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DNA damage and metal accumulation in four tissues of feral *Octopus vulgaris* from two coastal areas in Portugal

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ABSTRACT

The alkaline comet assay has been employed for the first time to estimate the basal DNA damage in the digestive gland, gills, kidney and gonads of *Octopus vulgaris*. Octopuses were captured in two coastal areas adjacent to the cities of Matosinhos (N) and Olhão (S), Portugal. The area of Matosinhos is influenced by discharges of the Douro River, city of Porto, industries and intensive agriculture, while Olhão is an important fisheries port. Previous works point to contrasting metal availability in the two coastal areas. Among the analysed tissues digestive gland presented the highest levels of Zn, Cu, Cd and Pb. Tissues of specimens from Matosinhos exhibited high levels of Cd and from Olhão enhanced Pb concentrations. The DNA damages in digestive gland, gills and kidney were more accentuated in specimens from Matosinhos than from Olhão, suggesting a stronger effect of contaminants. Elevated strand breakages were registered in digestive gland, recognised for its ability to store and detoxify accumulated metals. The DNA damages in kidney, gills and gonads were lower, reflecting reduced metal accumulation or efficient detoxification. The broad variability of damages in the three tissues may also mirror tissue function, specific defences to genotoxicants and cell-cycle turnover.

1. Introduction

Among the molecular components of the cell, DNA is an important target of environmental stress in organisms (Frenzilli et al., 2001). Various environmental contaminants are known mutagens. Damage to DNA may lead to mutations, strand breaks, altered bases (Shugart, 2000) and eventually carcinogenesis and other health disorders (Kurelec, 1993). It may result in severe consequences at individual, species and ecosystem level (Klobucar et al., 2003). Therefore DNA damage has been considered in toxicity testing.

Single-cell gel electrophoresis (SCGE or comet) assay has become a widespread technique for detection of DNA damage induced by xenobiotics, e.g. Cd (Desai et al., 2006; Fourie et al., 2007), Hg (Tran et al., 2007), organic compounds (Costa et al., 2008). The alkaline version of the assay has proven to be a simple and reliable method for the quantitation of total DNA fragmentation as a result of the formation of single-and double-strand breakage, xenobiotic–DNA adducts and alkali-labile sites (e.g. unstable altered nucleotides; Singh et al., 1988). The comet assay has been used in a wide range of aquatic organisms, such as marine diatoms (Desai et al., 2006), bivalve molluscs (e.g. Jha et al., 2005; Desai et al., 2006) and fish (e.g. Ahmad et al., 2006; Costa et al., 2008), for the biomonitoring of coastal environments. Most of studies deal with one and/or a limited number or combinations of contaminants, and thus research in aquatic ecosystems with complex mixtures and interactions of metals and other contaminants is still missing. Moreover, to our knowledge this technique has not been applied to assess DNA damage in cephalopod tissues.

The common octopus, *Octopus vulgaris*, is a sedentary cephalopod inhabiting coastal waters and thus susceptible to be exposed to contamination (Mangold, 1983). Local environmental conditions influence metal accumulation in tissues and several studies have proved the ability of these organisms to accumulate high levels of essential and non-essential elements, especially in digestive gland (e.g. Miramand and Guary, 1980; Miramand and Bentley, 1992; Bustamante et al., 1998a, b; Raimundo et al., 2004, 2005; Napoleão et al., 2005). However, only few data exist regarding tissue-level effects of accumulation (Bustamante et al., 2002; Raimundo et al., 2008).

The aim of this study was to examine whether DNA strand breaks (DNA-SB) in digestive gland, gills, renal appendages (herein called kidney) and gonads of the common octopus, *O. vulgaris*, reflect the accumulation of Zn, Cu, Cd and Pb. The selected tissues for this work are recognised to mirror the input,

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elimination or storage of trace elements. This hypothesis was tested in feral animals captured in two areas of the Portuguese coast with contrasting availability of those elements.

2. Material and methods

2.1. Samples

Twelve common octopuses, *O. vulgaris*, were collected from commercial catches in November 2007 in two coastal areas of Portugal: off Matosinhos ($n=6$) and off Olhão ($n=6$; Fig. 1). The Matosinhos coastal zone is drained by Douro, an important Iberian river. The Douro estuary is surrounded by the city of Porto and metropolitan area with industries and the riverine margins by intensive agriculture (Araújo et al., 2002). Toxicological studies were performed with fishes from the Douro estuary (e.g. Ferreira et al., 2006, 2008). However, a survey in the coastal area has reported high levels of Cd and Cu in the water column particularly in winter (Caetano and Vale, 2003) and slight enhancement of DDT compounds and PCBs (Quental et al., 2003). The southern zone (Olhão) is influenced by small rivers crossing the Iberian Pyritic Belt with ores containing large quantities of Zn, Cu and Pb, minor Cd content and traces of Ni (Palanques et al., 1995; Elbaz-Poulichet and Leblanc, 1996). This geological feature has been shown to affect Pb concentrations in octopus tissues (Raimundo et al., 2009). The sampled organisms were kept on ice until laboratory use (maximum 3 h). Then each individual was weighted and mantle length and gender determined. The specimens were immediately dissected, digestive gland (without rupture of the outer membrane), gills, kidney and gonads of each organism being totally removed.

2.2. Analytical methodology

2.2.1. Metals

Metals were analysed in lyophilised, grinded and homogenised samples after digestion with a mixture of HNO_3 (sp, 65% v/v) and H_2O_2 (sp, 30% v/v) at different temperatures according to the method described in Ferreira et al. (1990). All lab ware was cleaned with HNO_3 (20%) for two days and rinsed with Milli-Q water to avoid contamination. Three procedural blanks were prepared using the same analytical procedure and reagents, and included within each batch of samples. