ROLE OF CIGARETTE SMOKE IN CHRONIC KIDNEY DISEASES

Summery of Ph.D. theses

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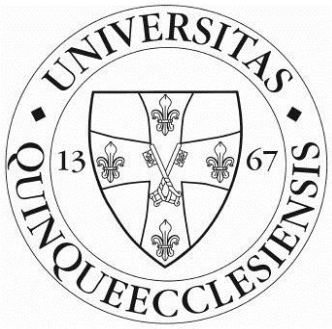

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ABBREVIATIONS

Ach	acetylcholine
ATP	adenosine tri-phosphate
$BaCl_2$	barium chloride
BMI	body mass index
$CaCl_2{\cdot}2H_2O$	calcium chloride
$Ca_v1.2$	L-type voltage-dependent calcium channel
CKD	chronic kidney disease
CO	carbon monoxide
CO_2	carbon dioxide
CS	cigarette smoking
CSB	cigarette smoke buffer
DM	diabetes mellitus
DNP	diabetic nephropathy
eGFR	estimated glomerular filtration rate
GFR	glomerular filtration rate
GSH	glutathione
HbA_{1c}	glycosylated hemoglobin
HR	heart rate
ING	idiopathic nodular glomerulosclerosis
K_{ATP}	ATP-sensitive potassium channel
KCl	potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
KW	Kimmelstiel-Wilson
LiCl	lithium chloride
MAP	mean arterial pressure
mg/c	milligram per cigarette
Mg_2SO_4	magnesium sulfate
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
NCX	Na^+-Ca^{2+} exchanger
NGS	nodular glomerulosclerosis
NO	nitric oxide
non-diab NGS	non- diabetic nodular glomerulosclerosis
non-KW	non-Kimmelstiel-Wilson diabetic nephropathy
ODQ	oxadiazolo-quinoxalin-1
RAAS	renin-angiotensin-aldosterone system
RI	resistance index
SD	standard deviation
SH group	sulph-hydril group
SOD	superoxide dismutase
TEA	tetraethylammonium
wCS	water-soluble components of cigarette smoke

INTRODUCTION

GENERAL ROLE OF SMOKING IN CHRONIC KIDNEY DISEASE AND DIABETIC NEPHROPATHY

The fight against cigarette smoking is a global challenge. Worldwide 1.2 billion people smoked in 2000, a number that is projected to increase to 1.6 billion by 2030. Tobacco currently causes an estimated 5 million deaths annually and if the actual trends continue, the number of deaths will be doubled by 2030. Chronic cigarette consumption is harmful in both active and passive smokers and it has a role in the initiation and progression of certain chronic kidney diseases (CKD), type 2 diabetes mellitus and in the progression of diabetic nephropathy and cardiovascular complications of diabetes mellitus (DM). It is also evident that chronic kidney failure raises the risk of cardiovascular morbidity and mortality, thus tobacco use can be considered as a factor which induces or aggravates processes that diminish life quality or even shorten life expectancy. Noteworthy is the „memory for smoking" of the organism, namely the deleterious effects of tobacco consumption do not last only until the cessation of cigarette smoking but even years after.

Tobacco consumption is involved in the initiation and progression of the most common causes of CKD e.g. diabetic nephropathy, ischemic nephropathy, nephrosclerosis, IgA nephropathy - the most frequent primary glomerulonephritis - or autosomal dominant polycystic kidney disease - the most common cystic kidney disease and some less frequent diseases like lupus nephritis. Smoking causes insulin resistance – thus increases the risk of metabolic syndrome and type 2 diabetes. Both in type 1 and type 2 diabetes smoking increases the risk of the initiation and progression of nephropathy. The pathomechanisms of these diseases are complex and not yet fully understood, therefore each step can be crucial for the outcome of the disease for each individual. We would like to underline the facts and theories that support the fight against an addiction that can destroy one's life moreover the life of the smokers family, colleagues or other "innocent" bystanders.

