Intestinal adsorption of uraemic toxins: a new strategy for anaemia management?

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Resistance to erythropoiesis-stimulating agents (ESAs) is an increasing problem influencing the successful management of anaemia in patients with chronic kidney disease (CKD). Hyporesponsiveness to ESAs may occur at the start of the treatment or subsequently and it may be transient or persistent. The importance of this issue is evidenced by the association of hyporesponsiveness with adverse outcomes in both haemodialysis and non-dialysis CKD patients [1–5]. Furthermore, the importance of detecting ESA hyporesponsiveness is also underlined by the fact that in the USA, hyporesponsive patients (those receiving epoetin at a dose >450 IU/week) consumed 52.5% of the total ESAs prescribed [6]. Despite the clinical relevance of ESA hyporesponsiveness, no standardized definition of this phenomenon has been produced. Different criteria have been proposed for defining initial hyporesponsiveness such as the Hb increase induced by the initial ESA dose [2–4], the ratio between ESA dose and patient Hb level, the so-called resistance index [7, 8], or the prescription of high-ESA dose (>450 U/kg/week IV epoetin or 1.5 µg/kg of SC darbepoetin) [1, 9]. More recently, KDIGO Guidelines classified ESA hyporesponsiveness as ’initial’ (if there is no increase in Hb concentration from baseline after the first month of ESA treatment on appropriate weight-based dosing) or ’subsequent’ (if there is need for two increases in ESA doses up to 50% beyond the dose at which the patient had been stable in order to maintain a stable Hb level) [10]. The lack of a universally accepted definition has produced various estimates of prevalence of ESA hyporesponsiveness in haemodialysis patients ranging from 7.3 to 17.6% [11]; similar…

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figures have been suggested for non-dialysis patients (14–15%) [4, 12]. However, these estimates did not discriminate between chronic and acute (and therefore reversible) ESA hyporesponsiveness. Very recently, Gilbertson et al. [13] compared three different definitions of hyporesponsive dialysis patients (total ESA dose, ESA dose per kg of body weight, or ESA dose per Hb level), with a careful methodology based on a 4-month observation period that allowed for the discrimination between acute and chronic hyporesponsiveness. They found a prevalence of 4.5%, and 15% of patients with chronic and acute ESA hyporesponsiveness, respectively.

Several causes may induce ESA hyporesponsiveness (Table 1) although the two most common are the development of iron deficiency and the onset of an inflammatory state that impairs the response to ESAs [7, 14, 15]. Intercurrent infection, in fact, is the most frequent reason why patients become temporarily (and reversibly) ESA-resistant. Mineral bone disorders are also strictly correlated to ESA hyporesponsiveness [16, 17]. In CKD patients, accumulation of uraemic toxins represents a further potential cause of ESA resistance. Indeed, some protein-bound molecules and in particular indoxyl sulphate (IS) have been involved in the pathogenesis of anaemia through increased suicidal erythrocyte death, the so-called eryptosis, mainly because of stimulation of extracellular Ca++ entry with increased suicidal erythrocyte death, the so-called eryptosis, with subsequent stimulation of cell shrinkage and expression of phosphotidyl serine on the cell membrane surface that provides the phagocytic signal [18]. Furthermore, IS could have a pathologic role in inappropriate erythropoietin production by disrupting the oxygen-sensing system both in vitro and in vivo, by suppressing in a dose-dependent manner the transcriptional activity of hypoxia inducible factor-1 (HIF-1) [19, 20].

Uraemic toxins play a role in endothelial dysfunction and cardiovascular disease (CVD) and possibly in the progression of renal disease in patients with CKD and are independently associated with mortality. Among these, the most pathophysiologically relevant compounds are the larger 'middle molecules' (protein-bound molecules) due to the difficulty of their dialytic removal. Overall, more than one hundred compounds have been identified and specific treatments aiming at reducing their circulating levels have been tested.

