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Egyptian versus natural mummification: tracking the differences in loss of tissue antigenicity

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Abstract

Previous reports of difficulties using immunohistochemical methods on mummified tissues attributed the problems mostly to the antiquity of the material. We examined mummified examples of contemporary human tissues (carotid endarterectomy specimens) obtained for research purposes, using 1) desiccation as an example of natural mummification, and 2) natron treatment (Egyptian mummification) followed by desiccation in controlled simulated desert conditions lasting up to 20 months and various reconstitution regimens. The remaining untreated tissues, fixed routinely in formalin and processed to paraffin blocks, served as a control. Additionally, we examined a contemporary human sural nerve bundle mummified in the Egyptian manner by the LIU-UMAB Mummy Project. All tissues were subjected to the same immunohistochemical procedures. There were differences in the degree of antigenicity between matching samples (desiccated only and natron treated) when comparing the same antigens. Thus, the initial mummification procedure has a crucial effect on the preservation of tissue antigenicity.

Introduction

Mummies fascinate the general public as well as scientists. Surprisingly, molecular biology methods were often more successful in assessing mummified human remains than histology and immunohistochemistry. The difficulties in successful usage of immunohistochemical methods were mostly attributed to the antiquity of the material. Immunohistochemistry is a well established method used for decades as a diagnostic tool for assessing tissue samples. It extends the scope of histochemical stains which allow only for recognising the type of tissue but not specific cell or matrix components. It is based on a combination of chemistry and immunology, using specific antibodies to disclose the presence of target antigens in tissues. The role of immunohistochemistry in paleopathology is recognised but there are only few published records of its use (Wick, 1980; Krypczyk, 1986; Fulcheri, 1995, 1999; Hoyle, 1997; Appenzeller, 2000).
The aim of this study was to perform carefully designed controlled experimental mummification of contemporary human tissues and examine the differences in antigenicity resulting from different methods of mummification.

Material and methods

Our material consisted of ten experimentally mummified fragments of human carotid artery endarterectomy specimens (Fig. 1a) and a fragment of the sural nerve (Fig 2a) from a contemporary mummy from LIU-UMAB Mummy project, University of Maryland.
We have an ongoing study using histo- and immunohistochemical methods to assess cellular and matrix changes in atherosclerotic lesions in human carotid endarterectomy specimens removed during surgery. The material is obtained with patients' consent and approved by the local Ethics Committee for scientific investigation. In 10 selected cases fragments of the arterial wall were subjected to experimental mummification (Fig. 1). Tissue was dissected for: 1) mummification in natron (Fig 1c,c'), 2) mummification by desiccation to mimic desert conditions (Fig 1b), 3) control tissue routinely processed to paraffin blocks, and 4) the main bulk of the lesion processed for the assessment of atherosclerotic lesions. Mummification was achieved at 45°C and after 35 days tissue samples were transferred to controlled simulated desert conditions (in a laboratory oven designated for this purpose), kept in desiccated state 18 to 20 months, than rehydrated and processed to paraffin blocks. All samples were subjected to

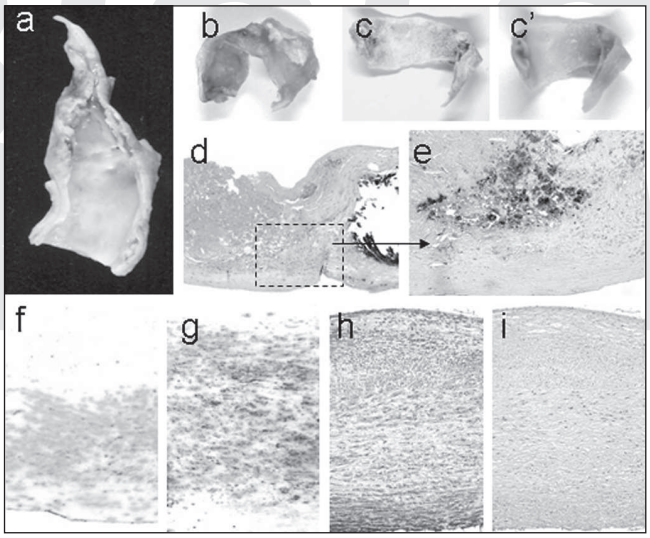

Fig. 1 - Experimental mummification. Contemporary carotid artery (endarterectomy specimen) (a). Two adjacent fragments of the artery (B-d) mummified by desiccation (b) and mummified in natron, (c) and after rehydration (c'). H&E stained cross-section of the naturally mummified artery (d) and stained for macrophage marker CD68 (e). naturally mummified artery stained weakly for a-smooth muscle actin (?-SMCA) (f) and natron mummified artery showing better staining (g). For comparison – control artery stained for ?-SMCA (h) and a section incubated with non-immune IgG only (i).

the same processing regime and the same histo- and immunohistochemical staining procedures.
In 1992, Bob Briers and Ronald S Wade attempted replication of 'state of the art' mummification process of the XVIII dynasty at the University of Maryland. The donor, an elderly male in his late seventies died of massive myocardial infarction. Natron (naturally occurring mixture consisting mostly of sodium carbonate and bicarbonate, used for mummification by ancient Egyptians) was imported for the study from the Wadi Natrun in Egypt and embalming process was replicated according to ancient texts (Herodotus, fifth century B.C.). The procedure was carried out for 35 days (as described by Herodotus) and the body weight loss through desiccation was almost 50%. Internal organs removed from the body were mummified separately and their histological examination and rehydration in Ruffer's solution and staining for several histochemical stains was a topic of a separate publication (Zimmerman, 1998). A detailed description of the embalming procedure was given in an earlier report (Brier, 1997). We have obtained a 5 cm. length of the sural nerve from this mummy for histochemical and immunohistochemical evaluation (Fig 2a).

