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Review

Inflammatory Pathways of Sulfonamide Diuretics: Insights into SLC12A Cl^- Symporters and Additional Targets

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Key Words
Thiazides • Thiazide-like • Loop-diuretics • SLC12A • Inflammation

Abstract
Thiazide, thiazide-like, and loop diuretics are primarily known for inhibiting members of the SLC12A family of Cl^- transporters, which include the Na^+Cl^- cotransporter (NCC), $Na^+K^+2Cl^-$ cotransporters (NKCC1 and NKCC2) and K^+Cl^- symporters (KCC1-4). While the main pharmacological effect of these diuretics is diuresis, achieved by promoting the excretion of excess water and salt through the kidneys, they have intriguing pharmacological effects beyond their traditional ones which cannot be solely attributed to their effects on renal salt transport. Of particular interest is their role in modulating inflammatory processes. These diuretics appear to exert both pro- and anti-inflammatory effects, potentially by influencing various pathways involved in immune responses. For example, NKCC1 has been implicated in the regulation of pro-inflammatory cytokines, such as interleukin-1β (IL1β), interleukin-8 (IL8) and tumor necrosis factor α (TNFα), which are critical mediators of immune cell activity during inflammation. The underlying mechanisms through which NKCC1 contributes to inflammation may involve key signaling pathways, such as that mediated by the nuclear factor kappa B (NFκB). This pathway is crucial for the activation and assembly of the inflammasome, as well as for regulating the phagocytic activity of immune cells. In addition, NKCC1 can control (or be controlled) by reactive oxygen species and oxidative stress, which contribute to the pathogenesis of various inflammatory conditions as well. Diuretics may help mitigate inflammation-related tissue damage by scavenging reactive oxygen species and boosting antioxidant defenses, thereby restoring redox balance in inflamed tissues. Despite these intriguing effects, the precise molecular pathways through which thiazide, thiazide-like and loop diuretics may modulate inflammatory responses remain poorly understood and warrant further investigation. This aspect of their pharmacological profile highlights their potential for therapeutic use beyond the scope of traditional diuretic functions.

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Introduction

Inflammation, often perceived negatively today, is frequently associated with damage and disease [1, 2]. This view is not necessarily tied to the word's etymology (from the Latin inflammare, meaning "to set on fire") but rather stems from a loose understanding of its biological role. We often overlook the concept established in 1859 by Rudolf Virchow, which emphasized that inflammation is a natural, typically beneficial response that frequently results in restitutio in integrum i.e., the restoration of the tissue to its original state [3]. Virchow noted that inflammation does not occur without irritation (irritatio) and that the affected organ experiences functional impairment (functio laesa). He also described several physiopathological outcomes triggered by the initial insult. These include increased cell volume due to active nutrient and water uptake, hypertrophy, cellular division leading to proliferation and swelling, often accompanied by the local accumulation of inflammatory cells, fatty degeneration and edema. Such processes, when chronic, can sometimes result in irreversible cell damage and death. This series of events, for instance, mirrors our modern understanding of the pathophysiology and progression of atherosclerosis, a chronic inflammatory condition characterized by endothelial dysfunction (functio laesa) and associated hypertension, dyslipidemia, hyperglycemia and insulin resistance (irritatio). It is now widely accepted that the first event leading to local vascular damage in atherosclerosis in response to those irritants, is the recruitment of monocytes, monocyte-derived macrophages and T cells which initiate and sustain local inflammation. This eventually leads to lipid accumulation within cells (e.g., foam cells) and in extracellular spaces. Over time, these factors promote local proliferation of smooth muscle cells, accumulation of connective tissue, and thickening and hardening of the blood vessels i.e., hallmarks of atherosclerosis (and inflammation) [4].