AST-120 is an inert binding compound acting as an intestinal adsorbent, constituted by fine spherical particles ∼0.2–0.4 mm in diameter composed of porous microcrystalline carbon; AST-120 is engineered to have a large surface area and uniform adsorbing capacity for various uraemic toxins including IS by adsorbing indole in the intestines, and consequently stimulating its excretion into faeces. Oral administration of AST-120 is effective in reducing in a dose-dependent manner the plasma concentration of IS by 3–39% [21].

AST-120 is widely used in Japan, Korea and Taiwan for the treatment of CKD patients to delay the progression of CKD. This effect is induced by a reduction of plasma levels of uraemic toxins, such as IS. Indeed, organic anion transporters in the proximal tubule permit IS uptake that stimulates TGF-β1, a profibrotic cytokine, in the renal parenchyma and induces oxidative stress via the promotion of free radical production and the reduction of superoxide scavenging activity. These mechanisms ultimately lead to glomerulosclerosis, interstitial fibrosis and tubular cell injury. Observational studies consistently showed a significant impact of AST-120 on reducing either GFR decline or ESRD rates [22, 23]. Conversely, RCTs provided conflicting results. Owada et al. [24] reported that, in 26 CKD patients followed for 12 months, AST-120 administration concurrent with protein restriction was more efficacious than low protein diet alone in slowing down the progression of CKD by inhibiting accumulation of IS. In contrast, in a 12-week RCT enrolling 156 CKD patients, AST-120 did not significantly modify creatinine clearance despite a dose-dependent reduction of IS [21]. Finally, the largest RCT published so far also provided negative results [25]. In this trial, 460 CKD patients with a mean estimated GFR of 22 mL/min were treated with AST-120 at dose of 6 g/day for 1 year or placebo; no difference was found between AST-120 and placebo in terms of renal event rates (ESRD or doubling of serum creatinine) even though the GFR decline showed in significantly lower results in patients treated with AST-120 than in the placebo group [25]. These data raise the question of whether the possible protective effects of AST-120 may require a prolonged treatment or whether the therapy should be started in an earlier phase of CKD. The ongoing Evaluating Prevention of Progression in CKD (EPPIC) trials being conducted in North America/Latin America and Europe, enrolling over 2000 subjects, will provide definitive answers on the renoprotective effect of AST-120.

Besides the progression of renal disease, the effect of AST-120 has been assessed also on CVD. Indeed, in patients with congestive heart failure and moderate CKD (serum creatinine 1.3–2.0 mg/dL), symptoms of heart failure, parameters of renal function and atrial natriuretic peptide as well improved after treatment with AST-120 added to conventional therapy for 24 months. In addition, in the subgroup of patients requiring hospitalization, the length of hospital stay and number of hospital admissions decreased significantly with AST-120 treatment in comparison with the 2-year period prior to initiation of therapy [26].

In an RCT including 50 patients with nondiabetic CKD, treatment with AST-120 for 24 months decreased carotid artery intima-media thickness and pulse-wave velocity (a measure of arterial stiffness) in comparison with patients who did not receive AST-120 [27].

In another study, AST-120 treatment at daily dose of 6 g for 24 weeks improved endothelial dysfunction in 40 patients with CKD stage 3–5 and was associated with a decrease of both IS plasma levels and markers of oxidative stress (ratio of oxidized/reduced glutathione levels) [28]. Administration of AST-120 was associated with a significant increase in flow-

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<th>Table 1. Possible causes of ESA hyporesponsiveness</th>
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<td>Absolute or functional iron deficiency</td>
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<td>Lack of dialysis adequacy</td>
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<td>Hyperparathyroidism</td>
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mediated endothelium-level vasodilation in the brachial artery of 40 patients with CKD [27].

As reported above, it is a matter of fact that despite adequate iron supplementation and ESA use, there are still patients who are unable to reach the foreseen Hb target range. Thus, we should chart new territories for overcoming the ESA hyporesponsiveness, including sorting out the myelo-inhibition of red cells production by uraemic toxins.