Large epidemiological cross-sectional studies (IRSA, PREVEND) have shown that in the general population there is a higher glomerular filtration rate (GFR) and an elevated risk for albuminuria or proteinuria in smokers compared to the non-smokers. However, in otherwise healthy individuals smoking a single cigarette causes a transient elevation in

blood pressure and a reduction in GFR, while the change in the normal range of albuminuria is not known. It was also suggested that glomerular pressure during smoking may be increased due to acute elevation of systemic blood pressure and changes of the renal autoregulation.

The prevalence of diabetes, predominantly type 2, is increasing worldwide, and diabetic nephropathy is the leading cause of end-stage renal failure. The basics of the clinical development and histological course of diabetic nephropathy are known, yet the exact pathogenesis remains uncertain. Kidney biopsies are relatively rarely performed in diabetic nephropathy, despite the fact that bleeding complications occur less frequently in diabetic nephropathy compared to other common kidney disorders. In general practice, the biopsy procedure in diabetic nephropathy is only indicated if a non-diabetic renal disease is suspected, and this situation probably occurs more often in type 2 than in type 1 diabetic patients. A recent consensus of pathologic classification of diabetic nephropathy deals with Kimmelstiel-Wilson (KW) lesion as a separate class (class III). The same histological pattern as that of KW lesion was also described in patients without the clinical evidence of diabetes mellitus, and named “idiopathic nodular glomerulosclerosis” (ING). ING is an extremely rare and distinct clinicopathologic entity, which is linked to hypertension and chronic cigarette smoking. Another name “smoking-associated nodular glomerulosclerosis” is also accepted for this entity, and is much more common in men than in women. There are several factors, such as smoking, hypertension, obesity, hyperlipidaemia, and renal insufficiency, that can contribute both to the development of ING and to the progression of diabetic nephropathy. However, among patients with KW, the exact prevalence of these conditions and altered laboratory parameters have yet to be described.

AIMS

1) **First, we aimed to test the hypothesis that cigarette smoking elicits acute changes in hemodynamic parameters and vasomotor tone of renal arteries in humans.**

   Investigation of the acute effect of either nicotinic or nicotine free cigarette smoke or sham smoking on the changes in the resistance index (RI) of segmental renal arteries; on the mean arterial pressure; on the heart rate.

2) **Second, we investigated the acute effects of water-soluble components of cigarette smoke (wCS) on the isometric tension of isolated rat segmental renal arteries in order to elucidate the underlying mechanisms.**

   a. **Testing the effect of different concentrations of nicotinic cigarette smoke and nicotine free cigarette smoke on isolated rat renal vessels.**
   b. **Evaluation of the potential contribution of the endothelium or carbon monoxide (CO) and/or nitric oxide (NO) in the smoking- induced relaxation of renal arteries.**
   c. **Elucidation of the role of free radicals, as known modulators of the vascular tone, in the relaxation caused by cigarette smoke.**
   d. **Investigation of the potential role of certain ion channels, $Ca_v1.2$ L-type calcium channels and the Na^+-Ca^{2+} exchanger, in the smoke-induced response of rat renal arteries.**

3) **Our third aim was to test the role of chronic cigarette smoking in male patients with diabetic nodular glomerulosclerosis.**

   Retrospective systematic revision of our native kidney biopsy databank between 2001 and 2011, and comparison of the smoking habits and other clinical settings in three groups:

      i. **men with type 2 diabetes mellitus and Kimmelstiel-Wilson lesion (KW)**
      ii. **men with non-KW diabetic nephropathy (non-KW; negative control)**
      iii. **male patients with non-diabetic nodular glomerulosclerosis (non-diab NGS; positive control).**

