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Human soft tissue preservation in the Cova des Pas site (Minorca Bronze Age)☆

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ABSTRACT

The preservation process of soft tissues in an archeological context is mainly unknown because they occur only in truly exceptional situations. The Cova des Pas is a Bronze Age site in Minorca where the special conditions enabled the preservation of some soft tissues associated with 66 individuals. This finding allows the study of the preservation process that took place by means of the analysis of the histological and chemical characteristics of the tissues. Our results show that the preservation mechanism was the adipocere, because the fatty acids profile shows higher concentration of saturated than unsaturated fatty acids. The evidence indicates that the kind of funerary ritual and the environmental conditions favored this preservation.

1. Introduction

References to preserved prehistoric human soft tissues in Europe are scarce. Hitherto, the most ancient tissue reported belongs to the Tyrolean Iceman (Tyrolean Ötztal Alps, Italy), that dates back to 5300 B.P. (Seidler et al., 1992) and has been preserved by cold conditions. Two semi-mummified individuals from Galera (Granada, Spain), (Molina et al., 2003), with an antiquity of 3500 years, were found in 1997. Also, in the prehistoric context, the site of Cova des Pas (Minorca, Spain, 3000 B.P.) can be added to this group due to the exceptional recovery of human soft tissues. The funerary complex of Cova des Pas is located in a cave in the Trebalúger ravine (south of Minorca). This is a small cave, approximately 7 m wide and 4.5 m long, located in the cliff wall about 15 m above the ground (Fullola et al., 2008). The collective burial contains a minimum of 66 individuals found in a strongly flexed position (Armentano et al., 2012). The first burials were deposited around 1100 B.C. at the end of the Bronze Age, although the largest number of inhumations took place between 900 and 800 B.C., during the first Iron Age (Van Strydonck et al., 2010). The funerary rite involved a primary inhumation of individuals, which presented maximum flexion of the upper and lower limbs. Furthermore, they were wrapped in shrouds and tied up with ropes. The corpses were deposited in several layers, piled up among previously buried individuals. According to Armentano et al. (2012), the good preservation of soft tissues (intracranial, intrathoracic, abdominal cavities and among others soft tissues) could be related to the overlapping of the corpses, since there were a large number of bodies buried in such a small space.

Soil analysis of the Cova des Pas showed the presence of high amounts of nitrates and sulfates, as well as calcium, iron and aluminum. The salts were associated with the presence of gypsum,

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quartz and sodium nitrate. The ions were the product of weathering of a clay mineral soil and calcite (Van Strydonck et al., 2010). Cabanes and Albert (2011) concluded that the presence of highly soluble minerals, such as sodium nitrate and gypsum, suggests stable dry conditions inside the cave. These minerals would have helped to absorb the humidity, facilitating the natural preservation of the corpses, the phytoliths and the vegetal remains.

Therefore, there are two possible explanations for the preservation of the organic remains in the Cova des Pas: a process of saponification, caused by the compacting and superimposition of individuals, and a process of mummification due to a dry environment.

Adipocere is the soap-like substance that can be formed from the neutral fats of decomposing bodies (Forbes et al., 2005a). Their formation is initiated by intrinsic lipases, which convert the triacylglycerides (TAG) into their corresponding saturated and unsaturated fatty acids, including myristic, palmitic and stearic acids (Liu et al., 2010). Hydroxyl forms are regularly identified in adipocere; however their presence appears to be dependent on the decomposition environment (Bereuter et al., 1996; Forbes et al., 2005b).

Although adipocere is typically regarded as a product of a damp environment, it can be formed in a variety of contexts, including dry environments and corpses in submersion (Quigley, 1998; O'Brien and Kuehner, 2007; Forbes et al., 2011; Ubelaker and Zarenko, 2011).

Nowadays, a "mummy" refers to any naturally or artificially preserved body, or soft tissue, where desiccation has prevented its decomposition (David, 1997). Dry environment, air circulation and elevated temperatures could lead to mummification of human tissue by means of desiccation (Makristathis et al., 2002). Taking into account that water is essential for the enzymatic activity and bacterial growth, as well as for arthropod colonization, dehydration of the tissues is a straightforward way to achieve mummification (Lynnerup, 2007).

The main goal of this work is to test both hypotheses of preservation, and to understand the taphonomical process responsible for the preservation of soft tissue. For this purpose, the characterization of these tissues through their microstructure and their fatty acids profiles was carried out.

