken en de bovengeschetste samenhang tussen de afname in proteinurie, glomerulaire filtratiesnelheid en filtratiefractie tijdens NSAID behandeling wijzen erop dat hemodynamische veranderingen, zoals daling van intracapillaire glomerulaire waterdruk en/of ultrafiltratie-coëfficient, ten grondslag liggen aan de tijdens NSAID's behandeling ontstane veranderingen in nierfunktie en eiwitverlies.

De effecten van NSAID's treden vooral op wanneer de nefrotische patienten zoutgedepleerd zijn en het renine-angiotensine systeem gestimuleerd is. De sterke relatie tussen de mate van renale prostaglandine-synthese remming en de verandering in glomerulaire filtratie-snelheid en eiwitverlies in deze patientengroep is in dit proefschrift beschreven. De bovenstaande dierexperimentele gegevens in beschouwing nemend is het zeer waarschijnlijk, dat NSAID's hun intrarenale hemo-dynamische effecten bewerkstelligen door interferentie in het delicate, hormonale evenwicht tussen het renine-angiotensine systeem en de prostaglandines, dat in deze groep van patienten de glomerulaire filtratie reguleert.

Vooral door Brenner en zijn medewerkers is naar voren gebracht dat langdurig verhoogde intraglomerulaire druk en flow leiden tot een snelle achteruitgang van de nierfunctie (de hyperfiltratie-hypothese). Aangezien wij veronderstellen dat NSAID's deze druk in patienten met een nefrotisch syndroom verlagen, hebben wij onderzocht of indometacine op lange termijn de nierfunktie van deze patienten in gunstige zin beïnvloedt. De retrospectieve analyse van 98 patienten met een nefrotisch syndroom op basis van verschillende nierziekten waarvoor geen effectieve behandeling bekend is, is weergegeven in hoofdstuk 4. Uit dat onderzoek blijkt dat de met indometacine behandelde patienten uiteindelijk een beter behoud van hun nierfunktie hebben dan niet-met-indometacine behandelde patien-

ten. Een univariantie-analyse toont dat een ernstige proteinurie en de door middel van een nierbiopsie gestelde diagnose membranoproliferatieve glomerulonefritis in deze groep van patienten de snelheid van nierfunktie-afname ongunstig beïnvloeden. De aanvangsbloeddruk en nierfunktie blijken geen effect te hebben op de snelheid van nierfunktie-afname. Het betere behoud van nierfunktie in de met indometacine behandelde patienten is des te opvallender, omdat zij aan het begin van het onderzoek veel meer proteinurie blijken te hebben dan de onbehandelde patienten. In het licht van de hyperfiltratie-hypothese is het opmerkelijk, dat vooral in patienten met een lage of normale serumcreatinineconcentratie het gunstige effect van indometacine op het nierfunktiebehoud blijkt. Het retrospectieve karakter van het onderzoek laat echter geen harde conclusies over de wenselijkheid van NSAID's in de behandeling van patienten met een idiopathisch nefrotisch syndroom toe.

De behandeling met NSAID's is niet de enige therapievorm die gepaard gaat met afname van proteinurie en behoud van nierfunktie bij patienten met een nefrotisch syndroom. Zowel uit dierexperimenteel onderzoek als uit klinische waarnemingen blijkt behandeling met angiotensine converting enzym-remmers (ACE-remmers), dipyridamol of eiwit-beperkt dieet - zij het in wisselende mate - te leiden tot vermindering van het eiwitverlies. Telkens lijkt ook een daling van de filtratiefractie op te treden, hoewel dit niet in alle drie behandelingsmethoden evengoed is gedocumenteerd. Het is vooral van het eiwit-beperkt dieet en van de behandeling met ACE-remmers bekend, dat zij de achteruitgang van nierfunktie in een aantal nieraandoeningen vertragen.

De overeenkomsten tussen de verscheidene behandelingsmethoden zijn groot. Verminderen zij de proteinurie in nefrotische patienten via een gemeenschappelijke pathofysiologisch mechanisme? Heeft dit op de lange duur een gunstige invloed op het behoud van nierfunktie van de nefrotische patienten? Deze vragen kunnen alleen worden beantwoord met behulp van prospectieve onderzoekingen waarin de effecten van deze behandelingsmethoden op de renale hemodynamiek, het eiwitverlies in de urine en het behoud van nierfunktie in deze groep van patienten vergeleken worden.