METHODS AND RESULTS

1. In vivo investigation of the acute effects of cigarette smoke on human renal arteries.

1.1. Materials and methods

Eight healthy male smoker volunteers with ages ranging 21 to 41 years were examined. The subjects smoked daily 17.7±6.9 cigarettes and the duration of smoking was 13.3±5.0 years. Renal disease was excluded, and all individuals were instructed to stop smoking 8 hours before the study. The subjects smoked one commercial available cigarette (tar: 10 mg/c, CO: 10 mg/c, nicotine: 0.8 mg/c) for 5 minutes. One segmental renal artery was chosen by a trained physician and the Doppler spectrum was detected. The resistance index [RI= (peak systolic velocity – end diastolic velocity)/peak systolic velocity] was measured in the same artery 3 minutes before, in every minute during, and 3 minutes after smoking the cigarette. The results 3 minutes before lighting and 3 minutes after finishing the cigarette and the lowest value during smoking were recorded. Also, systemic blood pressure and heart rate were measured in the same period. In another series of experiments on separate days the same protocol was repeated with the same subjects, but instead of the nicotinic cigarette first sham smoking, i.e., suction of a dummy cigarette, and second a nicotine-free –cigarette was used. The changes in the RI, mean arterial pressure (MAP) or heart rate (HR) during and after smoking were expressed as the percentage of the values before smoking. The study was approved by the local ethics committee (N°: 3344). All subjects gave written informed consent.

Statistical significance was calculated using Student's *t*-test in the human study and also in the subsequent animal experiments. All distributions were normal, data are means ± SD. The tests were performed with the SPSS program package, Version 15.0 (SPSS, Chicago, IL, USA), considering P values of 0.05 or less to be significant.

1.2. Results: human study

In healthy young men, smoking a nicotinic cigarette caused a transient and significant ($P<0.05$) reduction in the resistance index of a segmental renal artery.

Nicotine free cigarette smoking elicited a similar reduction in RI ($P<0.05$), as the nicotinic cigarette did.

Sham smoking did not elicit significant changes in these parameters.

Due to nicotinic cigarette smoke a transient elevation in MAP ($P<0.05$, and an elevation in HR ($P<0.05$) were also observed. However neither nicotine free cigarette smoking nor sham smoking changed MAP and HR significantly.

2. Ex vivo investigation of the acute effects of cigarette smoke on rat renal arteries.

In the previous study we have shown that cigarette smoking elicits acute and transient decrease in the resistance index of human renal arteries, which is indicative for a dilation of these vessels. In the present ex vivo experiments we investigated the acute effects of water-soluble components of cigarette smoke (wCS) on isolated rat segmental renal arteries in order to elucidate the underlying mechanisms.

2.1. Materials and methods

The modified method of Fésüs et al. was used. Cigarette smoke buffer (CSB) stock solution was prepared by passing the smoke of one commercially available cigarette through 5ml Krebs buffer (containing 119 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 1.2 mM Mg_2SO_4, 11.1 mM glucose, 1.6 mM $CaCl_{2*}2H_2O$, pH 7.4), for 5 min in order to trap its water-soluble components.

Adult (10-12 week-old, 300-350 g) male CFY rats were sacrified. After preparation of 2 mm long first order renal arteries (~150-200 μm in diameter), rings were mounted on two stainless steel wires (40 μm in diameter) in a Danish Multimyograph (Model 610M).

Vessels were bathed in Krebs buffer and gassed with 5% CO_2 and 95% O_2 at 37˚C, pH 7.4. The resting tension/internal circumference relationship for each vessel was determined, then after a normalization procedure vessels were allowed to stabilize for 30 min, and then isometric tension was continuously recorded. The contractile capacity was assessed by exposing the arterial segments to an isotonic 60 mM KCl solution. The presence of functional endothelium was assessed in all preparations by the ability of acetylcholine (ACh $3x10^{-6}$ M) to induce more than 50% relaxation of 60mM KCl precontracted vessels. When necessary, endothelium-denuded vessels were prepared by gently rubbing a hair through the lumen and verified by loss of response to acetylcholine. To give the vessels the possibility both to relax or to contract, rings were preconstricted

