SUPPLEMENTAL DATA 1

Adijang et al Nephrol Dial Transplant - 2008[40]

Model: study of aortic calcification (morphologic and immunohistochemic characterization of osteoblast related proteins (osteopontin, core binding factor 1 (Cbfal), alkaline phosphatase (ALP), osteocalcin), indoxylsulfate (IS) and organic anion transporter 1 and 3 (OAT 1 and 3); morphology of kidneys (glomerular area, mesangial area, MT-positive tubulointerstitial area, TGF-ßl-positive glomerular area and TGF-ßl-positive tubulointerstitial area).

Setting: in vivo in Dahl salt resistant rats and in salt sensitive Dahl rats, treated or not by administration PO of indoxylsulfate for 30 weeks (no IS treated salt resistant group)

Toxin: indoxylsulfate; PO administration to rats (200 mg/kg)

Concentration: 23.1 +/- 3.6 mg/L at the end of the administration period (30 weeks – lower before).

Albumin concentration: Not measured, as can be expected in rats.

Pathophysiologic changes:

> Increase in aortic thickness and calcification. Increase in osteoblast markers, IS and OATs. Increase of glomerular area, mesangial area, MT-positive tubulointerstitial area, TGF-ßl-positive glomerular area and TGF-ßl-positive tubulointerstitial in IS treated Dahl rats (n=10 – biochemical results in only 6)

Note: No salt resistant rats with IS administration

Adijang et al Biochem Biophys Res Commun - 2010[41]

Model: Study of cell senescence evaluated by immunohistochemistry of senescence-associated β-galactosidase (SA-β-gal), and senescence-related proteins such as p16INK4a, p21WAF1/CIP1, p53 and retinoblastoma protein (Rb).

Setting: in vivo in Dahl salt resistant rats and in salt sensitive Dahl rats, treated or not by administration PO of indoxylsulfate for 32 weeks.

Toxin: indoxylsulfate; PO administration to rats (200 mg/kg)

Concentration: In indoxylsulfate treated Salt sensitive Dahl rats, indoxylsulfate is continuously fluctuating between 15 and 20 mg/L between weeks 8 and 32, with the highest values around 20 exactly at these two moments. Values are somewhat lower in non-sensitive rats, between 14 and 9.

Albumin concentration: Not measured, as can be expected in rats.

Pathophysiologic changes:

> Increase in β-galactosidase (SA-β-gal), 16INK4a, p21WAF1/CIP1, p53 and retinoblastoma protein (Rb) in IS-treated Dahl sensitive rats as compared to Dahl-sensitive rats who were not treated by IS (n=8).–Also aortic caclicification and wall thickness more important than in non-IS treated rats.

Note: Dahl sensitive rats not receiving IS showed the same lesions but significanty less pronounced than the IS treated Dahl sensitive rats. Salt-resistant rats receiving indoxylsulfate showed no significant changes.

Bolati et al Am J Nephrol - 2011[38]

Model: Study of epithelial to mesanchymal transition in rat kidneys and cultured proximal tubular cells by immunohistochemistry, reverse transcription PCR and immunoblotting for epithelial markers E-cadherin and zonula occludens-1 and mesenchymal marker α-SMA. In vivo tests on normal and Dahl sensitive hypertensive rats.

Setting: in vitro and in vivo

Toxin: indoxylsulfate

Concentration: in vivo 9.4 +/-1.3 mg/L (normal rats) and 18.9 +/- 2.6 (Dahl rats). In vitro 250 μM (thus too high).

Albumin concentration: in vivo albumin as in living rats; in vitro 10% FBS

Pathophysiologic changes:

> Reduction of expression of E-cadherin and ZO-1 in both normal and Dahl rats treated by indoxylsulfate and enhanced expression of α-SMA in vivo (n= 6). Similar results in vitro (n= 3 to 5).

Note: -

Bolati et al BMC Nephrol 2013[50]

Model: In both wild type and salt sensitive hypertensive Dahl rats, IS-treated vs non-IS treated equivalent; study on proximal tubular cells; study of anti-oxidant regulators: erythroid-derived 2 like 2 (Nrf2); heme oxygenase-1 (HO-1; NAD(P)H quinine oxide reductase (NQO1); and of 8-hydroxy-deoxyguanosine (8OHdG), marker of ROS activation.

