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Sulforaphane and its methylcarbonyl analogs inhibit the LPS-stimulated inflammatory response in human monocytes through modulating cytokine production, suppressing chemotactic migration and phagocytosis in a NF-κB- and MAPK-dependent manner

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ABSTRACT

Sulforaphane [SF; 1-isothiocyanato-4-(methylsulfinyl)-butane], an aliphatic isothiocyanate (ITC) naturally derived from cruciferous vegetables and largely known for its chemopreventive potential also appears to possess anti-inflammatory potential. In this study, structural analogs of SF {compound **1** [1-isothiocyanato-4-(methylcarbonyl)-butane] and **2** [1-isothiocyanato-3-(methylcarbonyl)-propane]} containing a carbonyl group in place of the sulfinyl group in SF, were evaluated for their anti-inflammatory activities. In RAW 264.7 cells, the ITCs at non-toxic concentrations caused an inhibition of NO and prostaglandin E_2 (PGE_2) release through suppressing expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), as well as a reduction in matrix metalloproteinase-9 (MMP-9) expression, secretion and gelatinolytic activity. Further work performed on human monocytes isolated from blood of healthy donors revealed that the ITCs not only suppressed the expression and release of pro-inflammatory mediators IL-1β, IL-6, TNF-α and MMP-9, but also suppressed their antibody-independent phagocytic and chemotactic migratory abilities. These anti-inflammatory activities were mediated through suppression of the NF-κB and MAPK signaling pathways. In addition, the ITCs were revealed to interact with the cysteines in inhibitor of nuclear factor-κB kinase β subunit (IKKβ), which could contribute at least partly to the suppression of NF-κB signaling. In conclusion, results obtained in this study provide deeper insights into the anti-inflammatory properties of SF and its methylcarbonyl analogs and the underlying mechanisms. These compounds thus serve as promising candidates for clinical applications in controlling inflammatory conditions.

1. Introduction

Isothiocyanates (ITCs) are organosulfur compounds that are derived from glucosinolates present in cruciferous vegetables such as broccoli, cabbage, cauliflower, watercress and Brussels sprouts. Upon enzymatic hydrolysis of the various glucosinolate precursors by myrosinase in the crucifers or gut microflora, the respective ITCs bearing a common electrophilic –N═C═S moiety extending from an aromatic or aliphatic side chain are formed [1]. Epidemiological studies have demonstrated that higher consumption of cruciferous vegetables is associated with a reduced risk of cancer [2,3]. Indeed, the health benefits of consuming cruciferous vegetables have been attributed to a variety of micronutrients and phytochemicals including the ITCs; these constituents are reported to induce detoxifying enzymes, scavenge free radicals, alleviate inflammation, stimulate immune functions, inhibit malignant transformation, and regulate the growth of cancer cells [4]. There is therefore intense interest to examine the potential of ITCs as drug candidates. Among the naturally occurring ITCs, sulforaphane (SF), an aliphatic ITC derived from glucoraphanin in broccoli, has been most extensively studied for its chemopreventive potential. Importantly, the anti-inflammatory effects of SF and other studied ITCs have been found to be at least partly involved in mediating their chemopreventive potential [5,6].

Abbreviations: COX-2, cyclooxygenase-2; ECM, extracellular matrix; iNOS, inducible nitric oxide synthase; IκBα, inhibitor of nuclear factor-kappaB α subunit; IKK, IκB kinase; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappaB; PGE_2, prostaglandin-E_2; SDF-1α, stromal cell-derived factor-1α.