with 100nM epinephrine (series #1) which showed 60% contraction force of the 60mM KCl contraction. After reaching a stable contraction plateau, increasing doses of cigarette smoke buffer (1%, 5%, or 10% final concentrations) were administered into the vessels chamber. The rate of relaxation caused by the water-soluble components of cigarette smoke (wCS) was expressed as the percentage of the control (100%) vessel. Soluble guanylate cyclase inhibitor oxadiazolo-quinoxalin-1 (ODQ, 5 μM), inhibitor of the ATP-sensitive potassium (K_{ATP}) channel glibenclamide (10 μM), or large-conductance calcium-activated potassium channel blocker tetraethylammonium (TEA, 2 mM), were added to the vessel chamber 30 minutes before getting the epinephrine induced contraction plateau. Free radical scavengers, such as glutathione (GSH, 4 mM), catalase (1000 U/ml) or superoxide dismutase (SOD, 200 U/ml) were added both to the wCS solutions and to the vessel chamber 30 minutes before establishing the epinephrine induced contraction plateau.

In another series of experiments contractions evoked by modified depolarizing Krebs buffers were measured (series #2). After washing the vessels 3 times with Krebs buffer and waiting for 20 minutes $CaCl_2$ was substituted with $BaCl_2$ (3,2 mM) to obtain a control contraction. Then the preparations were washed 3 times with Krebs buffer and after another 20 minutes resting period the $BaCl_2$ contractions were repeated alone or in the presence of either 5% cigarette smoke buffer or the specific L-type calcium channel blocker nifedipine (10 nM). The peak responses to $BaCl_2$ in control conditions and in the presence of cigarette smoke buffer or nifedipine were measured and the ratio of the CSB/control and nifedipine/control contractions was calculated. The results were normalized by correcting the ratio with the percentage of 2 following “non-treated” $BaCl_2$-induced contractions.

In a third set of experiments (series #3), in the Krebs buffer, NaCl was substituted by equimolar LiCl to activate Na^+-Ca^{2+} exchanger. The protocol used was similar to series #2. Contractions were studied using either 1% cigarette smoke buffer or a specific blocking agent of the Na^+-Ca^{2+} exchanger SEA0400 (2 μM). These data were normalized as described in series #2.

All drugs were purchased from Sigma Chemicals (St Louis, MO, USA) except for SEA0400, which was a gift of Prof. Ferenc Fülöp, the head of the Institute of Pharmaceutical Chemistry, University of Szeged, Hungary.

The experiments were performed with the permission of the Hungarian Local Animal Experiment Committee (N°: BA02/2000-1/2008).

2.2. Results: animal experiments

In isolated first order branches of rat renal arteries, water-soluble components of nicotinic cigarette smoke elicited a dose-dependent relaxation compared to control.

Water soluble components of nicotine free cigarette had a similar effect.

Relaxation of isolated renal arteries by adding wCS into the chambers was still present after removal of the endothelium. We also found that relaxation of vessels to nicotinic wCS was not significantly affected by prior incubation of the vessels with agents interfering with known mechanisms of smooth muscle relaxation, such as oxadiazolo-quinoxalin-1, tetraethylammonium or glibenclamide.

In contrast, the hydrogen peroxide-scavenger catalase significantly diminished the relaxation of renal arteries caused by nicotinic wCS, whereas superoxide dismutase (SOD) significantly enhanced relaxation. Also, presence of reduced glutathione (GSH) significantly diminished the relaxation of vessels to nicotinic wCS.

Depolarizing Krebs solution caused a transient contraction of rat renal arteries, which was reduced by nicotinic wCS (5%). It is noteworthy, that the reduction was not different compared to the effects of the L-type calcium channel blocker nifedipine.

Use of a Na^{+}-Ca^{2+} exchanger activator Krebs solution also elicited a transient contraction in rat renal vessels. The Na^{+}-Ca^{2+} channel blocker SEA0400 reduced this contraction, similar to nicotinic wCS (1%).

3. Retrospective clinical study to investigate the role of chronic cigarette smoking in the development of diabetic nodular glomerulosclerosis (Kimmelstiel-Wilson lesion).