Setting: Dahl wild type, IS vs. no IS

Toxin: indoxylsulfate

Concentration: 9.4 mg/dL.

Albumin concentration: as in rats.

Pathophysiologic changes:

Only in IS treated wild type:

- Suppression of anti-oxidant Nrf2 and HO-1 and activation of 8OHdG vs. non-IS treated.
- According to the data for IS-treated Dahl sensitive rats no significant difference from non treated. Thus only wild type data relevant.

Note: Study contains in vitro data but not appropriate. Also animal study in a second model, Sprague-Dawley with CKD (thus not IS treated) with neutralization by AST-120

Chitalia et al Circulation - 2013[46]

Model: assessment of de-endothelialized vascular smooth muscle cells for tissue factor expression, activity, stability and posttranslational modification. Changes were associated to clot formation.

Setting: Uremic serum and IS induce tissue factor generation; this effect is related to clot formation. TF breakdown through ubiqination and this process is slowed down by indoxylsulfate. Experiments occurred in a flow loop mimicking coronary circulation.

Toxin: indoxylsulfate

Concentration: IS 25 mg/L.

Albumin concentration: Presumably-4 g/L, amail confirmation by author.

Pathophysiologic changes:

Activation of tissue factor generation (n=3). Effect seen from 4 hours on, continued over 24 hours. Also increasing effect with increasing dose of IS. SMC injury further enhances the effect.

Note: Same effect as with uremic toxins also seen with uremic serum; effect could be reduced by anti-TF neutralizing antibody. Also similar effect for uric acid and indole acetic acid.

Dou et al KI - 2004[26]

Model: endothelium (HUVEC)

Setting: in vitro

Toxin: indoxylsulfate

Concentration: 25-250 mg/L

Albumin concentration: 4%

Pathophysiologic changes:

Inhibited endothelial proliferation (5-bromo-2-deoxyuridine incorporation) (BrdU) (25-250 mg/L) (dose response) (n=4)

Inhibited endothelial wound repair after injury (videomicroscopy) (125-250 mg/L – if with albumin) (dose response) (n=?)

Note: similar results reported with p-cresol, but p-cresol is not present as such in the body {Martinez, 2005 339 /id;de, 2005 322 /id;Vanholder, 2011 321 /id}; no induction of endothelial apoptosis (annexin-V testing by flow cytometry). Effect less pronounced here in presence than in absence of albumin.

Dou et al J Thromb Haemost - 2007[27]

Model: endothelium (HUVEC)

Setting: in vitro

Toxin: indoxylsulfate

Concentration: 125-250 mg/L

Albumin concentration: 4% (g/dL)

Pathophysiologic changes:

> Induction of reactive oxygen species (ROS) measured by cytofluorometry (125-250 mg/L) (dose response) (n=10)
>
> Inhibition of NAD(P)H-oxidase neutralized this pro-inflammatory effect whereas inhibitors of xanthine oxidase, NO-synthase and mitochondrial RO production had no effect
>
> Indoxylsulfate strongly inhibited glutathione, one of the most important antioxidant systems in the cell. This test was not performed in the presence of albumin, though

Note: inhibition of the pro-oxidant effects by the antioxidants Vit C, vit E and N-acetylcysteine (NAC)

Faure et al J Thromb Haemost - 2006[28]

Model: endothelium (HUVEC)

Setting: in vitro

Toxin: indoxylsulfate

Concentration: 256 mg/L

Albumin concentration: 4% (g/dL)

Pathophysiologic changes:

> Increase in number of released endothelial microparticles as detected by enhanced annexin-V labeling measured by flow cytometry (n=8)

Note: -

Ito et al J Biol Chem - 2010[30]

Model: leukocyte endothelial interaction

Setting: in vivo studies after indoxylsulfate administration in drinking water in 5/6 nephrectomized mice; controls: 5/6 nephrectomized without indoxylsulfate

Toxin: indoxylsulfate

Concentration: 15.7 mg/L

Albumin concentration: no serum albumin mentioned but should be the usual ones for in vivo conditions

Pathophysiologic changes:

Interaction of leukocytes with endothelium of femoral arteries is enhanced in intravital microscopy studies (n=5)

Quantitative real-time PCR analysis also showed an increase of mRNA expression of E-selectin and the NAD(P)H oxidase subunits p47*phox* and p22*phox* vs normal rats and for E-selectin also vs nephrectomized rats not treated with indoxylsulfate (n=5). No (significant) changes however for ICAM-1 and VCAM-1

Note: the study contains an in vitro setting where no appropriate albumin is added and an in vivo setting in mice, where by definition appropriate albumin should be present. Injection of anti-selectin-E antibody neutralizes the interaction. Concentration IS low as compared to human uremia.