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Inflammation is a defense-intended immune response. While acute inflammation occurs transiently and resolves timely, chronic inflammation is persistent and poses an unintended threat. The latter is a dysregulated continuum of a multitude of events that include persistent infiltration of monocytes and macrophages at the site of inflammation and release of inflammatory mediators. Chronic inflammation has been implicated in several chronic diseases including auto-immune, cardiovascular, metabolic and neurological disorders, as well as certain cancers [7]. Current strategies employed to attenuate chronic inflammation are based on the use of conventional non-steroidal anti-inflammatory drugs (NSAIDs) or inhibitors of specific targets such as COX-2. However, these approaches provide only temporary symptomatic relief. Moreover, their usage has been associated with cardiovascular and gastrointestinal adverse effects [8–13]. Hence, there is an increasing need for alternatives. Given that inflammation results from the activation of several interconnected signaling cascades, employing agents that can intervene multiple steps in the chronic inflammatory response is deemed as a feasible anti-inflammatory strategy. ITCs, known to exert anti-inflammatory effects through targeting several signaling pathways, are thus promising alternatives. For instance, SF has been found to suppress the expression of inflammatory mediators through inhibition of the NF-κB [14] and MAPK pathways [15]. Broccoli sprout preparations rich in glucoraphanin have been demonstrated to reduce inflammation in the cardiovascular system and kidneys of spontaneously hypertensive stroke-prone rat [16]. Topical treatment with SF or SF-rich broccoli sprout extracts has also been reported in another study to protect against UVR-induced inflammation and edema in SKH-1 hairless mice [17]. The anti-inflammatory abilities of SF and other dietary ITCs have also been subsequently demonstrated in mouse or human skin by several research groups [15,18–20]. However, the inhibitory effects of ITCs on the inflammatory response in activated monocytes isolated from healthy human subjects are not well studied. Studies elucidating the NF-κB and MAPK pathways as molecular targets in these cells are also lacking.

In this present study, analogs of SF that contain a carbonyl group in place of the sulfinyl group in SF, namely compound **1** [1-isothiocyanato-4-(methylcarbonyl)-butane] and **2** [1-isothiocyanato-3-(methylcarbonyl)-propane], had been synthesized and evaluated for their anti-inflammatory activities in LPS-activated mouse macrophages and human monocytes. In relation to prototypical SF, compound **1** containing the same 4-carbon spacer as SF appeared to possess comparable suppressive effects on the LPS-stimulated expression of pro-inflammatory mediators including iNOS, COX-2, IL-1β, IL-6, TNF-α and MMP-9. Compound **2** containing a shorter 3-carbon spacer was marginally less efficacious. The migratory and phagocytic abilities of activated human monocytes were also inhibited by the compounds. These inhibitory effects were mediated at least in part through suppression of the activated NF-κB and MAPK signaling pathways. Importantly, the ITCs were revealed to modify cysteine residues in IKKβ, a crucial kinase component of the NF-κB pathway that triggers immune responses. In summary, this study provides further insight into the mechanisms underlying the anti-inflammatory effects of ITCs and the potential use of SF and its analogs against inflammatory diseases.

