Is an environmental nephrotoxin the primary cause of CKDu (Mesoamerican nephropathy)? PRO

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Chronic interstitial nephritis in agricultural communities (CINAC), also named chronic kidney disease of unknown origin (CKDu) or Mesoamerican nephropathy (MeN), is defined as a form of CKD affecting young men and less often women. Its etiology is unrelated to diabetes, glomerulonephritis, hypertension, or other known causes of CKD. CINAC patients live/work mainly in poor agricultural communities, often in hot tropical regions, and are exposed to potential toxic agrochemicals through work, by ingestion of contaminated food and water, and/or by inhalation. The epidemic dimension of CINAC was first observed in the 1990s in Sri Lanka and Central America and has since been found to be an important cause of CKD related deaths in an increasing number of countries.

CINAC patients have bilateral small irregular contoured kidneys with mutual size discrepancy of less than 1.5cm on ultrasound, as observed in advanced cases of analgesic nephropathy and aristolochic nephropathy. Almost all CINAC patients exhibit a proximal tubular lesion: tubular cell atrophy, basement membrane thickening, deficient proximal tubular cell (PTC) regeneration, loss of function, distal tubular proliferation/hypertrophy, and variable extents of interstitial fibrosis and cellular infiltration. Overt glomerular injury is rare in the early stages, while in later CKD stages, secondary glomerulosclerosis develops. By electron microscopy, PTCs demonstrate enlarged dysmorphic lysosomes (>1.2 µm) containing homogenous non-membrane bound electron dense rounded/irregular “aggregates” dispersed throughout the light to medium uniform electron dense lysosomal matrix (Figure 1). These features are also observed in a number of toxin-induced nephropathies.

Despite many similarities, a toxin-induced etiology has been considered mainly in Sri Lanka, whereas in Central America recurrent heat stress-induced acute kidney injury (AKI) has been hypothesized as predominant cause.

There are arguments disputing a major causal role of heat stress and dehydration in CINAC. The epidemic upsurge of CINAC was first observed in the 1990s in Sri Lanka and Central America shortly after the introduction and rapid increase in the usage of agrochemicals and replacement of labour-intensive buffaloes by mechanisation. During this sudden increase in prevalence, there were no major changes in ambient temperature and rainfall that could foster an epidemic scale increase in heat stress. The dehydration hypothesis is incompatible with the mosaic geographic distribution of CINAC endemic provinces in Sri Lanka, in view of a homogenous distribution of the agricultural population with the same climate, equal hours of sunshine and rainfall as well as type of agriculture. Furthermore, in contrast to El Salvador and Nicaragua, CINAC epidemics are not reported in the well-organized national public health
registry of Cuba, despite being a sugarcane-cultivating country with similar geo-climatic factors as the Central American region. There are also many individuals globally, e.g. those working in blast furnaces, miners working deep under the ground, who are exposed to the same harsh conditions as sugarcane workers but who have never developed rapidly progressive CINAC despite regular screening programs during active working periods and retirement.

In Sri Lanka and El Salvador, a number of studies show a chronic interstitial nephritis in woman comparable to the disease observed in male agricultural workers. Women, who stay at home, are less or negligibly exposed to heat stress and the harsh working conditions in the fields. Nevertheless, they develop CINAC, with a slower natural course as compared to their farming husbands. Only explicable through ingestion or inhalation of the same toxins present in the environment they share with their partners.

In an endemic area in Nicaragua, it has been shown that school children aged 12–18 years with no prior employment history have elevated urinary concentrations of the tubular injury markers neutrophil gelatinase-associated lipocalin (NGAL) and N-acetyl-D-glucosaminidase (NAG), indicating early tubular damage. Likewise, a high prevalence of CKD in children and adolescents has been reported in three agricultural CINAC endemic regions in El Salvador. This suggests the possibility of established early kidney damage prior to future occupational exposure to heat stress, dehydration or agrochemicals. In addition, the increased CKD related mortality pattern among women, children and adolescents in El Salvador and Nicaragua suggests that there are additional factors, beyond the hypothesised heat stress–dehydration mechanism, which point to the broader environmental context surrounding this epidemic. From the perspective of animal experiments, clear dehydration/heat stress alone during 4 weeks does not lead to the constellation of proximal tubular lesions as observed in CINAC patients.

An alternate theory advocates increased uric acid as causal factor. Although elevated serum levels of uric acid have been reported in 55-75% of CINAC patients, this is probably secondary to the reduced GFR as hyperuricemia is a prevalent finding in patients with CKD. Observed (slight) increases in serum uric acid are therefore unlikely to be a primary CINAC cause, and are not considered a criterion for diagnosis.