Itoh et al Anal Bioanal Chem - 2012[29]

Model: endothelium (HUVEC)

Setting: in vitro

Toxin: indoxylsulfate

Concentration: 29.9 and 57.2 mg/L

Albumin concentration: 4% (g/dL)

Pathophysiologic changes:

Increase in endothelial ROS production compared to control (no toxin) (n=3)

Note: p-cresylsulfate no effect in absence of albumin; concentration 37.1 and 109.1 mg/L

Koppe et al J Am Soc Nephrol - 2013[44]

Model: evaluation of several aspects of insulin resistance in vivo–in mice and in vitro on cultured adipocytes (modified fibroblasts) and C2C12 myotubes (equivalent to myocytes after modification of C2C12 myoblasts).

Setting: in vivo assessment in PCS treated vs control mice of glucose response to insulin, insulin-induced phosphorylation of PKB/Akt and activation of phosphorylation of ERK1/2 in gastrocnemius, adipose tissue size, ectopic lipid distribution; in vitro assessment in presence of PCS compared to control in adipocytes of lipogenesis and lipolysis, in myotubes of glucose uptake in response to insulin as well as elements of the insulin pathway by Western blotting and ERK1/2 phosphorilation .

Toxin: p-cresylsulfate.

Concentration: In vivo 5.01 +/- 1.59 mg/dl total and 0.82 +/- 0.24 free (total OK with clinical reality, free 2.5 times above mean uremic concentration). In vitro 40 mg/l (OK).

Albumin concentration: In vivo not measured, as can be expected in healthy mice (but protein binding in the expected range: 90.5 +/- 2.0 %). In vitro albumin 35 g/l BSA.

Pathophysiologic changes:

In vivo with PCS: decreased glucose response to insulin (n= 5-8), inhibited insulin-induced phosphorylation of PKB/Akt and activation of phosphorylation of ERK1/2 in gastrocnemius (n= 4-6), decreased adipose tissue size (n = 8), increased ectopic lipid distribution (n=8); in vitro in presence of PCS: in adipocytes decreased lipogenesis and increased lipolysis (n=4), in myotubes inhibition of glucose uptake in response to insulin together with changes in the following elements of the insulin pathway (Western blotting) - serine 473 phosphorylation of PKB/Akt, tyrosine-phosphorylation of IRS-1, serine phosphorylation (Ser 636) of IRS-1, p85 subunit of PI3K coimmunoprecipitation: (n=4-5), and ERK1/2 phosphorilation (n=6).

Note: Probenecid prevents PCS-induced disruption of insulin signaling pathways in C2C12 myotubes; the prebiotic AXOS decreased p-cresylsulfate levels and corrected metabolic changes.

Lekawanvijit et al Eur Heart J - 2010[35]

Model: Cardiac fibroblast collagen synthesis, cardiomyocyte proliferation (^{3}H-proline and ^{3}H leucine incorporation). In THP1 cells (substitute for monocytes – leukemia celline) production of cytokine TNF- α, IL-6, IL-1β mRNA expression estimated by PT-PCR. Furthermore, estimation of activity of MAPKinase and NFκB.

Setting: in vitro

Toxin: indoxylsulfate

Concentration: fibroblast collagen synthesis from 3μM on; on myocyte hypertrophy from 0.01 μM on (i.e. below the normal free concentration); TNF-α and IL-1β 100 μM (too high); IL-6 1 μM; MAPKinase and NFκB: 10 μM.

Albumin concentration: myocytes and fibroblasts in 10% FB); TMP-1 in 0.5% BSA.

Pathophysiologic changes:

> IS had pro-fibrotic (n=9 in triplicate), pro-hypertrophic (n=5 in triplicate)and pro-inflammatory effects (n=3). Decreasing IS might help in decreasing cardio hypertrophy in CKD. In THP1 cells increased production of TNF- α, IL-6, IL-1β mRNA expression estimated by PT-PCR. Furthermore activation of MAPKinase and NFκB (n= 3).