Among these toxins, IS, as discussed above, is of particular interest not only because of its potential capability to facilitate the progression of CKD, but also considering its effect in suppressing EPO mRNA expression [19, 20] and thus promoting ESA resistance. In this regard, it is interesting to note that haemodialysis patients with increased levels of IS needed a higher ESA dosage [29]. The fact that the oral adsorbent AST-120 is able to reduce IS plasma levels and has anti-oxidant activity and possibly a renoprotective effect [21–29] makes the investigation of this drug of interest for its potential effect in reducing ESA hyporesponsiveness.

Unfortunately, the data so far available to clarify this effect are scarce and therefore, the paper by Wu et al. [30], published in this issue of the journal, is appreciated. According to the authors, the exact pathway linking the accumulation of IS with anaemia is far from being elucidated and it is probably multifactorial. Indeed, experimental studies have shown that IS induces a sodium-potassium adenosine triphosphatase-dependent increase in oxygen consumption in proximal tubular cells, decreases renal oxygenation and consequently aggravates the hypoxia in the remnant kidney [30]. Furthermore, IS plays a role in modulating the transcriptional response of HIF-1 [20] and inhibits EPO-induced phosphorylation of the EPO receptor [31]. Therefore, there are clear data supporting the use of a drug able to reduce IS for improving anaemia correction in CKD patients, as was very clearly and reported in depth in the discussion section of the paper by Wu et al. [30].

This study has the important merit of trying to find a different approach for treating anaemia in already iron replete patients. The results of this paper, in fact, suggest the possibility of increasing the Hb levels without increasing the ESA dose in non-dialysis CKD stage 5 patients who in any case were able to respond to ESA treatment alone. We are well aware that from the therapeutic point of view, the real matter of concern is the true ESA hyporesponsiveness that makes patients severely anaemic and even transfusion dependent. Indeed, ESA hyporesponders are exposed to greater clinical complications (poor quality of life, transfusion-dependent infective and immunogenic risks) and higher costs. Unfortunately, the paper by Wu et al. [30] did not select patients with ESA hyporesponsiveness and included only ESA-naïve patients, therefore limiting the generalizability of their findings to the hyporesponsive-proven anaemia of CKD population.

The study results are intriguing considering that the achievement of the Hb target was three times more frequent in patients receiving AST-120 in the intention-to-treat analysis. However, it is not clear why there was such a relevant period effect of AST-120 in increasing the Hb levels when the drug was administered before the use of Mircera. The authors themselves speculated that the status of ESA naïve may account for this unexpected finding, being that the start of ESA therapy was associated with the use of larger doses. Indeed, Mircera doses were reduced by ~30% during both washout periods therefore supporting that randomized patients were clearly not ESA hyporesponsive.

Moreover, the low gastrointestinal tolerability of the drug may affect the compliance of the patients also considering that they usually receive many pills for treating the co-morbidities related to CKD. Indeed, >50% of patients experienced at least one treatment-related adverse effect, almost exclusively of gastrointestinal nature with a high number of drop out and withdrawn patients from the study (near 20%). This occurred despite the fact that the amount of the AST-120 administered (4.2–4.7 g/day on average) was lower than that originally planned in the study design (6 g/day).

According to these study results, AST-120 seems to be able to potentiate the effect of Mircera, particularly when AST-120 is given before the use of the ESA, leaving open the most important clinical question whether AST-120 is really able to revert the true ESA hyporesponsiveness.

The low gastro intestinal tolerability, even in a monocentric randomized control trial, should jeopardize the patient compliance especially in everyday clinical practice where the doctors, the nurses and more importantly the motivation of the patients is much less than the patient compliance in randomized clinical trials. Indeed, RCTs usually select those doctors, nurses and patients in the centres who are more motivated; this bias is particularly true when we are only referring to a single-centre experience.

