Ayurvedic medicine and NADPH oxidase: a possible approach to the prevention of ESRD in hyperoxaluria

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Saeed Khan’s longstanding commitment to investigating the pathogenesis of renal stones adds to our knowledge ever more with new evidence to support the hypothesis that exposure of the renal tubular cells to high oxalate and calcium oxalate (CaOx) crystals triggers an oxidative stress cascade that has a pivotal role in crystal retention in the kidney. In this Nephrology Dialysis Transplantation issue, Zuo et al. [1] clearly demonstrates in a model of hyperoxaluria that inhibiting reactive oxygen species (ROS) synthesis reduces tubular injury and prevents crystal retention in the kidney. In a way, they have completed 30 years of studies which began after the serendipitous observation reported by Baggio et al. [2] that idiopathic CaOx stone formers have tubular damage, as shown by their increased urinary excretion of proximal tubule enzymes.

Unfortunately, there is no valid model of human idiopathic calcium oxalate nephrolithiasis (ICN), although there are many models of hyperoxaluria. Mild hyperoxaluria, i.e. an oxalate excretion of up to 60 mg/24 h, is frequently observed in ICN patients [3], but the urinary excretion of oxalate is much higher in the rare secondary hyperoxalurias and the even rarer primary hyperoxalurias, generally exceeding 100 mg/24 h.

The experimental models used by many groups (including Khan’s laboratory) to investigate CaOx stone pathogenesis reflect, in our opinion, the conditions of primary and secondary hyperoxalurias rather than the more common, mild hyperoxaluria. In these experimental models, crystals are actually observed intraluminally in the upper nephron and downstream, and they are deposited mainly in the interstitium. While neither of these findings have ever been described in ICN patients, they are typically seen in the nephrocalcinosis of primary and secondary hyperoxaluria.

For the above reasons, we believe that the results of the investigations performed by Khan et al. in models of severe hyperoxaluria pertain to a different scenario from ICN. The findings reported by Baggio et al. most likely derive from a different pathogenic sequence. We have advanced the hypothesis that ICN has a two-hits pathogenesis: the first hit is a tubular injuring factor which facilitates CaOx crystal formation in the presence of a mild hyperoxaluria (second hit), possibly by delivering cell debris [4]. This corresponds with many findings in ICN and is consistent with the Randall’s plaque theory of lithogenesis—probably the most rational explanation for stone formation in the kidney: according to this theory, stones grow in the renal pelvis on Randall’s plaques, not in the tubular lumen.

Of course, the possibility of the tubular injury observed in ICN patients (the first hit) being caused by an oxidative stress (which is easy to envisage in these patients) cannot be ruled out. For instance, there is a known association between metabolic syndrome and hypertension with renal stones, and both conditions are associated with oxidative stress.

In this sense, inhibiting ROS synthesis, as in the experiment conducted by Zuo et al. [1], could theoretically be useful in ICN too, although it is very difficult in practice to ensure compliance with preventive treatments for conditions such as ICN, in which recurrent episodes are unpredictable and may be few and far between [5]. It would be much easier, of course, if an ICN patient needs to be treated for another chronic condition with a drug that has an antioxidant action too. This is the case of hypertensive ICN patients treated with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, or of hypercholesterolaemic ICN patients treated with statins… two birds could be caught with one stone because the above agents can also prevent ROS synthesis [6].

We therefore do not think the findings reported by Zuo et al. are relevant to the pathogenesis or to the treatment of ICN, although they are very important in shedding light on the pathogenesis of nephrocalcinosis in primary and secondary hyperoxalurias. As mentioned previously, these are rare conditions, although secondary forms of intestinal origin may be rising in prevalence as a consequence of bariatric surgery [7].

Both primary and secondary hyperoxaluria are associated with CaOx stones, but the crucial risk in both is end-stage renal disease (ESRD), which is almost unavoidable in the inherited, primary forms [8], and it is becoming more and
Having said as much, we think that the most interesting and promising aspect of the paper from Khan’s group concerns the use of apocynin (a natural, non-toxic agent) for inhibiting ROS synthesis to prevent renal damage occurring in hyperoxaluria. Apocynin is a synthetically-derived phytochemical component of *Pierorhiza kurroa*, a native plant growing in the mountains of India, Nepal, Tibet and Pakistan, and is used in traditional Indian medicine (Ayurveda). Apocynin is considered an efficient and selective inhibitor of the NADPH oxidase complex in phagocytic cells. The mechanism of inhibition involves the impairment of the translocation to the membrane of the cytosolic component p47phox of the NADPH oxidase complex [10]. NADPH oxidases were originally considered as enzymes expressed only in phagocytic cells involved in host defences and innate immunity, but recent evidence has shown that there is a family of seven NADPH oxidases (the NOX family) [11], which are key sources of ROS in many different organs, including the vasculature and the kidney.

Since NADPH oxidases have been shown to contribute to the pathogenesis of several disorders, apocynin has been investigated in many experimental models, also in the field of nephrology and in relation to hypertension. Apocynin reduces hypertensive animals’ blood pressure [12]. In a cisplatin-induced nephrotoxicity model, apocynin treatment led to improvements in the renal damage, the high serum creatinine levels and the urinary excretion of protein and tubular enzymes [13].