Given this particular context and broad understanding of inflammation, it is reasonable to hypothesize that hypertension is directly linked to inflammation, an association that is now accepted [5-8]. Hypertension is a well-established risk factor for cardiovascular disease [9] and a key component of the metabolic syndrome (MetS), a cluster of independent risk factors for type 2 diabetes (T2D) and cardiovascular mortality. These factors include insulin resistance, glucose intolerance, obesity and dyslipidemia [10, 11]. Moreover, MetS is characterized by chronic tissue inflammation [12, 13], likely driven by subtle or pronounced activation of inflammatory mediators, dysfunctional macrophages, neutrophils and lymphocytes [14], oxidative stress [15] and impaired vascular function [16-19]. Consequently, antihypertensive medications, like sulfonamide diuretics, may directly or indirectly influence pro- or anti-inflammatory responses [20, 21]. Specifically, thiazides (e.g., hydrochlorothiazide, one of the most commonly prescribed drugs), thiazide-like diuretics (e.g., chlortalidone, metolazone, indapamide) and loop diuretics (e.g., furosemide, bumetanide, torsemide, ethacrynic acid), are frequently prescribed to manage hypertension and associated conditions [22, 23]. Thiazides and thiazide-like diuretics are primarily used for hypertension, while loop diuretics are preferred in more severe cases, particularly when rapid diuresis is necessary. Within these therapeutic frameworks, sulfonamide diuretics may contribute to clinical outcomes by directly or indirectly modulating local and/or systemic inflammatory responses.

The known pharmacological targets of thiazides, thiazide-like and loop diuretics include members of the SLC12A family of ion transporters, which comprise seven cation-chloride symporters. These are SLC12A1-7 [24]; SLC12A8, a nicotinamide mononucleotide carrier [25]; and SLC12A9, a polyamine transporter with the potential to interact and inhibit SLC12A2 [26, 27]. These transmembrane proteins share similar molecular structures [28-32] and most of what we know about their physiological function is related to their ability to move Cl^-, Na^+, and/or K^+ across cell membranes to quickly regulate cell volume and maintain the balance of ions within cells [24]. Partially based on that, SLC12A family members are typically categorized based on their ion transport function i.e., the $Na^+K^+2Cl^-$ cotransporters NKCC1 (SLC12A2) and NKCC2 (SLC12A1), the Na^+Cl^- cotransporter NCC (SLC12A3), and

the K^+Cl^- cotransporters KCC1 (SLC12A4), KCC2 (SLC12A5), KCC3 (SLC12A6), and KCC4 (SLC12A7) [33, 34]. While there are numerous splice variants produced by SLC12A genes [35, 36], it is generally accepted that many of them are, to varying extents, sensitive to specific sulfonamide diuretics. Notably, sulfonamide diuretics exert their established pharmacological effects by inhibiting the transport function of NCC and NKCC2, respectively, in the kidneys [24, 37]. Since these two cotransporters are abundantly expressed in that organ [38, 39], NCC and NKCC2 are usually regarded as kidney-specific, thiazide- and bumetanide-sensitive cotransporters, respectively [40-44]. Nevertheless, it is now relatively clear that thiazides, thiazide-like and loop diuretics may have clinically important effects independent of their renal targets [45-49]. In fact, bumetanide at low and furosemide at high concentrations are potent inhibitors of the ubiquitously expressed NKCC1 variants (NKCC1a and NKCC1b) [50, 51] and furosemide, in particular, effectively inhibits KCC1, KCC2, KCC3 and KCC4 [52-56], although at lower concentrations than those required to inhibit NKCCs [57-62]. Importantly, while NCC and NKCC2 have a wider tissue expression pattern than originally thought [63, 64], they are typically found at much lower levels compared to those in the kidneys [65-73]. Therefore, their pharmacological and physiopathological relevance is often perceived irrelevant, and consequently, the physiological and pharmacological roles of extra-renal NCC and NKCC2 remain understudied.

