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The anti-inflammatory activity of natural allophane

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ABSTRACT

This paper presents evidence of the novel anti-inflammatory properties of natural allophane collected from New Zealand, Japan, and Ecuador. Allophanes were assessed by (i) the mouse-ear edema method using 12-O-tetradecanoylphorbol-13-acetate (TPA) as inflammatory agent; and (ii) the myeloperoxidase (MPO) enzymatic-activity method. After 4 h, applying 1 mg ear^{-1} allophane conveyed edema inhibition (EI; $p \leq 0.01$) in up to 39%, while MPO content inhibition (CI) values surpassed 60%. Pearson's correlation analysis between EI and MPO data showed that edema was mediated by the migration of neutrophils at $t = 4$ h ($p < 0.05$), but not at $t = 24$ h. The lack of variation in cellular migration with time was explained because of a reaction of zero-order kinetics. EPR spectra for allophanes showing higher anti-inflammatory activity denoted a broad signal centered at $g = 2$, and an intense spin–spin interaction, typical of a low-spin, octahedral Fe^{3+} environment ($S = 1/2$); and overlapping signals typical for Ni, with octahedral coordination, explained either by oxidation states $+1$ (Ni^{1+}), $+3$ (Ni^{3+}), or bulk Ni^{2+} ions.

1. Introduction

Allophanes are poorly crystalline hydrous aluminosilicates, identified as promising catalyst and catalytic carrier, with applications for processing inkjet printing; protecting dyes against ozone, or humectants (Desrousseaux and Poncelet, 2006; Creton et al., 2008); and purifying biodiesel (Sohling et al., 2009), among others. To the authors' knowledge, however, little is known on the effects of allophane on human health. A related study concluded that natural allophane induced lipid peroxidation (LP), the oxidative degradation of lipids in cell membranes (Cervini-Silva et al., 2014). Allophane consists of fine ($3.5 \leq d \leq 5.0$ nm), often hollow, spherical, and short-range order (Van Olphen, 1973; Brigatti et al., 2013), arranged in curved-octahedral and silica-tetrahedral layers, containing varying contents of Al and Si (Wada, 1989). LP by allophane was concluded to relate to Fe associated to surfaces, particularly Fe^{3+} soluble species stemming from surface-bound Fe^{3+} or small-sized Fe^{3+} refractory minerals over structural Fe^{3+} located in tetrahedral or octahedral sites of phyllosilicates or bulk Fe oxides (Cervini-Silva et al., 2014). This paper reports on the anti-inflammatory properties of allophanes, expanding on previous observations for halloysite (Cornejo-Garrido et al., 2012; Cervini-Silva et al., 2013a, 2015). Halloysite presented anti-inflammatory properties comparable to indomethacin. Furthermore, halloysite (1000 ppm) precluded LP ($IC_{50} = 2023$ ppm). Differences in biological reactivity are sought to characterize allophane and halloysite, provided differences in particle unit size, ca. 40 and 6000 Å, and cation exchange capacity at pH = 6, 10 and 5 meq Na^{+}/100 g, correspondingly (Theng et al., 1982). Using minerals to develop alternative technologies for human health care has become a thriving research area (e.g., Robertson, 1996; Viseras and López-Galindo, 1999; Carretero, 2002; Sahai and Schoonen, 2006; Gomes and Pereira-Silva, 2007; Williams et al., 2008; Schulz et al., 2014).

2. Materials and methods

2.1. Sources of allophane

Allophanes were obtained from a soil near Te Kuiti (ca. 42,000 year B.P.; Allo-1; Theng et al., 1982) and the rhyolitic Rotoehu tephra deposit in New Zealand (Allo-2); and Kitakami, Iwate in Japan (Allo-3; Van der Gaast et al., 1985); and Santo Domingo de los Colorados in Ecuador (Allo-E; Kaufhold et al., 2009, 2010a). The later source of

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allophane is of particular interest because it covers an area of >4000 km^2 and >5 m thick (600 m.a.s.l.; Kaufhold et al., 2010a; Cornejo-Garrido et al., 2012).