However, since apocynin has recently been shown to lower ROS levels in non-phagocytic cells by acting as an antioxidant rather than as a NADPH oxidase inhibitor [14], the above results most likely stemmed from a generic antioxidant activity of the drug. This impression is supported by the observation that the most prominent renal NADPH oxidase is NOX 4, which does not seem to require p47phox and is therefore unresponsive to apocynin [11].

As mentioned earlier, renin–angiotensin system inhibitors are also capable of (indirectly) inhibiting NADPH oxidase activation and the oxidative cascade. More importantly, they reveal very similar activities to those of apocynin in a model of hyperoxaluria–nephrocalcinosis [15–17]. They probably take effect on different targets in the oxidative cascade, however, as suggested by a recent experiment in a model of streptozotocin-induced diabetes, which disclosed that associating ramipril with apocynin resulted in the greatest decrease in extracellular matrix accumulation [18].

We do not have any genuinely effective agents for preventing nephrocalcinosis in primary hyperoxaluria. In 20–30% of primary hyperoxaluric patients, pyridoxine can reduce endogenous oxalate synthesis and oxaluria, but it generally only delays the onset of ESRD. Other measures, i.e. administering potassium citrate and increasing the urinary volume, are merely ancillary. The only really useful thing to do is to plan bariatric intervention carefully [19]. For intestinal hyperoxalurias, we have even fewer therapeutic options and probably the only useful thing to do is to plan bariatric intervention carefully [20]. Trials on new treatments are underway (see ClinicalTrial.gov, search for ‘hyperoxaluria’), e.g. on the use of *Oxalobacter formigenes*, a unique oxalate-degrading bacterium normally occurring in the human gut, or of the recombinant mutant form of the *Bacillus subtilis* oxalate decarboxylase. The breakdown of oxalate in the intestine may help to reduce oxaluria, and this could prove very useful in intestinal hyperoxaluria, though in primary hyperoxaluria it is unlikely to suffice because the main component of oxalate in urine is of endogenous origin. The use of chemical chaperones (e.g. betaine) to restore the defective enzymatic activity is also about to be investigated in a trial on primary hyperoxaluria. New approaches under investigation, i.e. hepatocyte cell transplantation and recombinant gene therapy for enzyme replacement, are still in very early stages [21].

Hyperoxaluria-induced ESRD is a very harsh clinical condition because patients develop severe hyperoxalaea and oxalosis, i.e. systemic CaOx crystal deposition, with very severe outcomes. Even the most efficient extracorporeal treatments cannot remove oxalate adequately and renal transplantation alone is almost invariably doomed to failure because of recurrent nephrocalcinosis. The experiment conducted by Zuo et al. [1] demonstrates that targeting ROS formation with apocynin might be an interesting treatment for hyperoxaluria–nephrocalcinosis conditions. Since apocynin is a very efficient antioxidant with a very low LD50 [13], it may have a unique advantage over other drugs, such as statins and renin–angiotensin system inhibitors. Rather than inhibiting the oxidative cascade downstream, it might naturally be more rewarding to take action upstream. Since NADPH oxidases are proving to be major sources of ROS in many tissues and many pathological settings, the ideal target for preventing renal damage in severe hyperoxaluria would be the NOX (and preferably NOX 4) enzymes in the renal tubular cells [22]. Unfortunately, the use of very selective, highly active NOX inhibitors is still in the early stages of research [22].

Only one clinical trial involving oral apocynin has been published to date [23]. Apocynin was combined with other plant products in an over-the-counter health supplement for treating asthma at a dose of ∼4 mg/kg of body weight a day. Only trends towards a clinical improvement favouring active treatment were seen in patient-centred outcomes. This dosage was quite low, however, by comparison with the doses used in experimental models, e.g. 15 mg/kg/day [14] and 100 mg/kg/day [1,13]. There are no known side effects of apocynin and it has been shown to have a very low toxicity (LD50: 9 g/kg) after oral administration in mice [13], so it seems to have a very favourable safety profile. It is important to confirm in other hyperoxaluria models, the favourable effect of apocynin and to verify that apocynin acts as an effective antioxidant in the human renal tubular cells too. Afterwards, we believe that a trial on apocynin for patients with primary and secondary hyperoxaluria for the prevention of ESRD is warranted.

Conflict of interest statement. None declared.

ADAMTS13—more than just TMA and TTP

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Abstract

ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type-1 motifs 13) has been shown to be of major pathophysiological importance for thrombotic microangiopathy (TMA) in the setting of thrombocytopenic purpura (TTP) when either lacking (inherited TTP) or if antibodies against ADAMTS13 are present (acquired TTP). A potential pathophysiological role of ADAMTS13 has also been postulated in other diseases i.e. myocardial infarction, atrial fibrillation and diabetic angiopathy.

Recent data provides evidence, however, for a completely different role of ADAMTS13 in vascular physiology and pathophysiology. In the present issue of Nephrology Dialysis and Transplantation, Bockmeyer et al. documented a physiological expression of ADAMTS13 in arteriolar...