Tissue processing

Our previous experience with mummified tissue showed that using Ruffer's solution diminished our chances of successful immunohistochemical staining. We therefore modified rehydration and fixation processes and used varied concentrations of buffered Tween 20 or Triton X (Sigma-Aldrich Co., St Louis, MO) instead. The tissues were fixed in 4% paraformaldehyde in phosphate buffer pH 7.4

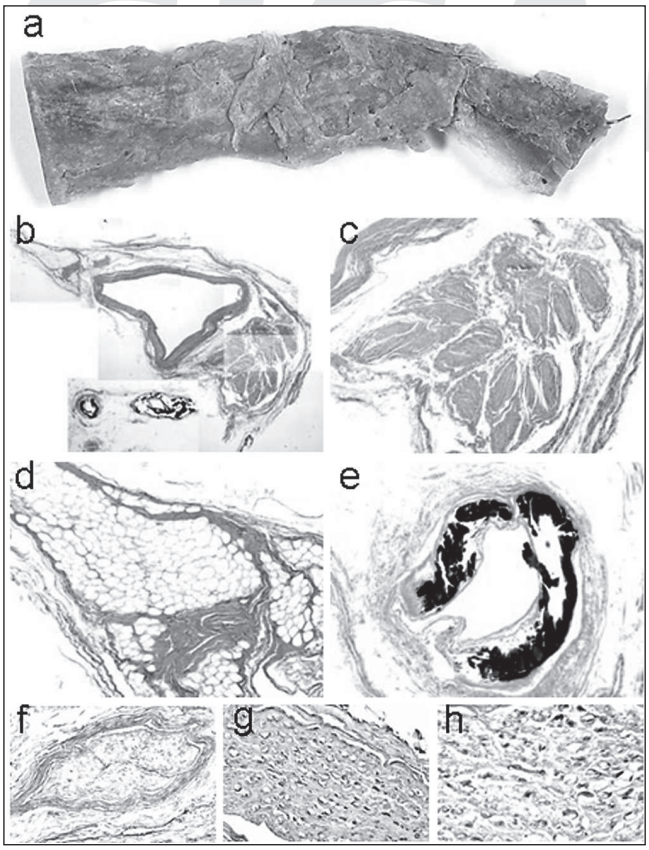

Fig. 2 - Experimental mummification. LIU-UMAB Mummy project. Sural nerve bundle (a). Rehydrated material processed to paraffin blocks and cut at 5 ?m for histological assessment. Composite micrograph showing full cross-section (b) and higher magnification of the nerve (c), adipose tissue (d) and media calcification of the artery (e). Immunostaining for collagen type IV and ubiquitin PGP9.5 (g&h).

and processed by hand though 4 changes of ethanol, 4 rapid changes of Histoclear (National Diagnostics, Atlanta, GA) and paraffin wax (one of us [MJ] experimented previously with different clearing agents and found Histoclear particularly useful for processing the mummified tissue). Our experience with processing mummified tissues showed that the combination of small size of the biopsy and processing by hand ensured the best possible duration for processing the tissue to paraffin wax, minimised shrinkage and helped to achieve good results with immunohistochemical staining.

Histochemistry

Haematoxylin and Eosin (H&E), Miller stain for elastic fibres, Picrosirius red, Masson's Trichrome, Toluidine blue and PAS stain were used.

Immunohistochemistry

A panel of monoclonal and polyclonal antibodies was used to test preservation of antigenicity of mummified tissues with contemporary (non-mummified) carotid arteries used as controls. We tested tissues for collagen type I and IV, elastin, α-smooth muscle actin and macrophage marker CD68. Different antigen retrieval methods (and combinations of

methods) have been used to disclose antigens in tissue (enzymatic pre-digestion with trypsin, pepsin, proteinase K and a panel of proteases, microwave and water-bath pre-treatment with citrate buffer pH 6.0 and 9.0).