In all dispersions, the <2-μm fraction was first separated by sedimentation under gravity, and later sodium-saturated and washed-free of excess electrolyte, either by centrifugation or dialysis against distilled water.

2.2. Electron-paramagnetic resonance (EPR)

EPR determinations were conducted with a JEOL JES-TE300 spectrometer, operated at X-Band fashions at 100 kHz modulation frequency, and a cylindrical cavity in the TE_{011} mode. The external calibration of the magnetic field was conducted using a JEOL ES-FC5 precision Gaussmeter. The acquisition and manipulation of spectra were performed using ES-IPRITS/TE EPR spectra, and recorded as the first derivative. Parameters, including *g* factor values, were also evaluated (Weil et al., 1994).

EPR determinations were acquired in 10 mg of solid sample using a quartz tube at room temperature. For all spectra collection, the spectrometer settings were: center field = 335.0 mT; microwave power = 1 mW; microwave frequency = 9.44 GHz; sweep width = ±250 mT; modulation width = 0.079 mT; time constant = 0.1 s; amplitude = 125; sweep time = 120 s; and accumulation = 1 scan. Signal intensity was determined as a double integral. Then, the obtained data was normalized.

2.3. Anti-inflammatory activity of allophane

Determinations of (i) the mouse-ear edema using 12-O-tetradecanoylphorbol-13-acetate (TPA) as inflammatory agent and (ii) MPO enzymatic activity were conducted as described elsewhere (Cornejo-Garrido et al., 2012; Cervini-Silva et al., 2013a,b, 2014, 2015). The quantification of MPO enzymatic activity is used in inflammation models as an enzymatic marker specific for migration and cellular infiltration (De Young et al., 1989). This is particularly the case for neutrophils bearing high contents of MPO (Bradley et al., 1982).

The enzymatic activity of MPO was determined from right-ear biopsies either 4 or 24 h after exposure to TPA-containing samples. The enzymatic activity of MPO was quantified colorimetrically using a BioTek microplate reader (EL×808) at λ = 450 nm. The activity of MPO was expressed as optical density per biopsy (OD/biopsy). All experiments were conducted in quadruplicates.

3. Results and discussion

3.1. TPA and MPO models

Applying 1 mg indomethacin inhibited the edema production in up to 82% after 4 h, surpassing control groups significantly ($p < 0.01$; Table 1 (top) and Fig. 1). Edema inhibition (%) increased with time. At $t = 4$ and 24 h, EI values for Allo-1, Allo-2, Allo-3, and Allo-E corresponded to 24 ± 3%, 39 ± 6%, 23 ± 3%, and 33 ± 3%; and 40 ± 7%, 39 ± 8%, 49 ± 9%, and 44 ± 8%, respectively. On this light, ΔEI, defined as ΔEI (%) ≡ EI (%, 4 h) − EI (%, 24 h), decreased according to: Allo-3 > Allo-1 > Allo-E ≫ Allo-2. The invariance of ΔEI for Allo-2 with time was consistent with a strict, short-term anti-inflammatory activity.

3.1.1. MPO

The enzymatic activity of MPO was the highest in TPA-containing samples and showed no variation in indomethacin samples with time (Table 1, bottom). Samples Allo-2 (51.1%) and Allo-E (60.3%) showed the highest MPO content inhibition (CI) values. Unlike EI, CI decreased with time, regardless of allophane (Fig. 2).

Furthermore, a Pearson's correlation analysis between EI and MPO data showed that edema was mediated by the migration of neutrophils at 4 h ($p < 0.05$), but not at 24 h. Furthermore, the lack of variation in cellular migration with time was explained because of a reaction of zero-order kinetics.

Table 1
Time-dependent anti-inflammatory activity of allophane as determined by the TPA and MPO models.[a].