Note: neutralization by inhibition of RWJ-67657 and U0126, linked to p38 and MEK1/2 pathways

Odamaki et al Nephrol Dial Tranplant - 2004[52]

Model: In vitro study of albumin production by isolated hepatocytes.

Setting: this is a study of several different compounds. The indoxylsulfate experimenyts were performed in the presence and absence of albumin

Toxin: indoxylsulfate

Concentration: 50 and 100 mg/L.

Albumin concentration: 4 g/dL.

Pathophysiologic changes:

Increase in albumin production.

Note: is in fact not a toxic but a positive effect . No difference with and without albumin .

Schepers et al Nephrol Dial Transplant - 2007[31]

Model: leukocyte function (whole blood)

Setting: in vitro (healthy volunteers)

Toxin: P-cresylsulfate

Concentration: 121.0 mg/L

Albumin concentration: no serum albumin mentioned but should be the usual ones for in vivo conditions

Pathophysiologic changes:

> Increase of oxidative burst activity of baseline leukocytes (monocytes and lymphocytes) (n=10); granulocytes trend but not significant

Note: Concentration P-CS slightly higher than accepted maximum

Shimizu et al Life Sci - 2012[37]

Model: Study in proximal tubular cells on the effect on the expression of Monocyte Chemotactic Protein-1 (MCP-1), a pro-inflammatory agent responsible for recruiting leukocytes into tubulointerstitial tissue. This study was performed in salt sensitive hypertensive Dahl and wild type rats to whom indoxylsulfate was administered; the rats were not made renal insufficient. Furthermore also in vitro study in tubular cells of the effect on activation of ERK, p38, JNK, NF-κB and p53. In addition, study of the effect in absence and presence of an antioxidant and inhibitors of the above mediators.

Setting: in vitro and in vivo

Toxin: indoxylsulfate

Concentration: in vivo, in normal rats given indoxylsulfate 9.4 +/- 1.4 mg/L, in Dahl rats 18.9 +/- 2.8 (appropriate thus). In vitro 250 µMol (thus too high).

Albumin concentration: in vivo, serum albumin as can be expected in rats, in vitro 10% FBS

Pathophysiologic changes:

> Induction of expression of MCP-1 at relevant concentrations in indoxylsulfate treated rats, both normal (n= 5) and Dahl (n= 5). All other experiments at inappropriate concentrations.

Note: indoxylsulfate administered per os (200 mg/kg in drinking water).

Shimizu et al Am J Physiol Cell Physiol - 2013[42]

Model: In vitro and in vivo study on renal tubular cells: expression of angiotensinogen and cAMP response element-binding protein (CREB).

Setting: in vivo in Dahl salt sensitive rats for expression of angiotensinogen.

Toxin: indoxylsulfate: PO administration to rats (indoxylsulfate concentration: 9.4 mg/L +/- 1.3.

Concentration: In vivo: indoxylsulfate concentration: 9.4 mg/L +/- 1.3. In vitro experiments (all the rest) were at too high concentrations (250 µM) for protein-poor medium (10% FBS).

Albumin concentration: Not measured, as can be expected in rats.

Pathophysiologic changes:

> Increase in agiotensinogen expression (n=8).

Note: -

Shimizu et Am J Nephrol - 2013[43]

Model: evaluation in proximal tubular cells of the p53-TGF-β_1-Smad3 pathway, involved in inducing fibrosis.

Setting: In vivo study on proximal tubular cells of salt resistant and salt sensitive Dahl hypertensive rats. Also in vitro studies but with only 10% FBS and for that protein concentration too high indoxylsulfate (250 µM).

Toxin: indoxylsulfate;-PO administration to rats (indoxylsulfate concentration: 9.4 mg/L +/- 1.3.

Concentration: In vivo: indoxylsulfate concentration: 9.4 mg/L +/- 1.3. In vitro experiments (all the rest) were at too high concentrations (250 µM) for protein-poor medium (10% FBS).

Albumin concentration: Not measured, as in rats.

Pathophysiologic changes:

> Only morphologic changes (increase TGF-β1 and Smad3 positive area). No statistical data. (n=8).

Note: -

Shimizu et al Life Sci - 2013[47]

Model: assessment of renal regulation of ICAM-1, playing a role in attracting monocytes and macrophages.