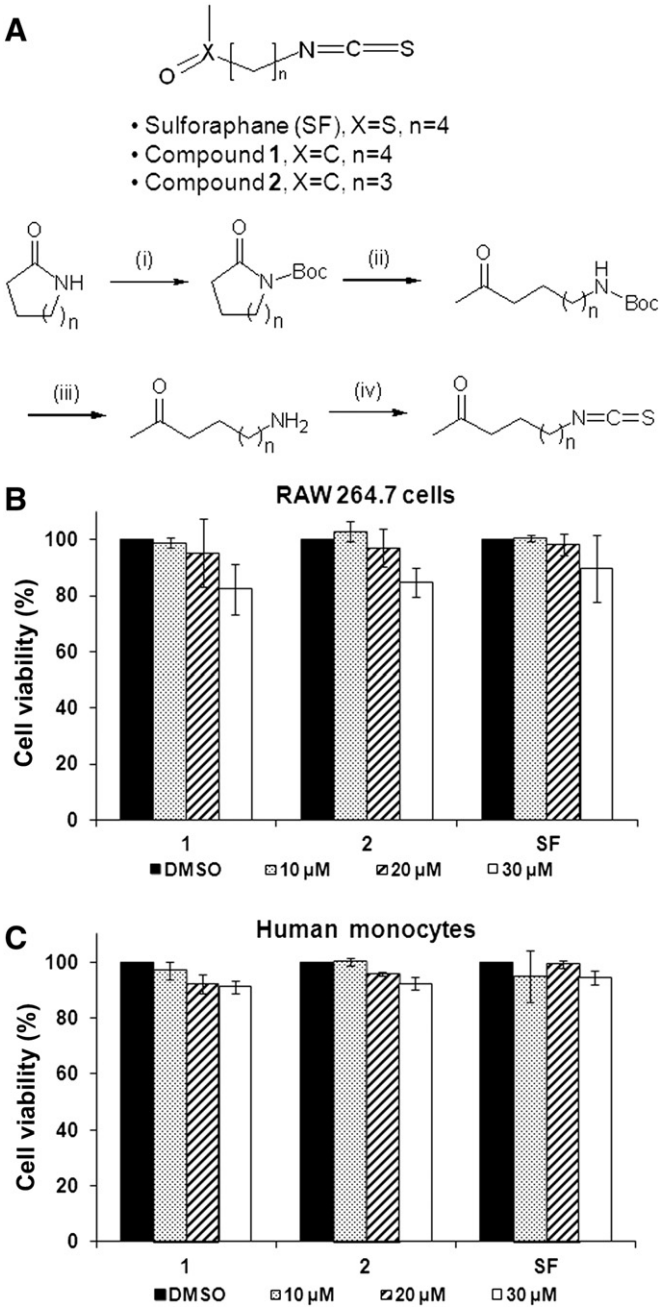

Fig. 1. Effects of ITC analogs on the viability of RAW 264.7 cells and human monocytes. (A) Structures and synthesis of ITC analogs. Reagents and conditions: i) DMAP, acetonitrile, Boc_2O, RT, 4 h; ii) MeMgI, THF, −78 °C, 3 h; iii) 37% HCl, RT, 30 min; iv) $CSCl_2$, 5% NaOH, RT, 30 min. (B) Viability of RAW 264.7 cells following ITC treatment at indicated concentrations for 24 h was assessed by MTT cell viability assay. (C) Viability of human monocytes following ITC treatment at indicated concentrations for 24 h was assessed by Trypan blue exclusion assay. Data are expressed as % viability to that of untreated controls.

2. Materials and methods

2.1. Chemicals and reagents

To prepare ITC analogs **1** and **2**, the respective starting material 2-piperidone and 2-pyrrolidone was reacted with 4-dimethylaminopyridine and di-tert-butyl dicarbonate (Boc_2O) in acetonitrile for 4 h at room temperature. The resulting product was stirred in the presence of methyl magnesium iodide and tetrahydrofuran for 3 h at −78 °C, followed by hydrolysis of the formed product with 37% HCl at room temperature for 30 min to form the primary amines. The primary amines were next reacted with thiophosgene in 5% NaOH for 30 min at room temperature to yield ITC analogs **1** and **2**. The structures of compound **1** and **2** and their synthetic route are shown in Fig. 1A. The compounds were characterized by proton and carbon nuclear magnetic resonance (NMR) and infrared (IR) and the purities as determined by reverse-phase HPLC were found to be at least 95%. Compound **1** was purified as a viscous oil (yield 15.6%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.68–1.72 (m, 4H), 2.16 (s, 3H), 2.47–2.52 (t, 2H, J = 6 Hz), 3.51–3.55 (t, 2H, J = 6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 207.9, 44.9, 42.5, 29.9, 29.3, 20.6. IR (neat, cm^{-1}): 2947 (sp^3 C–H), 2094 (N═C═S), 1712 (C═O). Compound **2** was purified as a viscous oil (yield 19.4%: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.91–