In this study we reviewed our clinic's native renal biopsy databank between 2001 and 2011 and we formed three groups of male patients: (i) all men with type 2 diabetes mellitus and Kimmelstiel-Wilson (KW) lesion; (ii) all diabetic men with non-KW diabetic nephropathy (non-KW); and (iii) all male patients without any carbohydrate metabolic disorder but with non-diabetic nodular glomerulosclerosis (non-diab NGS). Afterwards we compared among these groups the smoking habits and the retrospective available clinical

data that have an impact on the development of diabetic nephropathy and nodular glomerulosclerosis.

3.1. Subjects and methods

All native renal biopsy specimens of male patients (n=644) available at the 2nd Department of Internal Medicine and Nephrological Center of the University of Pécs from 2001 to 2011 were reviewed retrospectively. Type 2 diabetic patients with either KW lesion or other classes of diabetic nephropathy and patients with non-diab NGS were further analyzed. The obtained samples were examined by light microscopy, immunofluorescence, and electron microscopy. Type 2 diabetes mellitus and KW were defined following the literature by: (i) the evidence of diabetes mellitus, (ii) the histological finding of nodular mesangial sclerosis in at least one glomerulus (without global glomerulosclerosis in more than 50% of the glomeruli), and (iii) the exclusion of chronic membranoproliferative glomerulonephritis, chronic thrombotic microangiopathy, amyloidosis, monoclonal immunoglobulin deposition disease, fibrillary glomerulonephritis, and immunotactoid glomerulopathy. In the non-KW group, there were type 2 diabetics who met the criteria both of diabetes mellitus and diabetic nephropathy, not including KW lesion. The non-diab NGS group represented men with NGS but without any carbohydrate metabolic disorder proven by an oral glucose tolerance test, HBA_{1c} or fasting plasma glucose. Apart from a thorough review of the histological descriptions, factors potentially responsible for either the worsening of diabetic nephropathy or the development of NGS were also analyzed. These recorded clinical settings (age, body mass index (BMI), duration of diabetes mellitus, prevalence of hypertension (RR>140/90Hgmm), duration of hypertension, percentage of renin-angiotensin-aldosterone system (RAAS) blocker treatment, serum cholesterol, serum triglyceride, glycemic control (HbA_{1c}), and estimated glomerular filtration rate (eGFR) at the time of kidney biopsy were compared between the groups. The smoking habits of the different groups were analyzed by thorough revision of the patients' medical documents and a standardized telephone questionnaire was also performed. Both diabetic and non-diabetic patients were divided into two groups: ever-smokers and non-smokers. Cigarette consumption was expressed in pack-years (1 pack-year = consumption of 20 cigarettes / day for 1 year).

Statistical significance was calculated by using analysis of variance (ANOVA), chi square or Kruskal-Wallis tests as appropriate. Multiple group comparisons were carried out using ANOVA and Bonferroni's post hoc tests. All distributions were normal, with the exception of serum cholesterol, triglyceride, eGFR and pack-years. Data are expressed as mean ± SD. The tests were performed using the SPSS program package, Version 17.0 (SPSS, Chicago, IL, USA), considering P values of 0.05 or less to be significant.

3.2. Results: retrospective clinical case – control study

Among male patients who underwent a native kidney biopsy in the period between 2001 and 2011 in our nephrological center (n=644), the proportion of type 2 diabetic men with diabetic nephropathy was 9.5% (n=61), 2.3% (n=15) with KW and 7.2% (n=46) with non-KW. The percentage of non-diab NGS was 1.1% (n=7), including two patients (0.3%) with ING and five (0.8%) with non-diab NGS of different known origins.

Members of all three groups (KW, non-KW, non-diab NGS) did not differ from each other in several parameters: they were middle-aged (mean age: 56±1, 56±9, 55±1 years, respectively; p=0.935), obese (BMI: 30±5, 31±5, 28±6 kg/m^2; p=0.538) patients with hypercholesterolaemia (5.2 {4.0-6.3}, 5.5 {4.3-7.2}, 5.7 {4.7-9.9} mmol/l; p=0.500), hypertriglyceridaemia (2.0 {1.4-2.7}, 2.4 {1.6-3.1}, 3.4 {1.1-3.6} mmol/l; p=0.784), impaired renal function (eGFR, 25 {13-48}, 42 {20-70}, 42 {23-77} ml/min; p=0.483), high prevalence of hypertension (93, 87, 86%; p=0.782), and a long duration of hypertension (11±8, 13±10, 11±6 years; p=0.948;).

