INTRODUCTION

Globally, about 700 million patients suffer from chronic kidney disease (CKD). Most patients with advanced CKD develop renal anemia at some point, a complication associated with reduced quality of life and increased morbidity and mortality. Erythropoietin (EPO), the main erythropoiesis-stimulating hormone, is produced primarily by the kidney. In CKD, EPO deficiency is frequent and a well-documented cause of renal anemia. Other contributing factors are absolute and/or functional iron deficiency, inflammation with increased hepcidin levels, and shortened RBC life span. Augmenting red blood cell (RBC) production is a widely applied treatment strategy, so renal anemia is usually managed with erythropoiesis-stimulating agents (ESAs; similar in effect to endogenous EPO), iron supplements, and—more recently—hypoxia-inducible factor prolyl hydroxylase inhibitors, drugs that increase EPO production, improve iron availability, and reduce hepcidin levels.2

Since the steady-state number of circulating RBCs depends on the balance between RBC formation and RBC death, a shortened RBC life span is considered a contributor to renal anemia. In healthy individuals, the RBC life span is around 120 days. Shortened RBC life span is observed in most patients with advanced CKD; for example, in patients on hemodialysis, the average RBC life span is shortened to around 50 to 70 days.3,4 This suggests that interventions that systematically increase the RBC life span in CKD patients may alleviate anemia, leading to a larger steady-state RBC pool and thus, higher blood hemoglobin concentrations. As a side effect, such interventions would likely reduce the overall amount of ESAs needed to maintain adequate hemoglobin levels, as suggested by physiology-based mathematical simulations of anemia treatment.5

HYPOTHESIS

The Piezo1 hypothesis of renal anemia

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Abstract

Erythropoietin deficiency is an extensively researched cause of renal anemia. The etiology and consequences of shortened red blood cell (RBC) life span in chronic kidney disease (CKD) are less well understood. Traversing capillaries requires RBC geometry changes, a process enabled by adaptions of the cytoskeleton. These changes are mediated by transient activation of the mechanosensory Piezo1 channel, resulting in calcium influx. Importantly, prolonged Piezo1 activation shortens RBC life span, presumably through activation of calcium-dependent intracellular pathways triggering RBC death. Two Piezo1-activating small molecules, Jedi1 and Jedi2, share remarkable structural similarities with 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), a uremic retention solute cleared by the healthy kidney. We hypothesize that in CKD the accumulation of CMPF leads to prolonged activation of Piezo1 (similar in effect to Jedi1 and Jedi2), thus reducing RBC life span. This hypothesis can be tested through bench experiments and, ultimately, by studying the effect of CMPF removal on renal anemia.
While known for decades, the etiology of shortened RBC life span is still not well understood. Over 60 years ago, blood cross-transfusion experiments between subjects with and without CKD demonstrated that not RBC characteristics per se but rather the “uremic milieu” shorten RBC life span. Since then, research revealed that a rise in intracellular RBC calcium (icCa$^{2+}$) triggers a sequence of events that eventually result in eryptosis, a type of apoptosis that occurs in RBC. Eryptosis can be triggered by several factors that enhance icCa$^{2+}$, including osmotic stress, small molecules, and reactive oxygen species.

In RBCs, Ca$^{2+}$ influx under mechanical stress is regulated by Piezo1, a phylogenetically old and highly conserved mechanosensitive cation channel located on several cell types, including mammalian RBC. Physiologically, Piezo1 is activated by mechanical forces brought about by the transit of RBC through capillaries and narrow slits between endothelial cells in the spleen. The activation of Piezo1 results in Ca$^{2+}$ influx and increase of icCa$^{2+}$, which is short-lived (seconds); a Ca$^{2+}$ ATPase located in the RBC plasma membrane swiftly extrudes icCa$^{2+}$. The transient icCa$^{2+}$ rise mediates rapid reversible changes in cytoskeletal flexibility, a process critical for the RBC’s ability to traverse narrow anatomical structures. icCa$^{2+}$ also activates a K+ channel (Gardos channel), leading to hyperpolarization and a loss of K+, Cl−, and water, resulting in RBC shrinkage.

Given the critical role of Piezo1 in RBC physiology, it is not surprising that mutations affecting its structure may give rise to clinical phenotypes. For example, several Piezo1 gain-of-function mutations that result in delayed channel inactivation and prolonged Ca$^{2+}$ influx have been linked to dehydrated hereditary stomatocytosis, a non-immune congenital hemolytic disorder. Interestingly, three small molecules—Yoda1, Jedi1, and Jedi2—also decelerate Piezo1 inactivation and lengthen Ca$^{2+}$ influx. It is unknown if these molecules exist in vivo. However, it is notable—and central to our hypothesis—that the active center of Jedi1 and Jedi2 shares remarkable structural similarities with 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) (Figure 1), a 240 Da metabolite of furan fatty acids and a protein-bound uremic retention solute normally cleared by the healthy kidney. In CKD patients, CMPF levels are increased 5 to 15-fold compared to healthy subjects.

### 1.1 The hypothesis and its implications

The structural similarities of the active moieties between Jedi1, Jedi2, and CMPF as well as the fact that CMPF levels are elevated in CKD led us to hypothesize that (a) CMPF extends Piezo1 activation and Ca$^{2+}$ influx that triggers eryptotic pathways (Figure 2); (b) elevated CMPF levels in CKD reduce RBC life span and thus contribute to renal anemia; (c) lowering CMPF levels in CKD patients will improve anemia (Figure 3).

### 1.2 Testing the hypothesis

Our hypothesis can be tested through its implications on a clinical level but also mechanistically by exploring interactions between CMPF and Piezo1 on a molecular level.