In addition to their pharmacological profile, the ion carrier function of diuretic-sensitive symporters is, in turn, fine-tuned by phosphorylation of key residues in SLC12A proteins, mostly driven by the OSR1/SPAK (Oxidative Stress Responsive 1/Ste20-related proline/alanine-rich kinase) and WNK (With-No-Lysine kinases, WNK1-4) signaling cascades [74]. These kinases are widely expressed and play a role in multiple physiopathological processes. They are also exquisitely sensitive to fluctuations in intracellular K^+ and Cl^- concentrations [74, 75], and to inflammatory mediators [76-80]. These processes can, in turn, influence the expression level, pattern or function of diuretic-sensitive symporters. For instance, SPAK/OSR1-mediated activation of NKCC1 aggravated inflammatory responses after injury [81, 82], whereas NKCC1 inhibition reduced intracellular NFκB phosphorylation [83, 84] and activated local inflammatory cells [81, 85]. In microglia and macrophages, elimination of NKCC1 correlated with increased inflammasome priming, local production of IL1β [86] and efferocytosis [87]. This is relevant, as IL1β production requires assembly and activation of inflammasomes [88]. In addition, this and other interleukins/cytokines such as IL6 and TNFα play important roles in the inflammatory responses by activating NFκB-dependent pathways [89].

Inflammatory mediators, including nerve growth factor (NGF), bradykinin, prostanoids (e.g., prostaglandin E2), and ATP released from injured cells, along with cytokines like IL1β, IL6, TNFα, and interferon γ (IFNγ), as well as reactive oxygen species, can significantly modulate the expression and function of various Cl^- transporters and channels. These mediators influence cellular processes by altering ion flux and transporter activity, as suggested by studies on different cellular environments [90-93]. One example is NKCC1, whose expression and function are particularly sensitive to inflammatory mediators. Indeed, NKCC1 modulation has been observed in a wide range of cell types, including sensory neurons [94-97], where inflammation affects pain signaling and sensitivity [98]. Additionally, this cotransporter plays a critical role in non-neuronal cells, such as colonocytes and intestinal cells [95, 99-103], where it contributes to gut inflammatory conditions [104]. Beyond these roles, NKCC1 activity is modulated by inflammatory mediators in synoviocytes, the cells that line the joints, contributing to inflammatory joint diseases like rheumatoid arthritis [105]. It also impacts microglia [83, 106], the immune cells of the brain, linking NKCC1 to neuroinflammatory processes [98]. Other affected cell types include endothelial cells [107], which regulate blood vessel function during inflammation and choroid plexus epithelial cells [76, 108], which play a key role in cerebrospinal fluid production and brain homeostasis. Overall, the widespread impact of NKCC1 modulation by inflammatory mediators across these diverse cell types underscores its importance in both neural and systemic inflammatory responses.

Reactive oxygen species have also been reported to modulate thiazide- and loop diuretic-sensitive transporters. For instance, H_2O_2 [95, 100], superoxide [109], nitric oxide (NO) and NO-related species [110] can modulate NKCC1 and NKCC2 expression and function [111-115] as well as that of NCC [79]. NO also inhibited Cl^- transport in renal tubular cells by mechanisms likely related, at least in part, to NKCC2 [79, 112, 116, 117], and regulated expression levels of KCC1, KCC3 and KCC4 in primary rat vascular smooth muscle cells in a protein kinase G-dependent manner [118-121]. Therefore, sulfonamide diuretics may have clinically significant effects beyond diuresis i.e., by modulating both the transport and/or non-transport functions of their known targets expressed outside tubular cells, as well as that of different targets unrelated to the SLC12A family. In turn, SLC12A members can respond to various inflammatory stimuli. This response can be characterized either by increased or decreased expression and/or function, thereby impacting the local effect of sulfonamide diuretics as well as cell volume, ion composition and osmolality in a wide range of cells and pathophysiological conditions. Although, the reverse relationship i.e., the role that SLC12A family members may play in modulating local or systemic inflammatory responses remains a gap in our knowledge, several reports have recently suggested a potential direct causal relationship between NKCC1, NCC, high Na^+ and hyperosmolality in a variety of inflammatory responses [122-125] mediated by local cells, T cells or macrophages [71, 87, 126-132]. Therefore, it is plausible that osmotically sensitive SLC12A members may play a direct role in the modulation of local and systemic inflammatory processes.

In this review, we will examine the current evidence linking the use of sulfonamide diuretics to anti-inflammatory and/or pro-inflammatory responses in diverse clinical settings. Additionally, we will explore potential mechanisms involving SLC12A members in regulating these responses, particularly in the context of chronic inflammatory processes associated with hypertension and MetS.

Anti/inflammatory responses to sulfonamide diuretics: the evidence

Thiazides

The potential anti-inflammatory effects of thiazide diuretics (or any medication with anti-hypertensive effect) are closely intertwined with their well-established anti-hypertensive properties, making it challenging to distinguish between the two. Hypertension is strongly linked to chronic inflammatory processes [133] and often coexists with obesity, insulin resistance, dysglycemia and dyslipidemia i.e., the components of MetS [134-137]. These conditions are in turn associated with increased tissue inflammation [12, 138] and elevated levels of inflammatory markers, including TNFα, C-reactive protein (CRP), IL1β, IL6, and IL8 [139]. As a result, it has been suggested that hydrochlorothiazide treatment may modulate inflammatory responses in hypertensive individuals [140], particularly those with components of MetS [141] or T2D [142]. However, while hydrochlorothiazide reduces hypertension, its chronic use may also exacerbate insulin resistance, glucose intolerance, ectopic fat deposition and dyslipidemia [143]. These undesired "side effects" could contribute to the progression of inflammatory-related metabolic abnormalities and increase the risk of developing T2D. Thus, while thiazides may effectively normalize hypertension, the benefits of lowering blood pressure might be counterbalanced, to some extent, by negative effects on metabolic health and inflammation irrespective of a potentially direct anti-inflammatory effect of the drug.

Chronic inflammatory processes are typically assessed by measuring one or few biomarkers including the classic acute-phase reactants [e.g., CRP and serum amyloid A (SAA)], cytokine production (e.g., TNFα, IFNγ, IL1β, IL6, IL8, IL10 and IL12 and their receptors), altered macrophage function and several biomarkers of vascular dysfunction [12, 144-148]. Notably, a comparative trial of hydrochlorothiazide and other anti-hypertensive medications resulted in no detectable CRP changes in patients with T2D [142], in hypertensive patients

with inflammation [149], or in patients with mild/moderate hypertension [150]. In addition, short-term use of hydrochlorothiazide was not associated with anti-inflammatory benefits in newly diagnosed hypertensive patients [141]. Therefore, normalizing blood pressure with hydrochlorothiazide alone may have limited anti-inflammatory effects, as reflected in markers of low-grade inflammation such as SAA, CRP [136, 151, 152], or arterial stiffness [153]. Nevertheless, when used alone or in combination with other antihypertensive medications, some trials did report a general anti-inflammatory effect, suggested by reductions in CRP levels, but not in other biomarkers of inflammation, in hypertensive patients with MetS, either with [142] or without T2D [140, 154, 155]. Hence, the perceived extent of hydrochlorothiazide's anti-inflammatory actions in the clinical setting remains uncertain as it appears to depend on the specific inflammatory markers being examined, background inflammation and the metabolic status of the individuals being tested.

Thiazide-like diuretics

Unlike hydrochlorothiazide, thiazide-like drugs such as chlorthalidone and indapamide have not been clearly associated (yet) with worsened metabolic parameters or an increased risk of T2D in the management of hypertension [143]. On the contrary, these drugs, either used alone or in combination with other treatments, have revealed an overall beneficial anti-inflammatory effect, including reductions in CRP levels among patients with refractory [156] or mild hypertension [140]. This has been supported by studies showing improvements in various markers of oxidative and inflammatory tissue damage in hypertensive rats, with or without T2D [157-160]. Consequently, normalizing blood pressure with these diuretics may reduce inflammation and oxidative stress independently of the metabolic status or background inflammation. However, it should be noted that chlorthalidone has also been linked to elevated plasma CRP levels and impaired endothelial function in poorly controlled hypertensive patients with heart failure [161]. Therefore, despite the apparently positive overall anti-inflammatory effects, there may be specific physiopathological circumstances in which these drugs could contribute to negative inflammatory outcomes.

Loop-diuretics: Furosemide and Bumetanide

There are clinical studies that have indirectly examined the anti-inflammatory effects of these diuretics in humans with chronic inflammatory conditions. For instance, inflammatory bronchoconstriction associated with asthma was shown to be alleviated by inhaled furosemide in multiple clinical trials by mechanisms assumed to be locally anti-inflammatory [162-173]. In the case of bumetanide, the evidence suggesting a potential anti-inflammatory action comparable to that attributed for furosemide in humans or in preclinical models is scarce. However, bumetanide did reduce the inflammatory and phagocytic responses of human macrophage cell lines in response to lipopolysaccharide (LPS) in vitro and inhaled nebulized bumetanide rapidly attenuated LPS-induced acute tissue inflammation and lung injury in mice [174, 175]. Importantly, systemic bumetanide or inhibition of WNKs also delayed macrophage-mediated inflammatory resolution of LPS-induced lung injury in mice [87], suggesting that the diuretic may have the potential to promote or sustain chronic local inflammatory responses, despite its initial acute anti-inflammatory effects.

Anti/inflammatory responses to diuretics: the mechanisms

Hydrochlorothiazide and thiazide-like diuretics

Contrary to the general perception, the anti-hypertensive effects of these two classes of diuretics are far more complex than commonly assumed, involving multiple mechanisms that remain largely mysterious and surprisingly unrelated to their well-known renal targets

or diuretic action [176, 177]. Far less known are the molecular pathways by which thiazides and thiazide-like diuretics might directly influence inflammatory responses in hypertensive patients. In this regard, it has been proposed that, in most circumstances (see [161]), these diuretics may improve endothelial function and reduce local levels of inflammatory markers independent of the effect of blood pressure [178]. Conversely, better control of blood pressure and subsequent reduction in cardiovascular risk could counteract some background inflammatory processes in hypertensive individuals with MetS, T2D or other underlying low-grade chronic inflammatory conditions. Similarly, while hydrochlorothiazide can protect the kidneys by lowering blood pressure, it can also cause general electrolyte imbalances and hyperuricemia [179], or even local renal damage that might lead to local or disseminated inflammatory responses.

In fact, high, likely toxic concentrations of hydrochlorothiazide have been linked to renal tubular apoptosis, peritubular inflammation and renal interstitial macrophage recruitment in preclinical models [180, 181]. Moreover, similar to the chronic effect that bumetanide has on the distribution of NKCC1 in cultured cells [182], or that of furosemide on tubular NKCC2 [183], treatment with thiazides resulted in the formation of autophagosomes and redistribution of NCC from the apical plasma membrane to all over the tubular cells [181] likely precluding expected pharmacological responses. In turn, renal tubular cells, along with macrophages [88] can release IL1β in response to high glucose levels to promote and sustain local inflammation in the kidneys of obese and diabetic animal models [184] thus adding an extra layer of complexity to the inflammatory response. Lower doses of hydrochlorothiazide, however, have demonstrated an apparent beneficial anti-inflammatory effect in the kidneys of aldosterone-induced hypertensive rats [185]. Additionally, these lower doses have been shown to prevent T-cell accumulation in the kidneys and aortas of humanized mouse models of hypertension [186] and to reduce T-cell infiltration, local inflammation and arterial stiffening in the aortas of angiotensin-II-induced hypertensive mice [187]. Therefore, while high doses of hydrochlorothiazide may contribute to renal inflammation and tissue damage, lower doses may exert protective anti-inflammatory effects in hypertensive animal models without T2D or MetS, highlighting a contextual and potential dose-dependent impact of these class of diuretics on renal inflammation.

Along these lines, thiazides were also shown to reduce renal macrophage infiltration and slow renal disease progression [188]. However, hydrochlorothiazide did not affect local in vivo or in vitro levels of TNFα [189, 190] or IL1β production from neutrophils [191]. Instead, the diuretic inhibited T-cell accumulation in tissues, particularly in the thoracic lymph nodes, aorta and kidneys, in both animal models and hypertensive patients [186, 192]. Although hydrochlorothiazide (and chlorthalidone) lowered blood pressure, left ventricular hypertrophy and proteinuria, they did not impact reactive oxygen intermediates or the expression/release of chemo-attractants in blood vessels [193]. Similarly, bendroflumethiazide treatment had no effect on renal TNFα, IL6 and TGFβ1 levels in mice [194]. Notably, hydrochlorothiazide reduced IL17A, which is involved in small artery remodeling and associated to hypertension in mice [195]. Additionally, indapamide reduced oxidative stress and inflammation in the renal cortex by decreasing NFκB activation and TGFβ1 expression [196]. At any rate, it remains challenging to determine whether these effects are primarily due to the blood pressure-lowering properties of these diuretics or a direct influence on local cells, such as T-cells and the release of their associated cytokines, which are known contributors to local inflammation [197-199].

Interestingly, the finding that macrophages, vascular smooth muscle cells and endothelial cells express a thiazide-sensitive NCC [71] suggests a potential site for hydrochlorothiazide to directly modulate local inflammatory responses under physiopathological conditions. Supporting this, NCC expression in these cells is upregulated in response to pro-inflammatory cytokines such as TNFα, IL1β and IL18. Along these lines, it has been shown that IL18 and IL1β production by NCC-expressing tubular epithelial cells contribute to hypertension, renal inflammation, fibrosis and macrophage recruitment in hypertensive and diabetic animal models [184, 200]. Moreover, macrophage and/or tubular NCC may act as a receptor for

IL18, potentially contributing to the modulation of local inflammatory responses [71]. Therefore, while the hypotensive effects of hydrochlorothiazide may play a role in reducing inflammation, there is growing evidence that this diuretic may also have direct effects on vascular and inflammatory cells, particularly through NCC modulation, which could influence local inflammation independently of its blood pressure-lowering action [201].

In addition, by lowering blood pressure, hydrochlorothiazide may indirectly reduce the stress on blood vessel walls, potentially mitigating vascular inflammation over time, while exerting direct effects in vascular cells and local inflammatory cells [71, 202]. These vasodilatory and local anti/inflammatory actions of hydrochlorothiazide may be mediated by mechanisms that have historically been overlooked. These include actions through carbonic anhydrases [48, 203, 204], large-conductance K^+ channels [205, 206], $Ca2^+$-activated K^+ channels [207-209], K^+ channels [210] and other uncharacterized mechanisms, some of them potentially related to NCC [211]. Specifically, there are at least sixteen known variants of carbonic anhydrases, which are widely expressed, sensitive to sulfonamide diuretics and involved in various inflammatory processes [212-219]. Therefore, it is plausible that some of the anti/inflammatory effects of hydrochlorothiazide are mediated by these enzymes and ion channels. Clearly, this concept could be extended to thiazide-like drugs and other sulfonamide diuretics as well, thus suggesting a broader mechanism of action for this class of drugs [196, 220-223].

Furosemide and bumetanide

The mechanisms by which aerosolized furosemide exerts acute anti-inflammatory effects *in vivo* [163-172] are not well understood. Some studies have shown that inhaled furosemide reduces local levels of pro-inflammatory cytokines, such as IL6, IL8 and TNFα, in both patients and animal models with respiratory and inflammatory conditions [167, 224-226]. This suggests that furosemide may have direct or indirect anti-inflammatory properties. Along these lines, furosemide has been proposed to reduce contact hypersensitivity and modulate immune responses mediated by rodent macrophages and B-cells *ex vivo* [227, 228]. However, these effects could be partially attributed to the secondary normalization of blood pressure, as other non-diuretic antihypertensive drugs have also been shown to reduce macrophage activation in animal models of hypertension [229, 230]. Nevertheless, consistent with the observation that high concentrations of furosemide can suppress macrophage activation [174, 231] and inhibit migration of primary neutrophils *in vitro* [232], the diuretic has been reported to reduce LPS-induced pro-inflammatory cytokines, such as IL6 and TNFα in macrophage-like cell lines leading to general anti-inflammatory phenotypic changes in these cells [233]. In addition, high concentrations of furosemide reduced LPS-stimulated production of TNFα, IL6 and IL8 from blood mononuclear cells to levels comparable to that found with equimolar concentrations of hydrocortisone [234, 235]. However, it is important to mention that low concentrations of sulfonamides, including furosemide, failed to modulate cytokine production from macrophage-like cell lines in response to LPS, at least in the short term [236].