The initial proteinuria was high in all the three groups. There was a similarity in the KW and non-diab NGS groups (KW: 3.7±1.7 g/day; non-diab NGS: 5.8±4.2g/day; p=1.0 {for the logarithmic values, Bonferroni}) and it was somewhat lower in the non-KW group (non-KW: 2.3±1.6g/day). There was no statistical difference in the initial proteinuria between the KW and non-KW groups (p=0.153 {for the logarithmic values; Bonferroni}). The difference only just reached significance between the non-KW and non-diab NGS groups (p=0.042 {for the logarithmic values, Bonferroni}). The percentage of RAAS-blocker treatment, as a confounding factor both in the course of diabetic nephropathy [123] and non-diab NGS [50], did not differ significantly among the three investigated groups (KW: 100, non-KW: 87, non-diab NGS: 100%; p=0.222). Between the two diabetic groups, the duration of

diabetes (KW: 11±6, non-KW: 10±7 years; p=0.617) and glycemic control (HbA_{1c}: 6.5±1, 6.0 ±1%; p=0.88) was similar.

However, among type 2 diabetic men with KW, the majority (13/15= 87%) were smokers, unlike the non-KW group (16/46= 35%; p=0.001 vs. KW) but similar to the non-diab NGS group (7/7= 100%; p=1.0 vs. KW).

Cigarette exposure also differed among the groups and showed the same pattern as smoking prevalence (KW: 15 {6-30} pack-years; non-KW: 0 {0-21} pack-years; non-diab NGS: 30 {16-33} pack-years; p=0.010 non-KW vs. KW; p=0.008 non-KW vs. non-diab NGS). Moreover, there was no difference in cigarette consumption between the two groups with NGS (p=0.185).

DISCUSSION

The salient findings of the present *in vivo* investigation of the acute effects of cigarette smoke on *human renal arteries* and *ex vivo* investigation of the acute effects of cigarette smoke on *rat renal arteries* are two-fold. 1) In healthy individuals smoking of a nicotinic or a nicotine free cigarette causes a transient reduction in the resistance index of the segmental renal arteries. 2) Water-soluble components of nicotinic or nicotine free cigarette smoke elicit dose-dependent relaxations of rat renal arteries. These effects were not affected by removal of the endothelium, inhibition of the soluble guanylate cyclase, blocking of large-conductance calcium-activated potassium channels or ATP-sensitive potassium channels. However, the relaxations were reduced by catalase and enhanced by superoxide dismutase.

Previous studies have shown that human airway blood flow is transiently increased after smoking a cigarette. Also, Doppler ultrasound revealed an increased diameter of the portal vein during smoking. Based on these findings, we hypothesized that wCS cause relaxation of renal vessels, which together with the elevated mean arterial pressure during smoking and both endothelial cell injury and endothelial dysfunction could elicit hyperfiltration and increased albuminuria in smokers. Accordingly, in humans we studied resistance indices (RIs) of segmental renal arteries during smoking, known to reflect alterations in vessel diameter. The potential influence of the parasympatho-mimetic

activation due to deeper inhalations was excluded by sham smoking. It is known that tachycardia can lower the RI; however, our data shows that the non-nicotinic cigarette caused similar reductions in RI as the nicotinic cigarette did, without elevation in heart rate. In the present study, we provided clear evidence for a transient decrease in RI during cigarette smoking, which is indicative of dilation of the examined segmental renal arteries and/or more distal renal resistance vessels.

