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Research paper

Anti-inflammatory, antibacterial, and cytotoxic activity by natural matrices of nano-iron(hydr)oxide/halloysite

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This manuscript reports on the effects of natural Fe-halloysite matrices on infiltration and migration of neutrophils (polymorphonuclear (PMN) leukocytes), which, after the skin, constitute the primary protection of organisms against pathogens. Speciation of mineral Fe was quantified before and after treatment with citrate-bicarbonate-dithionite (CBD). Infiltration and migration of inflammatory and immune effector cells, and cell viability were quantified using the 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and myeloperoxidase (MPO) enzymatic activity methods, and the Griess assay. Halloysite was collected ~2 km from Opotiki, Bay of Plenty, New Zealand. HRSEM images confirmed typical morphological features proper of spheroidal Hal (S-Hal). Mössbauer spectroscopy of S-Hal confirmed the presence of Fe, octahedrally coordinated in the form of substituted Fe(III), magnetically ordered goethite or ferrihydrite. HRTEM images showed the presence of small-size domains of Fe (~3-nm) predominantly in the form of ferrihydrite. EPR analyses of S-Hal (0–5000 ppm) before and after reacting with desferrioxamine-B confirmed the fast release of Fe from the nanodomains of ferrihydrite. Early inhibition of edema by S-Hal doubled that by CBD treated Hal (*t*-S-Hal), explained because labile Fe (2-*L*-ferrihydrite) enhanced the 4-h anti-inflammatory response. On the other hand, prolonged inhibition of edema by S-Hal and *t*-S-Hal compared, consistent with the release of Fe from the Hal structure. The presence of S-Hal or *t*-S-Hal related to the inhibition of MPO content. After 4 h, the inhibition of MPO content by S-Hal or *t*-S-Hal compared to that by commercial indomethacin (*ca.* 80%). S-Hal or *t*-S-Hal showed high inhibition of MPO contents shortly after exposure, but decreased sharply afterwards. On the other hand, tubular Hal (T-Hal) caused an increasing inhibition of MPO with time, explained because clay structure restricted the kinetics and mechanism of MPO inhibition. Evidenced showed that the release of mineral Fe related to infiltration and migration of inflammatory and immune effector cells, expanding the knowledge that metal ions affect inflammatory responses. Finally, dose–response experiments confirmed that the inhibition of edema and cell viability were surface-mediated. Natural clay reservoirs are complex in composition, therefore identifying the molecular mechanism(s) regulating cell migration and infiltration becomes necessary prior to recommending their use for healing purposes.

1. Introduction

Halloysite (Hal), a clay mineral member of the kaolin group, occurring in the thousands of tons in soil volcanic environments, has been reported to as an effective anti-inflammatory comparable to commercial indomethacin (Cornejo-Garrido et al., 2012; Cervini-Silva et al., 2013a, 2015a) but little information has become available on how chemical composition in Hal may alter immune response(s). In particular, metals

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Table 1
List of acronyms and definitions used in this study.

Term (presented in alphabetical order)	Acronyms	Observations
Edema inhibition	EI	Calculated using Eq. (1)
Edema mass determined for samples exposed to TPA only and TPA plus indomethacin or Hal (S-Hal, *t*-S-Hal, or T-Hal)	A	Defined in Eq. (1)
Edema mass determined for samples exposed to TPA plus indomethacin or Hal (S-Hal, *t*-S-Hal, or T-Hal)	B	Defined in Eq. (1)
Effector cells		A cell made to react by sth outside the body[a]
Hal		Halloysite
Infiltration		Refers to infiltration of inflammatory cells such as neutrophils around blood vessels
Neutrophils	PMN	Polymorphonuclear leukocytes
Specific surface area	σ_s	Units: $m^2\ g^{-1}$
Spheroidal halloysite	S-Hal	–
Spheroidal halloyiste after treatment with citrate-bicarbonate-dithionite (CBD)	*t*-S-Hal	–
Tubular halloysite	T-Hal	–
12-*O*-tetradecanoylphorbol-13-acetate	TPA	TPA method described in Monks et al. (1991)

[a] Phillips et al. (2010).

can affect inflammatory responses. For instance, metal ions (e.g., Cu, Ni, and Zn) stimulated polymorphonuclear (PMN) leukocytes to move slower than reference cells (incubated with *N*-formyl-methionine-leucine-phenylalanine; FMLP), attributed to the average circularity of metal-ion stimulated cells, during a cell track, which was higher than for FMLP-stimulated cells (Hunt et al., 1992). Depending on the coordination environment, metals can act as pro- or anti-inflammatory. For instance, while Ni prompted inflammation (Dirsch et al., 1998) mineral Ni did not (Cervini-Silva et al., 2015b). Natural allophane enriched with Ni inhibited myeloperoxidase contents in up to 60% (Cervini-Silva et al., 2015b).