2. Materials and methods

Nineteen samples belonging to 9 individuals were analyzed. They were, thus, representative samples of several soft tissues of different individuals located in different zones of the cave. The histological analysis was carried out on 15 samples belonging to these 9 individuals. They included soft tissues from intracranial, intrathoracic and abdominal regions, as well as some other tissues externally adhered to bones, and also bony tissue itself. Eleven samples were subjected to biochemical analysis (fatty acid profile) (Fig. 1) (Table 1).

2.1. Histological processing

Small pieces of each specimen (8–10 mm) were rehydrated in Sandison solution (Sandison, 1955) for 1–2 h. For bony samples, the tissue was decalcified for 1 h in 5% nitric acid prior to rehydration. Samples were then fixed in 10% formalin and immersed in multiple baths of increasing concentrations of ethanol and xylene. The specimens were then embedded in paraffin wax and microsectioned at 3–5 μm. Given the uniqueness and scarcity of samples, the uses of more general and informative stains (Hematoxylin–Eosin and Masson Trichrome) were used first, in order to obtain a comprehensive view of the soft tissue.

2.2. Biochemical analysis

Gas chromatography-mass spectrometry analysis was performed using 70 mg of tissue taken from the different individuals. Lipid extraction was performed following the method of Makristathis et al. (2002). In brief, each sample was mechanically homogenized with a mortar, and lipids were saponified using 8 M sodium hydroxide and methanol (1:1). Afterward, methylation of the fatty acids was performed using 6 M aqueous hydrochloric acid and methanol (54:46), and was then extracted into n-hexane and t-butylethylether (1:1). The organic extract was cleaned up by adding 0.3 M sodium hydroxide. The extracts were subjected to analysis by GC and GC–MS. Experimental conditions for GC were as follows: gas chromatograph Hewlett Packard 6890 series II GC System Agilent Technologies; capillary column HP5- MS 30 m × 0.2 mm × 0.25 mm film thickness; FID detector. Chromatographic volume was 1 μL with an injection temperature of 275 °C. Program temperature was as follows: initial 120 °C 2 min, ramp of 2 °C/min until 220 °C; the second ramp was 10 °C/min until 270 °C, and final ramp was 30 °C/min until 300 °C, which was maintained for 15 min. Total run time: 73 min.

The FA analyzed were the most informative acids in adipocere formation (Takatori, 2001; Dent et al., 2004; Ubelaker and Zarenko, 2011).

2.3. Data analysis

In order to understand the relationship between the fatty acid profiles of the specimens, box-plots were represented and a principal components analysis (PCA) was performed. Ten selected fatty acids from specimens from our study were compared to data from 21 specimens preserved in different environments, and 17 fresh tissue specimens from previous projects (Varmuza et al., 2005) (Table 2). The fatty acid profile of the Cova des Pas (CdP) was also analyzed and compared between tissues. The statistical analyses were performed using Spss 15.0.

3. Results

3.1. Paleohistology

Macroscopically, the soft tissues from the Cova des Pas were brittle and dry, and easily pulverized under soft pressing. The intracranial samples were found adhered to the inner surface of the cranium on the decubitus area. These tissues were shapeless and shrunken, about 5 × 5 cm and *duramater* was not readily identified (Fig. 2). The intrathoracic tissues appeared totally collapsed in the decubitus zone of the costal margin. They formed a homogenous and flattened layer of about 10 × 7 cm and with a low weight (Fig. 3). In contrast, abdominal mass was located in the hypochondriac region. Tissues showed an amorphous shape and a homogenous aspect (6 × 4 cm) with a high density. Finally, the soft tissues attached to bones were distributed among all bony remains. They consisted of thin layers of variable sizes with a bright cover and salt deposits (Fig. 4).

Microscopically, intracranial soft tissue specimens showed the reticular and homogeneous eosinophylic background characteristic of brain tissues, indicating the presence of remnants of cytoplasmic structures (Eklektos et al., 2006). Furthermore, some larger spaces contained concentric structures, reminiscent of vessels inside the cortex. In some instances, abundant round empty spaces (20–40 μm) were observed, suggesting neuronal locations in the brain cortex (see Prats-Muñoz et al., 2012) (Fig. 5).

Some intrathoracic specimens showed typical characteristics of pulmonary parenchyma, since a thin connective tissue layer