Due to the clinical relevance of ESA hyporesponsiveness [1–5] and its economic impact [6], we should probably consider different strategies with different drugs. Unfortunately, some approaches have already been tested with inconclusive or negative results or in need of confirmatory studies. Oral pentoxifylline was given for 4 months to ESA hyporesponsive haemodialysis patients with a reduction of the production of TNF-alfa and IFN-gamma associated with an increase in Hb plasma levels [32]. In CKD-ND patients, the administration of pentoxiphylline was associated with a reduction of IL-6 plasma levels, and an increase of TSAT, possibly due to iron release from the reticulum endothelium by a pentoxiphylline-mediated modulation of hepcidin production [33]. Recently, several observational studies demonstrated that lower 25(OH)D3 and higher CRP levels are independently associated with lower Hb concentrations in both CKD-ND and haemodialysis patients [17, 34–36]. Furthermore, giving cholecalciferol to 158 haemodialysis patients resulted in a reduction of CRP and ESA dose [37], suggesting a possible effect of vitamin D analogue in preventing anaemia related to inflammation and ESA hyporesponsiveness.

Administration of L-carnitine, even though effective in reducing C-reactive protein, did not improve ESA resistance [38] while the use of ascorbic acid may result in an increase in Hb concentration and TSAT and decrease in ESA requirements [39] but it was associated with important side effects including the severe risk of oxalosis [40].

In dialysis patients, the use of an adequate dialysis dose and ultrapure dialysate and high-flux dialysis or haemodiafiltration...
seemed to facilitate the anaemia correction; however, nowadays, these approaches do not seem to add too much in the majority of cases, considering that adequate dialysis dose, ultrapure dialysate and convective treatment are already the gold standard in many centres.

In conclusion, owing to the multifactorial pathogenesis of ESA hyporesponsiveness, new potential strategies for management of ESA hyporesponsiveness are regarded with particular interest. The ongoing trials on novel therapeutic approaches for correcting anaemia of CKD patients, including hepcidin modulation, erythropoietin gene therapy and HIF stabilization, are being awaited with much interest, especially in ESA hyporesponsive patients.

Whether AST-120 may be considered as a further tool for treatment of ESA resistance requires further large trials focused on ESA hyporesponsive patients: the major concern is related to patient compliance considering the low gastrointestinal tolerability of this drug.

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CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or part, except in abstract format.


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Protein restriction: a revisited old strategy with new opportunities?

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When dialysis facilities were not widely distributed, protein intake had been a hot topic for many years to delay, for as long as possible, the need for dialysis in patients with advanced chronic kidney disease (CKD). The seminal paper by Giovannetti and Maggiore [1], which was published in the Lancet exactly 50 years ago, was a strong message for the nephrological community: many patients were given a low-protein diet with apparently great success, despite the lack of a true control group. Strong believers to this approach had to match with strong criticisms, mainly related to the risk of malnutrition, especially when in those days, protein-free foods had very low palatability, were difficult to find and were expensive. Hence, patients systematically tended to reduce the caloric intake far below that which was recommended and developed malnutrition. The situation was even worse when the low-protein diet was prescribed by nephrologists without adequate nutritional expertise. Often, signs of malnutrition from laboratory tests were misinterpreted as therapeutic success: serum creatinine (the standard method for evaluating renal function in those days) often decreased as a consequence of hypercatabolism, reducing muscle mass and creatinine production, and not as an improvement in renal function.

The popularity of this dietary approach dramatically decreased when dialysis facilities became largely available and, possibly as a reaction, starting dialysis earlier became an attractive approach to reduce morbidities and mortality in CKD patients, as proposed by Bonomini et al. [2]. However, again, there were strong limitations, such as the absence of a control group and the fact that the historical comparison did not take into account the lag time between the early and the late start of dialysis and just considered survival from the start of dialysis.

In the 1980s and early 1990s, the low-protein diet had become the mainstay of CKD management, with the idea that it could delay the progression of CKD in the long term. The popularity of the low-protein diet was also sustained by the