Results

Mummified carotid endarterectomy samples

All histochemical stains were successful although weaker than in contemporary tissues with the exception of nuclear staining with Haematoxylin which was very weak or virtually absent in natron treated tissue and slightly better in naturally mummified tissue (Fig 1d), while eosin staining was good in both. The tissue which was only desiccated showed better colour definition for all histochemical stains comparing to natron treated samples.
Immunohistochemistry: Collagen type I and IV were better in naturally mummified tissue; elastin was stained similarly in both types of mummification. The results of immunostaining for two cellular markers, α-SMC actin and macrophage marker CD68 are compared in Table 1. There

Case number	Natural mummification			Egyptian mummification (natron)		
	H&E	α-actin	CD68	H&E	α-actin	CD68
CAA1	+	+	+	±	+++	±
CAA2	++	+	+	+	++++	±
CAA3	++++	+	+++	++	+/++	+
CAA4	++	±	+++	+	++	++
CAA5	++++	+	++++	+++	+++	+
CAA6	++	very weak	++++	+++	+++	++/+++
CAA7	+++	+	++++	++	++	++
CAA8	++	±	++	+	+++	+
CAA9	++	+	++	+	+++	++
CAA10	++++	+	++++	++	++	+

Table 1 - Comparison of haematoxylin and eosin staining and immunostaining for α-smooth muscle actin and macrophage marker CD68 in 10 naturally and natron mummified carotid artery samples.

were differences between individual samples, but generally staining for CD68 was excellent in naturally mummified (Fig 1e) and poor in natron treated tissues and the staining for α-SMC actin showed a reversed trend (Fig. 1f,g).

Mummified sural nerve bundle

The rehydration of tissue was excellent (Fig 2b-e). Despite visible damage to the nerve at high magnification, the immunostaining for collagen type IV (Fig 2f) and neuronal marker PGP9.5 (Fig 2g,h) was acceptable comparing to control sections incubated with non-immune IgG (not shown).

Discussion

Previous experimental studies on mummification (Zimmerman 1972, 1977) utilised small fragments of tissues, which quite understandably showed better preservation than the mummies. Both natural mummification by desiccation and that achieved by placing the body in close contact with inorganic salts cause movement of water from deeper part to the surface (skin) and towards body cavities (in case of natron placed in abdominal and thoracic cavities). That process alone damages the tissue more in regions where there is a substantial mass of soft tissues over the bones. The movement of water though tissue and evaporation on the surface causes damage to the epidermis resulting in its detachment and desquamation from exposed surfaces of the body (Aufderheide, 2002). We realise that mummification of small fragments of tissue is not representative of the process of whole body mummification but, on the other hand, carotid arteries are located in a relatively superficial anatomical location where natron would desiccate them in relatively short time, similar to our experiments. The desiccation period after mummification was 18-20 months, a mere moment comparing to mummies but we think that the differences in antigenicity observed in our experiment give an insight into mummification process.
The LIU-UMAB mummy project material was examined 12 years after mummification, a much longer time than the time span of our experiment and the biopsy of the sural nerve was obtained specifically for this study. This allowed us to experiment with processing and staining techniques which we now employ for ancient mummified remains.

Conclusions

We have found differences in the degree of antigenicity between desiccation alone and natron treated matching samples when comparing the same antigens. Thus, the initial mummification method has a critical effect on the preservation of tissue antigenicity.
These results have bearing on investigation of ancient human remains, as it can be expected that the differences in antigenicity (as reflected by immunohistochemical staining), depend on the initial mummification process. Our study further indicates that, contrary to previously held views, the antiquity of the material may aggravate the problem but the age of the mummy is not a principal cause of failed immunohistochemical staining.
This is a preliminary study. We intend to continue mapping the differences between the experimentally natron treated and desiccated contemporary tissues. We further plan to

use the results to guide our planning for work on the mummified tissue collection.

Literature Cited

Appenzeller O, Hoyle CV, Santoro CM, Appenzeller M. 2000. Paleoneurobiology. Chungara, Revista de Antropologia Chilena 32:97-102.

Aufderheide AC. 2002. The scientific study of mummies. Cambridge: Cambridge University Press.

Brier B, Wade RS. 1997. The use of natron in human mummification: a modern experiment. ZAS 124:89-100.

Fulcheri E. 1995. Immunohistochemistry: a new outlook in histopaleopathology. Boll Soc Ital Biol Sper 71:105-10

Herodotus. 1920. Histories, book II. Cambridge, MA: Harvard University Press p 86-89.

Hoyle CHV, Thomas PK, Burnstock G, Appenzeller O. 1997. Immunohistochemical localization of neuropeptides and nitric oxide synthase in sural nerves from Egyptian mummies. J Auton Nerv Sys 67:105-108.

Krypczyk A, Tapp E. 1986. Immunohistochemistry and electron microscopy of Egyptian mummies. In: Science in Egyptology. Proceedings of the Science in Egyptology Symposia. David AR, Tapp E, editors. Manchester University Press

Wick G, Haller M, Timpl R, Cleve H, Ziegelmayer G. 1980. Mummies from Peru. Demonstration of antigenic determinants of collagen in the skin. Int Arch Allergy Appl Immunol 62:76-80.

Zimmerman MR. 1972. Histologic examination of experimentally mummified tissue. Am J Phys Anthropol 37:271-280.

Zimmerman MR. 1977. An experimental study of mummification pertinent to the antiquity of cancer. Cancer 40:1358-1362.

Zimmerman MR, Brier B, Wade RS. 1998. Brief communication: Twentieth-Century Replication of an Egyptian Mummy: Implications for Paleopathology. Am J Physical Anthropol 107:417-420.