MODULATORS AND MEDIATORS OF KIDNEY DISEASE PROGRESSION: NEW TARGETS OF PREVENTION AND TREATMENT

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MODULÁTORY A MEDIÁTORY PROGRESIE CHORÔB OBLIČIEK: NOVÉ CIELE PREVENCIE A LIEČBY

Background: **Hemodynamic (i.e. hyperfiltration) and metabolic (i.e. insulin resistance) changes are the targets of the present preventive measures of kidney disease progression. New horizons of molecular nephrology have extended the possibilities in the proliferation research.**
Objectives: **To review evidence on the significance of proliferative processes and the possibilities of interfering with proliferation.**
Methods: **A review of experimental and clinical studies elucidating the significance of thromboxane, platelet derived growth factor (PDGF) and transforming growth factor-β (TGF-β) for the proliferation and kidney disease progression.**
Results: **Proliferation participates in the development and progression of glomerulosclerosis and interstitial fibrosis. A number of growth factors and cytokines trigger and accelerate the progression of kidney diseases. A number of PDGF antagonists (i.e. simvastatin, heparin, trapidil, tertatol and low protein diet) attenuate the kidney disease progression.**
Conclusions: **Even the present knowledge enables to improve further the kidney disease treatment schedules.** ***(Tab. 3, Ref. 22.)***
Key words: kidney disease, proliferation, thromboxane, platelet-derived growth factor, transforming growth factor-β, glomerulosclerosis.

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Východiská: **Ovplyvnenie zmien vyvolaných v obličkách hyperfiltráciou nefrónov a inzulínovou rezistenciou sú cieľom súčasných preventívnych opatrení progresie chorôb obličiek. Nové horizonty molekulárnej nefrológie rozšírili možnosti výskumu proliferácie.**
Ciele: **Urobiť prehľad o proliferatívnych procesoch a o možnostiach ich ovplyvnenia.**
Metódy: **Prehľad experimentálnych a klinických štúdií objasňujúcich význam tromboxánu, trombocytového rastového faktora (PDGF) a transformujúceho rastového faktora-β (TGF-β) pre proliferáciu a progresiu chorôb obličiek.**
Výsledky: **Proliferácia participuje na vzniku a progresii glomerulosklerózy a intersticiálnej fibrózy. Viacero rastových faktorov a cytokínov spúšťa a akceleruje progresiu chorôb obličiek. Viaceré antagonisty PDGF (napr. simvastatín, heparín, trapidil, tertatolol a nízkobielkovinová diéta) spomaľujú progresiu nefropatií.**
Závery: **Už doterajšie poznatky umožňujú zlepšiť liečbu chorôb obličiek bezprostredne.** ***(Tab. 3, lit. 22.)***
Kľúčové slová: ochorenie obličiek, proliferácia, tromboxán, trombocytový rastový faktor, transformujúci rastový faktor-β, glomeruloskleróza.

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Hemodynamic mechanisms participating in kidney disease progression are the primary target of the present treatment schedules. However, new possibilities influencing metabolic and notably proliferative processes open new horizons of rationale prevention and treatment (5). Their overview will be outlined in the presented paper.

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Insulin resistance (IR)

Insulin belongs to the proliferative hormones and its proliferative action appears to be preserved even during IR**.** Because of the increased plasma insulin concentrations in IR patients the proliferation is stimulated. As a result, the improvement of insulin sensitivity attenuates also the proliferative pathways.

Almost all kidney disease patients in predialysis period suffer from IR, which is caused principally by the accumulated endproducts (3): At least 3 of them were isolated and defined in our laboratory:

Inhibitor of glucose utilization was isolated at the beginning of 70-ties by a tedious technique of classical liquid chromatography (6) and it was found to be a peptide. By crossover point

determination and other techniques its noncompetitive inhibition of P-fructokinase was defined (4).

Hippurate was a second inhibitor isolated by a more sophisticated HPLC methodology and its structure defined by mass spectrometry and nuclear magnetic resonance (18).

Pseudouridine, an endproduct of tRNA breakdown was a next inhibitor, isolated and defined in our laboratory (14). Additional inhibitors, such as 5-hydroxyindoleacetic acid (20) participated in IR of renal failure patients.

The mentioned and additional accumulated "uremic toxins" caused almost 100 % prevalence of insulin resistance in patients entering dialysis and its improvement in patients starting dialysis.