There are strong epidemiological data supporting toxin(s) as the cause of CINAC, although it is beyond the scope of this manuscript to discuss them all. A comprehensive study of the drinking water in Sri Lanka and two recent systematic reviews summarize the acquired knowledge of the last 30 years.
First, a recent study in Sri Lanka assessed the relationship between potential nephrotoxic elements arsenic, cadmium, lead, uranium, silica, strontium and fluoride in drinking water, and urine samples collected from individuals with and without CKDu in endemic areas, and from individuals without CKDu in nonendemic areas\textsuperscript{13}. All water samples – from a variety of source types (i.e. shallow and deep wells, springs, piped and surface water) – contained extremely low concentrations of potential nephrotoxic elements, and all were well below international drinking water guideline values.

Second, a systematic review of epidemiologic studies that addressed associations between any indicator of pesticide exposure and any outcome measure of CKD came to the following conclusions\textsuperscript{14}. Four studies, out of 21, with stronger designs and better exposure assessment (from Sri Lanka, India and USA) showed exposure-responses or clear associations, for different pesticides (glyphosate, organochlorine, alachlor, atrazine, metolachlor, pendimethalin, paraquat).

Third, a US cohort study of licensed applicators observed associations between ESRD and a considerable number of specific pesticides. Most interesting is the association with paraquat, among other pesticides, which also was implicated in ESRD among the wives of the pesticide applicators\textsuperscript{8,15}. Paraquat is one of the few pesticides with established acute nephrotoxicity after administration of high doses over a short time. The increased risk of ESRD related to intermitted paraquat use associated with other agrochemicals could be a consequence of episodes of clinical or subclinical AKI caused by nephrotoxic pesticides as suggested by others\textsuperscript{16}.

Fourth, a study in a CKDu endemic area in Sri Lanka found a significant association with overall pesticide application (OR 2.3, 95% CI 1.0-5.6) and use of glyphosate (OR 5.1, 95% CI 2.3-11.3), adjusted for age, sex, education, family CKD and exposure modifiers\textsuperscript{17}. It was the only one conducted in CKDu endemic areas that investigated a potential exposure-response relationship by combining questions on water intake from different sources in relation to water hardness and levels of the herbicide glyphosate detected in water. With drinking pipe water or reservoir water with soft water and with trace or no detection of glyphosate as the reference, drinking from serving wells with hard water and intermediate concentrations of glyphosate (median 0.6 $\mu$g/L) yielded an adjusted OR of 2.5 (95% CI 1.1-5.7). Drinking from abandoned wells with very hard water and highest concentrations of glyphosate (median 3.2 $\mu$g/L) yielded an adjusted OR of 5.5 (95% CI 2.9-10.3).
Fifth, a methodologically sound review concluded that there was no consistent evidence to support the association between CKD and heat stress-dehydration, whereas this was the case for agrochemicals\(^\text{18}\). While physiological/pathophysiological and mainly epidemiological reasoning, and some experimental animal studies support the concept of heat stress and dehydration as causes of chronic kidney damage, no solid evidence of this in humans is available, nor are there studies that show indisputably that they are the single or preponderant cause of the onset of CKDu. Chapman et al. however found consistent evidence for the adverse effect of agrochemicals on CKD, and in some studies, an association with end-stage renal failure\(^\text{18}\). In this meta-analysis, which included 13 studies from different regional areas, the overall effect was positive, and became significant when cross-sectional studies were removed.

Next to the epidemiologic support, several PTC changes further corroborate a toxin-induced etiology and suggest involvement of a particular pathway. Lysosomal morphology varies greatly in PTCs depending on different factors such as proteinuria, causes of tubular injury, etc. The CINAC lysosomes have a specific morphology, requiring electron microscopic examination at high magnification for accurate identification. The striking morphological similarities (PTC lysosomal lesions, tubular atrophy and fibrosis) observed in CINAC patients, calcineurin inhibitor (CNI) treated patients and some toxic nephropathies such as clomiphene, lomustine and lithium, suggest a common tubulotoxic etiology. Many of these nephrotoxic drugs exert direct or indirect modulatory effects on calcineurin, a phosphatase regulating activity of nuclear factor of activated T-cells (NFAT; involved in immunosuppression) and, interestingly, transcription factor EB (TFEB; involved in autophagy, lysosomal biogenesis, cargo and exocytosis)\(^\text{19–21}\). Although the involvement of NFAT in CNI toxicity has not been unequivocally proven and other pathways (e.g. TWEAK/FN14) may be involved, it remains clear that calcineurin mediated immunosuppression and nephrotoxicity are intimately linked\(^\text{22–24}\). Finally, it is important to note that other nephrotoxic models such as analgesic nephropathy, aristolochic acid nephropathy (i.e. rats receiving only this compound), cis-platinum and in almost all (7 out of 8) tenofovir cases do not show the CINAC and CNI associated lysosomal lesions, supporting the idea that a specific pathway or set of pathways is involved.