TPA model

	$t = 4$ h		$t = 24$ h		ΔEI (%)[c]
	Edema (mg)	EI (%)[b]	Edema (mg)	EI (%)	
Control	16.4 ± 0.6	–	15.3 ± 0.9	–	–
Indomethacin	2.9 ± 0.7**	82.4**	3.3 ± 0.5**	78.3**	4.1
Allo-1	12.4 ± 0.4**	24.3**	9.2 ± 1.03**	40.3**	−16
Allo-2	10 ± 1.1**	39.2**	9.3 ± 1.2**	39.2**	−0.05
Allo-3	12.6 ± 0.4**	23**	7.8 ± 1.2**	48.9**	−25.9
Allo-E	11 ± 0.3**	33.2**	8.6 ± 1.3**	43.9**	−10.7

MPO model

	$t = 4$ h		$t = 24$ h		ΔCI (%)[e]
	$OD_{450\ nm}$ biopsy^{-1}	CI (%)[d]	$OD_{450\ nm}$ biopsy^{-1}	CI (%)	
Basal	0.11 ± 0.03	–	0.2 ± 0.06	–	–
Control	0.63 ± 0.09	–	1.1 ± 0.08	–	–
Indomethacin	0.02 ± 0.006**,f	92.5**	0.06 ± 0.02**, *g*	90.7**	1.8
Allo-1	0.48 ± 0.09	24.4	0.99 ± 0.13	11.4	13
Allo-2	0.31 ± 0.07*	51.1*	1.0 ± 0.04	10.4	40.7
Allo-3	0.37 ± 0.08	40.4	0.96 ± 0.11	13.8	26.6
Allo-E	0.25 ± 0.06*	60.3*	0.79 ± 0.02*	29.1*	31.2

[a]Listed data represents the average ($n = 5$) plus or minus the standard error of the mean. Data analysis was conducted using the Dunnett's test. Magnitude values $p \le 0.5$ (*) and $p \le 0.01$ (**) were considered significantly different. [b]Edema inhibition (EI) was expressed in percent (%). [c]ΔEI (%) ≡ EI (%, 4 h) − EI (%, 24 h). [d]MPO content inhibition (CI) was expressed in percent (%). [e]ΔCI (%) ≡ CI (%, 4 h) − CI (%, 24 h). Positive ΔEI or ΔCI values referred to a higher activity at $t = 4$ h than at $t = 24$ h; and negative ΔEI or ΔCI values otherwise. Magnitude values for control samples corresponded to: [f]0.24 ± 0.02 at 4 h and [g]0.6 ± 0.06 at 24 h.

Next, the structure of allophane showing higher CI values was analyzed by EPR. Spectra for samples Allo-2 and Allo-E showed a wide range for *g* values (Figs. 3 and 4). A broad signal centered at $g = 2$, denoted an intense spin–spin interaction, typical of a low-spin, octahedral Fe^{3+} environment ($S = 1/2$), commonly found in iron oxides (Fe_2O_3; Kitagawa, 1973). However, spectra for Allo-2 and Allo-E differed. The former showed a broad, *pseudo*-isotropic signal in the center field, confirming dipolar coupling, along with small shoulders ca. $g = 4.3$ and 3.04. The latter denoted high contents of paramagnetic species (Fig. 4), with a high degree of asymmetry, and strong first-derivative

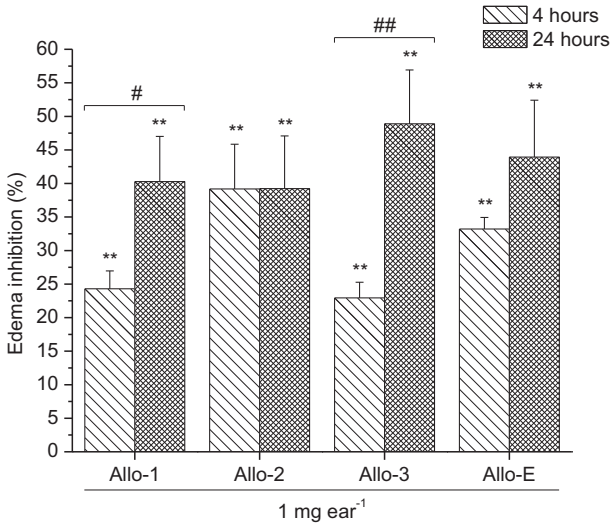

Fig. 1. Edema inhibition by natural allophane.