Setting: Next to human tubular cells (HK-2) studied in vitro, also in vivo studies were performed with Dahl rats receiving IS. Two strains were receiving IS: Dahl salt sensitive rats and wild type. Rats received IS (200 mg/kg) for 32 weeks.

Toxin: indoxylsulfate

Concentration: in vivo assessment: 9.4 mg/L in wild strain and 18.9 mg/L in Dahl hypertensive rats; in vitro: 250 µmol but without albumin, only Dulbecco. Thus only in vivo experiments relevant.

Albumin concentration: As can be expected in normal rats.

Pathophysiologic changes:

Assessment of ICAM-1 mRNA expression in both wild strain and salt sensitive rats treated with IS (n= 5) (also Dahl hypertensive rats not receiving IS showed higher ICAM-1). Dahl hypertensive treated with IS had higher expression than Dahl hypertensive and no IS.

Note: Inhibitors of NADPH oxidase, NF-κB and p53 suppressed the increase of ICAM-1 mRNA expression (but only in vitro).

Sun et al Nephrol Dial Transplant - 2012[34]

Model: Inflammatory gene expression in cultured renal proximal tubular cells

Setting: in vitro

Toxin: indoxylsulfate and p-cresylsulfate

Concentration: IS and PCS 1 and 5 mg/L (concentrations thus correspond to free fraction)

Albumin concentration: Serum starved cells

Pathophysiologic changes:

Stimulation of a whole series of pro-inflammatory genes (see below) (n= ?)

Note: The major genes from cytokines in the functional networks that were activated were Tgfb1, fasl, IL6/15, IL15, Csf1/3 and Cxcl10; those from the triggered intracellular signals were Stats, Smads, Nfkb2, Ikbkb, Bcl2 and Bax. Functional networks linked to Tgfb1: Col4a5, Cxc10, Fasl, Stat1 and Ikbkb.

Sun et al PLOS One - 2012[36]

Model: Study of activation of the renal renin–angiotensin–aldosterone system (RAAS) in proximal tubular cells and of renal tubular epithelial-to-mesenchymal transition (EMT) and of TGF-β, Snail, fibronection, α-Smooth muscle actin (α-SMA) and E-cadherin production as mechanisms in the induction of kidney fibrosis. Direct study of fibrosis by assessment of nephrosclerosis scores.

Setting: in vitro and in vivo

Toxin: indoxylsulfate and p-cresylsulfate

Concentration: in vitro studies were showing a positive effect from 1 mg/L on for both IS and PCS with a further dose responsive increase for 5 and 50 mg/L. The in vivo data (TGFβ, Snail, fibronectin α-SMA and E-cadherin) were collected after peritoneal injection in mice but plasmatic level was not specified.

Albumin concentration: in vitro PBS, % not specified; in vivo as in animals.

Pathophysiologic changes:

> Activation of RAAS , EMT , TGF-β , Snail , TGF-β , fibronectin , and α-SMA and decrease of E-cadherin production . Increase of nephrosclerosis , all with both IS and PCS (n=8).

Note: Neutralization effect with losartan.

Sun et al Kidney Int - 2012[39]

Model: study of kidney fibrosis, methylation of Klotho gene, Klotho expression.

Setting: in vivo in B-6 mice; in vitro on renal tubular cells

Toxin: indoxylsulfate and p-cresylsulfate; in vivo injected to mice

Concentration: for indoxylsulfate in mice treated by indoxylsulfate 8.55±0.37,mg/L, treated by p-cresylsulfate 5.61±0.60 mg/l (corresponding to a very moderate increase in concentration); for p-cresylsulfate in mice treated by pCS 1.82 +/-0.33 and in those treated by IS 0.66 =/- 0.04 which are in fact numbers corresponding to normal. In vitro 1, 5 and 50 mg/L (1 (pCS) and 5 (IS) OK for free fraction).

Albumin concentration: in vivo albumin as in living mice; in vitro no protein content specified.

Pathophysiologic changes:

> In mice (n= 8) induction of kidney fibrosis, hypermethylation of the Klotho gene and decreased Klotho expression; inhibition of DNA methyltransferase isoform 1 caused a decrease of methylation and increased Klotho expression in vitro.

Note: In mice treated by one toxin the other toxin rises as well, in absence of renal failure.