Next naturally addressed step was the timing of IR development in kidney disease patients. A group of patients with normal or just slightly decreased kidney function was evaluated by the method used previously in San Antonio Heart Study (10), i.e. oral glucose tolerance test with the simultaneous determination of glycemic, insulinemic and C peptide responses to glucose administration. Fifty percent of the evaluated patients suffered from IR even at the early stage with no accumulation of hippurate or pseudouridine, while the prevalence of IR in our population was about 15 %, in accordance with other international epidemiological studies. It was suggested, that besides the accumulated uremic toxins other factors related to IR participate in kidney diseases (8). They were just superimposed by the action of uremic toxins and improved by dialysis which at least partially decreased the accumulation of various metabolic endproducts.

Tab. 1. Neurohormonal modulators of proliferation.

Angiotensin II
Plasma renin activity
Aldosterone
Epinephrine and norepinephrine
Vasopressin
Atrial natriuretic factor
Insulin

A 48 % prevalence (21) of IR was found in a comparative study of essential hypertension, which was suspected to participate in IR also of kidney disease patients. However, no difference in blood pressure was found in IR and insulin sensitive (IS) kidney disease patients (8). Of course, all patients were treated for hypertension effectively. Thus, no definite answer could have been formulated. IR, undoubtedly an accelerating factor of athero- and glomerulosclerosis appears to be associated also with kidney diseases.

Neurohormonal modulation

Plasma concentration of various hormones increases during cardiovascular diseases, with the modulation of cardiovascular system but also other organs and tissues. Their accumulation in plasma was illustratively presented both in acute myocardial infarction (12) and chronic heart failure (9). The increased hormonal levels in plasma modulate not only the impaired myocardium, but the whole organism. This is true also for the opposite relationship: in experimental remnant kidney model the accumulation of platelet derived growth factor (PDGF) in the myocardial tissue was described (1). We do share a good overview on the neurohormonal modulation in cardiovascular diseases and experimental studies. Unfortunately just fragmental or indirect evidence was collected in other diseases, notably in human kidney diseases.

Angiotensin II (A II): On the basis of the experimental and clinical studies A II was suggested to trigger the neurohormonal modulation (17). Additional hormones (Table 1) were released secondarily. Moreover, the inhibition of A II production by ACEI decreased not only A II plasma concentration but also the plasma levels of the listed hormones. The relationship was even more complicated by the existence of two RAAS pools, i.e. plasmatic and tissue pools of A II production.

As a result also the effect of ACEI on IR was evaluated: At that time various studies published in literature came to discrepant conclusions: Either improvement or no change of IR during the ACEI treatment. The discrepancy was solved in 3 studies with enalapril, lisinopril and perindopril (16): only patients who were insulin resistant improved insulin sensitivity, but not those who were insulin sensitive. Thus, the discrepant findings of various research groups were caused by different composition of the studied groups. The studies with ACEI turned the attention to the hemodynamic, metabolic and notably proliferative effects of A II.

Neurohormonal mediation

Hormones modulate the cells by binding to specific receptors and intracellular production of messengers and mediators which fulfill autocrine or paracrine functions (15). Most of them are growth factors or cytokines. Much has been learnt on the dynamics of changes in a model of Anti Thy 1.1 experimental nephritis (11): After a single dose of Anti Thy 1.1 antibody a benign and transient glomerulonephritis develops. The first changes, developing already during the first day are the kidney accumulation of platelets, containing basic fibroblast growth factor (bFGF), PDGF and other factors accumulated in dense and α-granules. They are followed by the intensive mesangiolysis apparent up to 5 days in which time the proliferative processes predominate. The proliferation starts with the repopulation of mesangium by mesangial cells. The following production of extracellular matrix is stimulated by TGF-β. The most important mediators appear to be:

Thromboxane (TXA_2): Two pools of TXA_2 take place in organism: *a) Vascular pool*, i.e. TXA_2 synthesized in platelets and leukocytes. *b) Tissue pool:* several kidney cells synthetize TXA_2, notably mesangial and epithelial cells in glomeruli, collecting duct and renomedullar intersticial cells. The main stimulator of TXA_2 production is A II but also other hormones participate in the stimulation of TXA_2 synthesis. In fact, TXA_2 appears to be a mediator of various A II actions in the organism (7).

TXA_2 shares various effects important for renal disease patients (7). *a) Proliferative effects:* They were found and confirmed in various experimental studies and even in tissue culture cells. *b) Stimulation of mesangial matrix production:* TXA_2 stimulates fibronectin synthesis in mesangial cells and the production could be attenuated by the inhibition of TXA_2 synthesis.

Experimental studies: The inhibition of TXA_2 production (both vascular and tissue pools) by cyclooxygenase or thromboxane syn-

Tab. 2. Modulators of PDGF production and action in kidney diseases (21).