Together, these data lend support to the hypothesis that NKCCs (and/or KCCs) are involved, at least in part, in the modulation of the anti-inflammatory responses attributed to loop diuretics [237]. Specifically, the loss of NKCC1 has been shown to protect mice from acute lung inflammation, edema and injury caused by bacterial infections. This protection appears to result from impaired function of alveolar, lung epithelial, or endothelial cells, rather than from an effect on local inflammatory cells of hematopoietic origin [175, 238]. In addition, the inhibition of NKCCs using relatively low concentrations of bumetanide has been found to reduce acute lung inflammation in *ex vivo* experiments, possibly through the suppression of epithelial NFκB-dependent local production of TNFα [239]. However, in the longer term, systemic administration of bumetanide to mice after LPS-induced lung injury, at a time when inflammation is typically expected to have resolved, led to increased levels of IL1β, IL1α, IL6 and TNFα in bronchoalveolar fluid, by mechanisms associated with impaired

WNK1-OSR1/SPAK-NKCC1 signaling, which control phagocyte function and the local anti-inflammatory response [87], thus suggesting that the diuretic may actually prolong the inflammatory state.

Interestingly, TNFα is known to increase the expression of OSR/SPAK, a substrate of WNK kinases and a major regulator of NKCCs and KCCs [74], in an NFκB-dependent manner [240]. Moreover, NKCC1 deficiency leads to increased efferocytosis i.e., the process whereby apoptotic cells are cleared by phagocytes, whereas that of KCC1 reduces it [87]; and efferocytosis increase the expression of multiple genes including NKCC1, KCC1 and some of their upstream kinases [241]. Further, activation of NFκB promotes osmotic stress and cell swelling [242], which then activates WNK kinases [74]. Furthermore, the WNK-OSR/SPAK-NKCCs signaling pathway has been shown to play a crucial role in alveolar fluid clearance, mitigating inflammatory lung injury and edema in animal models [243]. Along these lines, the WNK4-OSR/SPAK-NKCC1 pathway was recently shown to modulate primary macrophage activation and reduce LPS-induced lung inflammation and injury in mice [81]. Therefore, collectively these findings suggest that bumetanide- and furosemide-sensitive NKCCs and KCCs modulate local inflammatory responses through a complex regulatory network involving osmotically- and K^+/Cl^--sensitive WNKs, NFκB-driven cytokine production, macrophage activation and neutrophil migration. Importantly, monocytes and T cells also express NKCC1 and other transporters of the SLC12A family [241, 244-248], whereas NKCC1, KCC3 (as well as Cl^-) have been implicated in neutrophil phagocytic activation [249-252]. Therefore, these data imply that loop-diuretics may play a direct role in the immunoinflammatory processes mediated by phagocytes at multiple levels.