Tsujimoto et al J Pharm Pharmacol - 2010[32]

Model: metabolic clearance of losartan – measurement of the metabolite EXP-3174 using pooled human liver microsomes

Setting: in vitro

Toxin: indoxylsulfate

Concentration: Indoxylsulfate is diluted in uremic serum at 3-300 µmol/L and then diluted into the medium with microsomes 10% vv. The concentration in normal serum is within the acceptable range. As the sample is diluted 1/10 the protein binding will certainly decrease, but the final concentration of 20 µmol remains well within the range also for free solute. An effect is seen at 300 µmol/L, thus 30 µmol/L diluted (as a matter of fact a bit too high for entirely free, but there is still some protein).

Albumin concentration: Final albumin concentration low but total concentration within the acceptable range of free concentration.

Pathophysiologic changes:

> Inhibition of losartan metabolism (dose response but only starting at the uremic range, n=3 or 4) (uremic range is the highest concentration of the dose-response)

Note: Studies also on other toxins; for the cresols, however, only p-cresol was studied (of course, very likely, some cresol reaches the liver)

Tsujimoto et al Ther Apher Dial - 2012[48]

Model: Investigation of pump systems involved in the non-renal clearance of pravastatin, in view of its accumulation in renal failure, in spite of it not being cleared by the kidneys: assessment of the role of uremic toxins and uremic milieu on the expression of OATP2B1 (at play in intestinal uptake of Pravastatin) and MRP-2 (at play in intestinal/bile secretion of Pravastatin) in Caco-2 cells (intestinal cell line) and of OATP1B1 and OATP2B1 (at play in intestinal and hepatic uptake of pravastatin) in HEP3B cells (hepatic cell line).

Setting: This is a pure in vitro study using HBSS (Hank's balanced salt solution) as medium. The latter contains no protein or albumin but experiments were done at low enough concentrations to be representative for free fraction.

Toxin: indoxylsulfate

Concentration: 20 µmol, which corresponds to free concentration.

Albumin concentration: 10% FCS.

Pathophysiologic changes:

Clear inhibition only in Caco-2 for OATP2B1 and MRP-2 (n=3). No consistent changes in HEP3B (only slight decrease for OATP2B1.

Note: inhibitory effect not only seen for IS but also for IAA, hippuric acid and cmpf. No significance of effect given. Two effects taken together seem contradictory.

Watanabe et al Kidney Int - 2013[45]

Model: evaluation of toxicity on renal tubular cells via activation of production of radical oxygen species (ROS).

Setting: in vitro assessment of activity of NADPH-oxidase; upregulation mRNA inflammatory mediators and TGF-β1 (associated with renal fibrosis) – cell cultures in K-SFM (keratinocyte serum free medium; no albumin); in vivo administration p-CS to 5/6 nephrectomized rats for 4 weeks with assessment of tubular damage and enhancement oxidative stress.

Toxin: p-cresylsulfate.

Concentration: in vitro: effect seen only at 100 and 500 µmol/L (thus too high in absence of protein in medium); no significant effect at 10 µmol/L (which is a correct concentration without protein). In vivo (5/6 nephrectomized rats): animals evolved with or without CKD till week 16, then one randomly selected CKD group received PCS up till 20 weeks, the other not. At week 16 (in all rats) PCS was between 2.0 and 2.99 µmol/L; at week 20 (non-PCS treated rats) PCS was 4.52 +/- 5.29 µmol/L; in the PCS treated rats this value was 32.57 +/- 19.94 µmol/L. 32 µmol/L is in the low human uremic range; 5 µmol/L is in the low normal range.

Albumin concentration: Not measured, as in rats.

Pathophysiologic changes:

> Only in vivo changes count: in the presence of added PCS, an increase of tubule degeneration, TGFβ1 activity, free radical production, and NADPH-oxidase activity were observed. (N=9)

Note: Effect neutralized by Knock-down of $p22^{phox}$ and p22 neutralized the effect pointing to the importance of NADPH-oxidase. Also suppression effect by administration of probenecid and NAC. Mechanism similar as for indoxylsulfate. After 4 weeks of PCS not only PCS levels but also Screa and urea were slightly but significantly higher (pointing to a possible broader general uremic effect in this group, but then still PCS-induced – although the differences on PCS are much more dramatic than those in urea or creatinine; changes just point to an impact on kidney function.