Stimulators	Inhibitors
PDGF	IL-1, IL-6
TGF-β	TNF-α
EGF	Trapidil
bFGF	Tertatolol
TNF-α	Simvastatin
Endothelin	Tyrphostin
LDL	Low protein diet
Thrombin	Heparin
PG $F_2\alpha$	

Tab. 3. Antagonists of TGF-β action (20).

Neutralizing TGF-β antibodies
Other potential TGF-β antagonists
Latency-associated peptide
Antisense oligonucleotides
Proteoglycans (decorin)

thase inhibitors and notably by thromboxane receptor antagonists attenuates the progression of kidney disease and proteinuria (13).

Clinical trials: Patients with diabetic nephropathy suffer from the increased renal production and urinary excretion of TXA_2. In accordance with findings in nondiabetic kidney diseases the inhibition of proliferation and proteinuria by inhibiting TXA_2 synthesis is probable. However, no controlled clinical trial has been performed yet.

TXA_2 besides the direct effects shares also indirect effects: It is the most important amplifier of platelet aggregation. However, during the aggregation a series of growth factors, mediators stored in platelet dense or α-granules, are released locally. Et least two of them will be mentioned.

Platelet derived growth factor (PDGF): PDGF is one of the most important growth factors with various biological effects. The most important effect appears to be the proliferative action on mesenchymal cells, but PDGF is also a mediator of other growth factors, such as EGF. PDGF stimulates the production of TGF-β in kidney mesangial, epithelial and endothelial cells. The relationship is rather complicated because there are three active isoforms, i.e. PDGF AA, BB and AB with their receptors PDGF-Rαα, ββ, αβ. Overlapping of action because of crossover binding makes the relationship even more confusing (19).

PDGF shares a positive feedback mechanism, i.e. minimal production immediately amplifies further PDGF production; PDGF production is stimulated or inhibited by various factors (Table 2). Some of them are exploited therapeutically. Unfortunately, no data are at the disposal about PDGF plasma or serum concentrations in diabetic subjects though there is a great probability of its increased concentration at least in plasma.

Transforming growth factor (TGF-β): It circulates in 3 isoforms, the most important being TGF-β1, circulating in plasma bound to latency associated peptide (LAP) and the determination of free and latent forms has been developed just recently. Its biological effects depend on its concentration: at low concentrations TGF-β1 inhibits cell proliferation and stimulates extracellular matrix production. in this respect it is the most important mediator of fibrogenesis participating in the development of glomerulosclerosis and intersticial fibrosis in kidney diseases (2,5). PDGF stimulates the TGF-β production and TGF-β can block PDGF's actions by regulation of PDGF receptor expression. TGF-β is also an immunosuppressant that is sonsiderably more powerful than cyclosporine in suppressing T cell function. The fibrogenic action of TGF-β could be summarized: *a)* It *autoinduces* its own production. *b)* TGF-β directly upregulates the genes of most matrix proteins leading to their increased synthesis. *c)* It *modulates the expression* of integrins on the cell's surface in a manner that facilitates attachment to the newly synthetized matrix. *d)* TGF-β *suppresses* the production of *proteases* that normally degrade and turnover the matrix. It also stimulates synthesis of *protease inhibitors.* On the other hand *proteases* are isoform-specific regulators of the binding of TGF-β to α_2-macroglobulin (22).

In diabetes, because of dominant feature of mesangial expansion in glomerulosclerosis, much attention was paid to TGF-β1 balance. Several pieces of evidence have been collected (2): *a) Immunohistochemical* detection of TGF-β1 accumulation in rat and human diabetic nephropathy in renal biopsy both in NIDDM and IDDM. *b) Cultured mesangial cells* synthetized more TGF-β1 at increased glucose medium concentration. *c) Mesangial hypertrophy* is induced when exposed to TGF-β1 in vitro. *d) TGF-β1 mRNA* was found to be expressed in glomeruli of kidney biopsy specimens both in NIDDM and IDDM. *e) TGF-β1* not only stimulates mesangial matrix expansion but it also inhibits its degradation.

An intensive research of TGF-β action has been started because of its dominant role in fibrogenesis. Various alternatives are presented in table 3. The most intensively studied antagonist appears to be decorin which is at the disposal already of recombinant origin. However, neither decorin nor other antagonists have been used yet in human studies.

Future research

Much has been learnt from the performed studies and the mediators with the highest impact have been defined. Their exploitation in clinical practice could markedly attenuate the prevalence and progression of kidney diseases. However, decisive factors, the dynamics and especially the relationship between various growth factors and cytokines have not been elucidated yet, because of methodological hindrances and expenses. Last, but not least, the controlled clinical trials are urgently needed.*

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