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Oxidant production by heme peroxidases (such as myeloperoxidase [MPO], lactoperoxidase, eosinophil peroxidase and peroxidasin) impacts host defense and macromolecular structure and has the potential to cause tissue damage. Marked increases in phagocyte MPO, capable of producing highly reactive hypochlorous acid (HOCl), have been noted in multiple chronic inflammatory diseases. However, measuring MPO-derived oxidants at the site of production in vivo is technically challenging. The rhodamine-based probe R19S, first described by X. Chen et al (Chem Commun 2011; 47:4373-4375), becomes fluorescent R19 after reaction with HOCl but does not react with superoxide, hydrogen peroxide or several other reactive species. Thus, R19S may be useful to study MPO activity in situ. We sought to characterize the potential responses of R19S to other heme peroxidase-derived oxidants to establish whether a fluorescent response is solely indicative of HOCl or may include others. We analyzed responses of R19S to HOCl (positive control), hydrogen peroxide (negative control), hypobromous acid (HOBr), hypothiocyanous acid (HOSCN), hypoiodous acid (HOI) and taurine chloramine (TauCl) at pH 5.5 and 7.5. A similar study was recently published by A.M. Albrett et al (J Biol Chem 2018; ahead of print). Our results show strong agreement with their study at neutral pH (with a response hierarchy of HOI > HOCl > HOBr > HOSCN), although the overall responses of HOBr and HOSCN relative to HOCl were greater. At pH 5.5, we observed an altered hierarchy, with HOBr and HOI responses diminished (mean of $14.0 \pm 0.5\%$ and $65.1 \pm 1.0\%$ of HOCl, respectively, vs. $83.2 \pm 2.3\%$ and $165.8 \pm 6.4\%$ at pH 7.5), while HOSCN response was increased ($93.3 \pm 5.1\%$ of HOCl, vs. $37.6 \pm 0.2\%$ at pH 7.5). Although TauCl did not cause fluorescence at neutral pH, modest response was detectible at acidic pH ($9.8 \pm 0.2\%$ of HOCl). These data illustrate that the environment of R19S determines its responsiveness to peroxidase-derived oxidants and should be considered when interpreting results.

https://doi.org/10.1016/j.freeradbiomed.2018.10.242

236

Mechanistic insights on the immune and antioxidant response of functionalized cellulose nanocrystals: Does surface charge matter?

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The interest in derivatives of cellulose nanocrystals (CNCs) for multiple biomedical application has been increasing in the recent years. CNCs is a versatile platform that shows variation in size and dispersion depending on the methods of extraction and preparations, which can impact their reactivity in biological systems. In addition, CNCs are suitable to functionalization with an array of polymers, generating chemically related nanomaterials with different morphologies, surface charges and reactivity. This diversity of physicochemical characteristics can lead to a various biological activities and potentially to undesirable effects, including a robust immune response. Previously, we reported that a cationic CNCs derivative, CNCs-poly(AEMA2), evoked substantial immunological response in mouse and human macrophages, by inducing the secretion of the inflammatory cytokine interleukin-1β (IL-1β). This effect was partially associated with mitochondria-derived reactive species (ROS). In this study we sought to understand the mechanistic differences regarding immunological responses evoked by functionalized CNCs and whether surface charges is associated with this effect. We investigated the effect of a cationic, CNCs-poly(AMPA), and an anionic, CNCs-poly(NIPAAm) derivative on the secretion of inflammatory cytokines, mitochondria-derived ROS and mitochondrial function and antioxidant response as well as on endoplasmic reticulum (ER) stress, in human and murine inflammatory cells. CNCs-poly(AMPA) evoked the greatest immunological response in LPS-stimulated murine cell line, while CNCs-poly(NIPAAm) showed a significant NLRP3 inflammasome-dependent and independent immunological response in non-stimulated human primary monocytes. In addition, CNCs-poly(NIPAAm) increased the generation of acidic vesicular organelles and mitochondrial-ROS as well as increases antioxidant enzymes in non-stimulated cells, while CNCs-poly(AMPA) affected mitochondrial function by decreasing the intracellular ATP. These differences on the mitochondrial function and ER stress response suggest a diverse mechanisms associated with their immune activity. Moreover, these effects may be related with the surface charges of the functionalized CNCs, and their likely interactions with biomolecules in the intra and extracellular milieu.

https://doi.org/10.1016/j.freeradbiomed.2018.10.243

237

Cell-based analysis of the immune and antioxidant response of the nanocarrier β-cyclodextrin conjugated with cellulose nanocrystals

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Cellulose nanocrystals (CNCs) have great potential in many areas of research, applications and future commercialization prospects. Recently, CNCs have emerged as attractive candidates for biomedical applications such as drug and gene delivery systems. Cyclodextrins (CD) are cyclic oligosaccharides with the unique ability to form inclusion complexes with drug molecules and as such, they have been commonly employed as materials in nanoparticle-based drug delivery systems. The conjugation of CNCs with β-CD has been previously reported for drug delivery applications but there are no studies on any potential immunological response of the surface modified CNCs with β-CD. The current study examined the potential immune and antioxidant response induced by CNCs grafted with β-cyclodextrin (CNCs-β-CD) in human monocyte cell line (THP-1) and mouse macrophage-like cell line (J774A.1) We analyzed the secretion of the pro-inflammatory cytokine, interleukin 1β (IL-1β), by ELISA and mitochondria-derived reactive oxygen species (ROS) using fluorescence cell imaging as well as examining examined the intracellular levels of proteins involved in the immune and antioxidant response by immunoblotting. Our results indicated neither a dramatic increase in the IL-1β secretion nor in the mitochondria derived ROS. We also observed no changes in the intracellular antioxidant response in THP-1 cells treated with different concentrations of CNCs-β-CD. Overall, CNCs-β-CD is non-immunogenic and does not induce an increased antioxidant response under the conditions tested and hence has the potential to be used as a drug delivery carrier.

https://doi.org/10.1016/j.freeradbiomed.2018.10.244

238

Antioxidants ameliorate hyperoxia-induced lung injury by inhibition of HMGB1