Several clinical study designs can be considered to explore the effect of CMPF removal on RBC physiology. We envision various stages predicated on in vitro and ex vivo experiments. First, the impact of technologies intended to remove CMPF on lowering its concentration needs to be evaluated. This can be done by assessing CMPF kinetics with and without these methods. Second, once confirmed that CMPF removal can indeed be enhanced in vivo, it will be important to assess RBC life span when comparing hemodialysis with and without CMPF removal interventions in place. Cross-over designs would be a preferred choice in that respect. Well-established means to estimate RBC life span have recently been complemented by the measurement of phosphatidylserine exposure on the RBC surface as a proxy of RBC survival. Eventually, a randomized controlled trial will be pivotal to assess the impact of CMPF removal on anemia management, including the utilization of erythropoiesis-stimulating agents.

The means to lower CMPF require careful considerations. As a weak organic base with a strongly lipophilic
character, CMPF binds to human serum albumin's binding site. A strong association between albumin and CMPF renders its removal by conventional hemodialysis ineffective. Consequently, to test our hypothesis, we propose the removal of CMPF by several means, considering three potential approaches. First, adsorptive techniques (Figure 4, panels A and B) with or without fractionated plasma separation. Adsorptive means can be used in combination with hemodialysis. When used in combination with plasma separation, the albumin-rich plasma fraction is brought in contact with sorbent material to deplete albumin-bound CMPF (Figure 4B). The cleansed plasma is then reunited with the blood cells to undergo conventional hemodialysis. Second, we propose an approach that combines displacement of CMPF using an exogenous binding competitor to increase its free fraction followed by adsorption (Figure 4C). Previous clinical proof-of-concept studies have demonstrated the utility of the displacer concept to enhance the dialytic removal of protein-bound uremic solutes. Third, albumin dialysis, a method used primarily in patients with liver failure to remove

![Figure 2](https://example.com/f2.png)

**Figure 2** Biochemical processes during RBC capillary passage. Top panel: Physiological role of Piezo1 during capillary transit of RBCs, modified from. Bottom panel: Posited prolonged activation of Piezo1 by elevated CMPF would trigger extended Ca\(^{2+}\) influx and subsequent eryptosis. In healthy individuals, the passage of RBC through capillaries leads to mechanical stimulation of the Piezo1 mechanoreceptor, subsequent transient Ca\(^{2+}\) influx, and increased RBC deformability and shrinking. Activation of the Gardos channel occurs in response to increased levels of icCa\(^{2+}\). This facilitates the export of K\(^{+}\), Cl\(^{-}\), and water. After the passage, Piezo1 is inactivated and icCa\(^{2+}\) extruded by a Ca\(^{2+}\) ATPase. Consequently, the RBC reverts to its pre-passage shape. In CKD patients, we hypothesize that elevated levels of CMPF prolong Piezo1 activation. A sustained activation of Piezo1 would then trigger Ca\(^{2+}\)-dependent eryptotic pathways and shorten the RBC life span.
FIGURE 3 Proposed novel role of CMPF and Piezo1 in renal anemia. In addition to the well-established role of erythropoietin deficiency in the pathogenesis of renal anemia, we hypothesize that elevated levels of CMPF will shorten RBC life span and thus contribute to renal anemia. The hypothesis predicts that CMPF removal, as a potential intervention, would improve anemia in CKD.

FIGURE 4 Schematic depiction of concepts for the removal of CMPF. Adsorption (panels A and B), displacement (panel C), and albumin dialysis (panel D), respectively. (A) With adsorption modalities, albumin-bound CMPF is brought in close proximity with a sorbent of mainly hydrophobic material composition and porosity allowing attraction forces to retain CMPF during its passage. With hemoperfusion, whole blood is passed through an adsorber cartridge. (B) Here, the albumin-rich plasma fraction (generated via plasma filter or centrifuge) is brought in contact with sorbent material. The cleansed plasma is then reunited with the blood cells. A combination with conventional dialysis is possible. (C) The displacement concept builds on the competition between a displacer and CMPF at its albumin binding site, resulting in an increased free, dialyzable CMPF fraction (adapted from Madero et al.17). (D) In albumin-dialysis, blood is passed through a membrane and dialyzed against an exogenous albumin solution. Free CMPF is cleared by diffusion across the membrane. The albumin-dialysate circuit is regenerated by passing through an adsorber cartridge.
albumin-bound and water-soluble substances, could be feasible, despite complex logistics, and high cost (Figure 4D).

Complementary to an intervention trial, bench studies can shed light on the molecular mechanisms that are central to our hypothesis. The interaction of CMPF with Piezo1 can be explored through the exposure of isolated and mechanically stressed RBC to different concentrations of CMPF in comparison to Jedi1 and Jedi2. Our hypothesis predicts that CMPF increases icCa2+ (Figure 3). Previous data have shown that icCa2+ is higher in uremic RBC and increases the eryptosis rate. Various techniques quantify icCa2+ kinetics, e.g., flow cytometry, confocal microscopy, and microplate-reader-based assays. Patch clamping is a powerful, straightforward electrophysiological tool to test the effect of CMPF on Piezo-1 channel properties. Several groups have used patch-clamp methods to demonstrate Piezo1 activation in RBCs.

In summary, we hypothesize that CMPF shortens RBC life span and aggravates anemia in CKD. If correct, this would imply that interventions that lower CMPF levels would systematically increase RBC lifespan in CKD patients, alleviate renal anemia and reduce ESA needs.

AUTHOR CONTRIBUTIONS
All authors contributed to the development of the hypothesis, literature search, and manuscript writing.

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