Evaluation of indication, implantation and protocol renal transplant biopsy specimens, revealed that the lysosomal CINAC lesion is acquired in association with sustained CNI exposure. The lysosomal lesion was found in less than 10% at implantation and up to 76 % of indication biopsies, whereas protocol biopsies at 6 and 12months after transplantation showed a prevalence of 50% and 67%, respectively.
The hypermetabolic PTCs, with their pronounced O₂ consumption/delivery ratio of 79% are highly susceptible to a repeated toxic/hypoxic insult (cadmium, aminoglycosides, cisplatinum, tenofovir, aristolochic acid...), particularly when there is increasing intracellular concentration of reabsorbed and secreted potential toxin(s) (e.g. aminoglycosides, cis-platinum, paraquat). A substantial number of hydrophilic pesticides (e.g paraquat, 2,4-D, pyrethroids) are eliminated by the kidney through glomerular filtration and proximal tubular reabsorption/secretion, and hence may concentrate in the PTCs²⁵. It’s well documented that these toxins generate reactive oxygen species inactivating calcineurin and induce cellular damage²⁶,²⁷.

The PTC damage and interstitial expansion/fibrosis in CINAC patients in the absence of overt glomerulosclerosis fits with insights developed by Grgic et al. that selective, targeted and repeated non-lethal injury of the PTC is sufficient to initiate maladaptive repair and drive the formation of interstitial fibrosis, loss of peritubular capillaries and secondary glomerulosclerosis²⁸. This is consistent with a study of López-Marín et al. reporting chronic tubulo-interstitial nephropathy (interstitial fibrosis, tubular atrophy) with secondary glomerular and vascular damage in Salvadorian agricultural communities²⁹. In addition, a prospective histopathologic study by Fischer et al. of 11 Nicaraguan patients with MeN, biopsied at their earliest clinical appearance, identified patchy tubular cell injury/atrophy and interstitial inflammation in the cortex and corticomedullary junction, without involvement of glomeruli, indicating that an infectious or toxic agent is a likely cause of renal injury¹⁶.

Tubular type proteinuria (i.e. low molecular weight proteinuria) and increased proximal tubular markers in the urine are observed in most cases of CINAC patients, further corroborating proximal tubular injury/damage¹,³⁰. Although moderate to- /overt proteinuria can be seen in some cases, particularly in association with advanced CKD³¹.

Summary
CINAC, MeN, CKDu from different regions (Sri Lanka, El Salvador, India and France) express the same morphologic lesions, epidemiological profiles and clinical manifestations indicating a comparable renal disease around the globe⁷. A CINAC histopathological constellation of lesions has been identified to be useful in routine investigation of renal biopsies of patients clinically suspected of CINAC, provided they meet the specific morphologic criteria⁷.
Renal biopsies demonstrating an increased prevalence of this renal CINAC lysosomal lesions are (up to now) associated with toxin-induced nephropathies. Although the nature of the toxin is not yet determined. However, epidemiological, experimental and pathological arguments point towards agrochemicals/pesticides. Heat stress/dehydration, when present, may contribute to the development and progression of CINAC towards end stage renal failure.

The striking parallel between renal biopsies of CINAC patients and CNI-treated patients suggest calcineurin pathway inhibition as a putative mechanism, although involvement of other pathways cannot be excluded. Interestingly, some pesticides have (direct or indirect) CNI activity and hence may have several biological effects in common with the classic CNI (cyclosporine, tacrolimus) such as CNI, Na⁺/K⁺-ATPase inhibition, electrolyte disturbances, immunosuppression (susceptibility to infections) and nephrotoxicity.

According to M. Haas on our recent study⁷,³²: “Although multiple questions remain, related to the pathways involved in this toxic nephropathy, to the possible treatment/reversibility of CINAC as well as its prevention, the study from Vervaet and coworkers represents an important step forward in our understanding of this devastating condition, and one that will undoubtedly stimulate additional investigation” and animate debates.

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Figure 1. Overview of the constellation of proximal tubular lesions observed in patients with CINAC/CKDu.

A) Proximal tubular cells containing many enlarged argyrophilic granules, demonstrated to be lysosomes\(^7\) (arrowheads; Periodic Acid Schiff Methenamine (PASM) staining). B) Affected proximal tubule demonstrating enlarged lysosomes, flattened atrophic epithelial cells with loss of brush border (solid arrow), apical blebbing and cell fragment shedding (open arrows; PASM staining). C) Affected proximal tubules display autofluorescent granules, a subset of which demonstrated to be positive for lysosomal markers\(^7\). (PT: proximal tubule, DT: distal tubule). D) The same section as in C immunohistochemically stained for the proliferation marker proliferating cell nuclear antigen. There were very few scattered proliferating epithelial cells in the affected PTs. In contrast, unaffected distal nephron cells demonstrated prominent proliferative activity. E-G) Proximal tubular cells with enlarged dysmorphic lysosomes (≥ 1.2 µm) containing homogenous non-membrane bound electron dense rounded/irregular “aggregates” dispersed throughout the light to medium uniform electron dense lysosomal matrix. G) Two hallmark lysosomes (white asterisks), accompanied by several smaller ones.