Investigation of isolated rat renal arteries showed dose-dependent relaxations in response to water-soluble components of cigarette smoke. Nicotine, the most investigated component of cigarette smoke is unlikely to be responsible for the observed response, since wCS of nicotine free cigarettes induced similar relaxations of vessels compared to wCS of nicotinic cigarettes. We have also found that nicotine free cigarettes did not elicit significant alterations in systemic BP and HR, findings which are consistent with the literature. We could also exclude the potential contribution of carbon monoxide (CO) because inhibition of soluble guanylate cyclase or large-conductance calcium-activated potassium channels – the main targets of CO – did not affect relaxation of vessels to wCS. In addition, previous human studies have shown that CO levels remain high even 30 minutes after smoking a single cigarette, whereas dilation of renal vessels occurred early and transiently (~5 min). Also, because removal of the endothelium or inhibition of the soluble guanylate cyclase by ODQ did not influence significantly the relaxation of vessels to cigarette smoke we could exclude the contribution of nitric oxide produced by endothelial nitric oxide synthase, as well.

It is known that free radicals found either in cigarette smoke or generated in the vascular tissue can modulate vascular tone. Hydrogen peroxide can induce dilation via endothelial and smooth muscle-dependent mechanisms. In the present study, renal arteries were incubated with 1-10% solutions of wCS, which is similar to the concentrations used by others. We found that the hydrogen peroxide scavenger catalase significantly diminished smoke-induced renal artery relaxation, whereas SOD, which dismutates superoxide to hydrogen peroxide, enhanced the relaxation. Thus, relaxation of renal arteries by wCS can be – at least in part – mediated by hydrogen peroxide present in the wCS-containing bath solution and/or produced by SOD in the vessel wall. Moreover, our findings that relaxations were present after removal of the endothelium suggest that primarily smooth muscle dependent mechanisms are involved. Reactive oxygen species

are known to regulate ion channels and transporters. For example, thiol oxidation has been reported to activate a redox-regulated vasodilator mechanism involving inhibition of Ca^{2+} influx through L-type calcium channels in coronary arteries. In the present study, GSH (an inhibitor of thiol oxidation) diminished the relaxation caused by wCS.

It is known that barium ions block potassium channels and induce smooth muscle depolarization with increased cation influx through voltage-dependent $Ca_v1.2$ L-type calcium channels. Accordingly, we observed that renal artery contractions induced by $BaCl_2$ were reduced by the $Ca_v1.2$ L-type calcium channel blocker nifedipine. Noteworthy, we observed similar inhibitory effects by 5% solution of wCS. The striking similarity of inhibition of barium-induced contractions by wCS and nifedipine suggest that voltage-dependent $Ca_v1.2$ calcium channels might be involved in the action of wCS. These data support the idea that inhibition of $Ca_v1.2$ L-type calcium channels occurs *via* a redox-regulated mechanism.

A modified isotonic Krebs buffer by replacing NaCl with equimolar LiCl causes a transient contraction in smooth muscle *via* Ca^{2+} influx through the Na^+-Ca^{2+} exchanger. In the present experiments, SEA0400 - a specific blocker of the Na^+-Ca^{2+} exchanger - elicited a reduction of Li^+-induced contraction to a similar extent as 1% solution of wCS, suggesting that Na^+-Ca^{2+} exchange mechanism may also play a role in the vasomotor action of cigarette smoke. It is of note that H_2O_2 induces oxidative modification of thiols on the Na^+-Ca^{2+} exchanger in cardiomyocytes.

Taken together, we suggest that hydrogen peroxide present in water-soluble components of cigarette smoke and/or formed from superoxide anion in the vessel wall could, in part, explain the relaxation of rat renal arteries to wCS, a mechanism, which may also operate in renal vessels of humans. It is also likely that $Ca_v1.2$ L-type calcium channels and the Na^+-Ca^{2+} exchanger are also involved in smoke-induced vasomotor response, but further investigations are needed to substantiate their role. The acute detrimental effects of cigarette smoke i.e. on renal hemodynamics mean a 5/ 10/ 40 times repetitive harm for the kidneys in a smoker who smokes 5/ 10/ 40 cigarettes a day. Epidemiological studies confirm the relationship between chronic cigarette smoking and both the initiation of CKD in the general population and the progression of chronic kidney disease in patients.