Yisireyili et al Life Sci - 2013[49]

Model: In Dahl salt sensitive hypertensive rats, investigation of heart weight, left ventricular weight, diameter of cardiomyocytes, parameters of cardiac fibrosis [Mason-trichrome, TGFβ1, α-SMA, type 1 collagen] and of oxidative stress [NADPH-oxidase (Nox4), malondialdehyde (MDA, 8-hydroxy-deoxyguanosine (8OHdG)]; finally also expression of anti-oxidant regulators, constituting nuclear factor [(erythroid-derived 2) like 2 (Nrf2); heme oxygenase-1 (HO-1).

Setting: In vivo study on Dahl hypertensive rats administered IS compared to same rats not administered IS

Toxin: indoxylsulfate

Concentration: 18.9 mg/L.

Albumin concentration: as in rats.

Pathophysiologic changes:

In IS-treated rats:
- Increase in diameter cardiomyocytes
- Increased fibrotic area; increased staining for TGFβ1, SMA, type-1 collagen,
- Increase of Nox4, 8-OHdG and MDA
- Decreased staining for Nrf2 and HO-1

Note: no effect IS in wild type rat; study contains no in vitro arm

Yamamoto et al Kidney Int - 2006[51]

Model: In vitro study of the effect of indoxylsulfate on smooth muscle cell proliferation.

Setting: although most experiments are in the absence of albumin, some have been performed in the presence of albumin

Toxin: indoxylsulfate

Concentration: 250 and 500 μM.

Albumin concentration: 4 g/dL.

Pathophysiologic changes:

Increased smooth muscle cell proliferation.

Note: data with and without albumin are expressed differently (fold-change vs. absolute values) making comparison difficult. Fold changes seem at least as prominent in the presence as in the absence of albumin.

Yu et al Clin J Am Soc Nephrol - 2011[33]

Model: Proliferation, senescence and production of nitric oxide and oxygen free radicals by HUVEC as a model of endothelial damage; cell proliferation: incorporation of ^{3}H-thymidine and by direct cell counting; cell senescence: in situ staining for SA-β-galactosidase

Setting: in vitro

Toxin: indoxylsulfate

Concentration: 1.25 to 125 mg/L; effect from 2.5 mg/L on

Albumin concentration: No mention of albumin

Pathophysiologic changes:

Inhibition of proliferation; enhancement of senescence; decrease NO production; increase ROS production (for 2.5 mg/L from the 3d hour on) (n= 4 in duplicate)

Note: This in vitro study is associated with an observational clinical study showing an in vivo improvement of endothelial function as demonstrated by flow-mediated endothelium-dependent vasodilatation (FMD) after 24 weeks of AST-120. This study arm was observational. There were no controls. All 40 patients received AST-120. Correction of in vitro effects by probenecid and the antioxidants NAC, rotenone and apocynin.

SUPPLEMENTAL DATA 2

	Total quality score
Dou 2004, KI[26]	2
Odamaki 2004, NDT[52]	2
Faure 2006, J Throm Haemost[28]	1
Yamamoto 2006, KI[51]	3
Dou 2007, J Throm Haemost[27]	4
Schepers 2007, NDT[31]	2
Adijang 2008, NDT[40]	3=2+1
Ito 2010, J Biol Chem[30]	4=3+1
Tsujimoto 2010, J Pharm Pharmacol[32]	1
Lekawanvijit 2010, Eur Heart J[35]	5
Adijang 2010, Biochem Biophys Res Commun[41]	3=2+1
Yu 2011, cJASN[33]	3
Bolati 2011, Am J Nephrol[38]	4=3+1
Itoh 2012, Ann Bioanal Chem[29]	0
Sun 2013, NDT[34]	2
Sun 2012, Plos One[36]	5
Shimizu 2012, Life Sci[37]	2=1+1
Sun 2012, KI[39]	5
Watanabe 2013, KI[45]	5=4+1
Tsujimoto 2012, Ther Apher Dial[48]	1
Shimizu 2013, Am J Physiol Cell[42]	2=1+1
Shimizu 2013, Am J Nephrol[43]	3=2+1
Koppe 2013, JASN[44]	5=4+1
Chitalia 2013, Circulation[46]	4
Shimizu 2013, Life Sci[47]	3=2+1
Yisireyili 2013, Life Sci[49]	4=3+1
Bolati 2013, BMC Nephrol[50]	4=3+1