The role of mineral Fe associated to Hal surfaces on immune responses is subject to current study, provided that mineral Fe plays a crucial role in biogeochemical cycles. Most recently, other authors concluded that mineral Fe(II) is primarily responsible for the anti-bacterial activity of soils (Williams et al., 2011). On the other hand, related studies reporting the effects of nontronites $[(Na_{1.05})[Si_{6.98}Al_{0.95}Fe_{0.07}][Al_{0.36}Fe_{3.61}Mg_{0.04}]O_{20}(OH)_4$, NAu-1, green color, Al-enriched; and $(Na_{0.72})[Si_{7.55}Al_{0.16}Fe_{0.29}][Al_{0.34}Fe_{3.54}Mg_{0.05}]O_{20}(OH)_4$, NAu-2, brown color, Al-poor, contains tetrahedral Fe] from Uley Mine, South Australia, and hectorite $[(Na_{0.80})[Si_{7.90}Al_{0.10}][Mg_{5.30}Li_{0.70}]O_{20}(OH)_4$, SHCa-1, contains calcite; Kibanova et al., 2009] purchased from the Source Clays Repository of the Clay Minerals Society, and matrices of Fe-minerals containing olivine, fosterite, and pyrrhotite (Cervini-Silva et al., 2013b) promoted oxidative stress *via* lipid peroxidation (LP; Supplementary text), initiated because of the surface production of hydroxyl radicals. In addition, minerals containing redox-active elements other than Fe (e.g., Pb) can also induce LP *via* Fenton-like reaction(s) (Cornejo-Garrido et al., 2011). However, contrary to mineral surfaces bearing redox-active elements (Kibanova et al., 2009; Cornejo-Garrido et al., 2011; Cervini-Silva et al., 2013b, 2014), Hal surfaces reportedly inhibited LP (Cornejo-Garrido et al., 2012; Cervini-Silva et al., 2013a, 2015a); corresponding EPR data showed no stabilization of radical intermediates and a lack of antioxidant activity by Hal surfaces (Cornejo-Garrido et al., 2012; Cervini-Silva et al., 2013a).

Natural Hal surfaces present various morphologies, from elongated or short tubes to platy or spheroidal particles (Joussein et al., 2005). In particular, spheroidal Hal, forming after the rapid dissolution of volcanic glass, is highly heterogeneous, owning surface depressions, disks, squat cylinders; a high abundance of discontinuities in layer stacking, between and along packets (Kirkman, 1977), and slit-shaped pores forming as a result of shrinkage of block of layers during formation (Kirkman, 1977; Joussein et al., 2005); varying degrees of crystallinity, with an increasing degree of disorder towards the interior of the particle (Joussein et al., 2005); and lower dehydration rates compared to other forms of Hal, causing a relative invariant cation exchange capacity (Joussein et al., 2005), while retaining positively-charged solutes over negatively-charged ones (Churchman and Theng, 1984).

This paper reports on the effects of mineral Fe on the anti-inflammatory activity of Hal spheroidal and the role of mineral Fe. A list of acronyms is provided in Table 1. Spheroidal halloysite (S-Hal) was collected ~2 km from Opotiki, Bay of Plenty, New Zealand. To assess the role of mineral Fe, S-Hal was treated using the citrate-bicarbonate-dithionite (CBD) method described elsewhere (Mehra and Jckson, 1960; Komadel, 2003; Stucki et al., 1988; Stucki, 2013; Ribeiro et al., 2009; Stucki et al., 2014). Furthermore, the results of immune response testing for spheroidal halloysite before (S-Hal) and after treatment (*t*-S-Hal) were compared against those obtained for highly-pure tubular

Table 2
XRF and Rietveld analysis for spheroidal, *t*-spheroidal, and tubular halloysite (S-Hal, *t*-S-Hal, and T-Hal).

Weight (%)											
Sample	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	MnO	MgO	CaO	Na_2O	K_2O	P_2O_5	LOI	Sum
S-Hal	45.2	0.4	28.8	4.8	0.0	0.2	0.1	0.4	0.1	0.1	20.2	100
t-S-Hal	46.0	0.4	25.8	5.0	0.0	0.1	0.1	1.9	0.0	0.0	20.7	100
T-Hal[a]	45.0	0.15	37.6	1.2	0.01	0.2	0.3	0.1	0.3	0.01	14.5	85.5

Mineralogical composition (%)							
	Halloysite	Amorphous material	Albite	Quartz	Alunite	Anatase	Fe_2O_3
S-Hal[b]	61.0	36.0	1.7	1.4	–[c]	–	–
t-S-Hal[b]	80.8	18.0	1.6	<1	–	–	–
T-Hal[a,d]	95	–	–	–	2.7	0.15	1.21

Surface area ($m^2\ g^{-1}$)	
S-Hal	169
t-S-Hal	132
T-Hal	64.5

[a] ±0.1 S.E. Data taken from Pasbakhsh et al. (2013).
[b] Sum = 100.
[c] – = below detection limits.
[d] Sum = 99.06.