The most common cause of CKD is diabetic nephropathy, however, it is not established yet if chronic cigarette smoking is only one risk factor for diabetic nephropathy or if it has a pivotal role in the development of certain stages of diabetic nephropathy.

In our single centre reptrospective study, we have described, for the first time, a very high prevalence of smokers (87%) among type 2 diabetic men with KW lesion in contrast to diabetic males with non-KW diabetic nephropathy of whom the prevalence of smoking was 35%. The latter is similar to the occurance of smoking in the general population. Other risk factors responsible for the progression of diabetic nephropathy were common both in patients with KW and in type 2 diabetics with other classes of diabetic nephropathy; however, only the smoking habit differed clearly between the KW and non-KW groups. Cigarette consumption was also significantly more frequent in the two separate patient groups with NGS (KW and non-diab NGS) compared to the group without (non-KW), so the group of patients with non-diab NGS served as a positive control. Interestingly, we have found chronic cigarette consumption not only in patients with ING, similar to the literature, but in all male patients with non-diab NGS of known origins, like monoclonal immunoglobulin deposition disease, amyloidosis, immunotactoid glomerulopathy and membranoproliferative glomerulonephritis. Several potential mechanisms arose for the explanation of smoking-induced kidney damage in diabetic nephropathy, and our study group strengthened the role of hyperfiltration. It is known that cigarette smoking acutely elevates blood pressure, and we described, that cigarette smoking also causes an acute relaxation of the renal arteries. The chronic alteration of the filtration barrier, because of endothelial cell injury, endothelial dysfunction and podocyte damage due to smoking, and the repetitive elevation of intraglomerular pressure and hyperfiltration might all play an important role in the patomechanism of NGS. These processes together could lead to the hyperfiltration and increased albuminuria observed in smokers without diabetes and with type 2 diabetes mellitus. We have found a parallelism between the severity of chronic cigarette consumption and the grade of proteinuria between the different groups. This suggests a role of tobacco consumption in the development of proteinuria and is in agreement with the literature. Our data suggest that, among the many progression promoters of diabetic nephropathy, chronic cigarette smoking could have a pivotal role in the development of KW lesion.

THESES

1) **In otherwise healthy male chronic smokers, cigarette smoking acutely reduces the resistance index (RI) of segmental renal arteries.**

2) **The reduction of the RI of segmental renal arteries due to cigarette smoke in healthy volunteers is transient and the process is not dependent on nicotine.**

3) **Water-soluble components of cigarette smoke – either nicotinic or nicotine free – elicit dose-dependent relaxations of rat isolated renal arteries.**

4) **The nicotinic cigarette smoke-induced relaxations of rat isolated renal arteries happen primarily due to smooth muscle dependent mechanisms and it is unlikely that either nitric oxide or carbon monoxide play a major role in this phenomenon.**

5) **The relaxations of rat renal arteries due to cigarette smoke are reduced by catalase and enhanced by superoxide dismutase, which suggest that cigarette smoke reduces the vasomotor tone of renal arteries in part via hydrogen peroxide, present in water-soluble components of cigarette smoke and/or formed from superoxide anion in the vessel wall.**

6) **It is likely that $Ca_v1.2$ L-type calcium channels and the Na^+-Ca^{2+} exchanger are also involved in smoke-induced vasomotor response of rat renal arteries.**

7) **Chronic cigarette smoking could play a pivotal role in the development of Kimmelstiel-Wilson lesions in type 2 diabetic men.**

PUBLICATIONS RELATED TO THE Ph.D.THESES:

Halmai R, Szijártó IA, Fehér E, Fésüs G, Molnár GA, Brasnyó P, Fülöp F, Gollasch M, Koller A, Wittmann I. Cigarette smoke elicits relaxation of renal arteries. *European Journal of Clinical Investigation.* 2011;41 (2):195-202. (impact factor (IF): 3.018)

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