Emerging inflammatory mechanisms: old concepts meet new ones

Several members of the SLC12A family of Cl^- loaders and extruders directly participate in the regulation of the intracellular Cl^- concentration ($[Cl^-]i$), which in most cells is kept above thermodynamic equilibrium making possible its electrogenic exit via Cl^- channels [253]. In fact, Cl^- ions are now recognized to play a significant role in cellular signaling, acting as an effector or even a second messenger in widely diverse biological processes (reviewed in [254]). Indeed, beyond its known influence on cell volume regulation, Cl^- ions impact the membrane potential and hormone secretion [255] as well as the balance of reactive oxygen species and the pH levels both inside and outside of the cell [256]. In addition, Cl^- plays a pivotal role in modulating the function of several key organelles, including endosomes, phagosomes and lysosomes [257, 258]. Further, fluctuations in $[Cl^-]i$ have been associated with a wide array of cellular functions including: i) regulation of gene expression [259, 260], such as that of IL1β [261]; ii) protein synthesis and/or function, including that of the transcription factor RUNX1 [262, 263], myeloperoxidase [264], the transient receptor potential melastatin 7 (TRPM7) [265] or the mechanistic target of rapamycin, complexes 1 and 2 (mTORC1-C2) [266]; iii) post-translational modifications, including that of WNK1, WNK4 and OSR/SPAK [75, 267, 268]; and iv) cell cycle progression, proliferation and differentiation [259, 266, 269, 270]. Importantly, inflammatory responses are initiated by local macrophages and relayed to other innate immune cells, requiring the coordinated activity of multiple mechanisms, including those previously mentioned [269, 271-274]. Given this complexity, it is not surprising that the SLC12A family of Cl^- symporters may play a direct role in modulating local inflammatory responses.

In fact, recent evidence suggests that both Cl^- and K^+ contribute to the regulation of inflammasomes (reviewed in [275]) i.e., multi-protein complexes that act as sensors or receptors within the innate immune system [276-280]. Inflammasomes are crucial in various inflammatory conditions, including MetS and T2D [281-283]. A key function of inflammasomes is the activation of caspase-1, an enzyme that cleaves pro-inflammatory cytokines, such as IL1β and IL18, into their active forms. This activation triggers inflammatory responses aimed to combat infections and respond to host-derived damaged

or misfolded proteins (i.e., irritatio). This response often leads to a form of inflammatory cell death known as pyroptosis, which serves to perpetuate inflammation until phagocytes can remove the irritants through efferocytosis [284-286]. Interestingly, activation of NKCC1 via K^+/Cl^--sensitive WNK-OSR1/SPAK signaling has been shown to delay the resolution of inflammation driven by the innate immune response [287]. This delay impairs the normal function of innate immune cells (i.e., functio laesa) which further contributes to prolonged inflammation.

Conclusion

Current evidence, although still limited, may suggest that members of the SLC12A family of Cl^- loaders and extruders may play direct and/or indirect roles in inflammatory processes by regulating local cell volume and ion homeostasis, which in turn is important for the functional regulation of inflammatory cells. This regulation also affects local cellular functions and responses including cytokine production, immune cell activation and phagocytic function. Specifically, NKCC1 and NCC have been linked to inflammation through their involvement in promoting local pro-inflammatory responses in endothelial cells and in cells of the innate immunity in different tissues. Therefore, under different physiopathological circumstances, pharmacological modulation of these cotransporters may help either mitigate inflammation, promote or sustain it, highlighting their potential role in modulating immune and inflammatory processes. Nevertheless, in the context of hypertension, MetS and T2D, untangling the potential anti- or pro-inflammatory effects of thiazide, thiazide-like and loop diuretics remains inherently complicated by the intricate relationships that exist between blood pressure, obesity, glucose intolerance, insulin resistance and chronic low-grade tissue inflammation. At the cellular and molecular levels, expanding our understanding of Cl^- ion signaling is essential for unraveling the molecular and metabolic alterations observed in inflammatory conditions where Cl^- transport is disrupted, as well as for better comprehending normal physiological responses such as inflammation.

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Author Contributions

The Author participated in the conceptualization, acquisition, analysis and interpretation of published data, drafted all versions of the manuscript and critically review the last one for intellectual content. The Author approved the final version of the manuscript for publication of its content.

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The author has no ethical conflicts to disclose.

AI Disclosure Staement

AI tools have not been used to create scientific content in this work. AI (ChatGPT) was only casually employed to corroborate standard English grammar and spelling; and in few instances, to rephrase some sentences for lexical diversity.

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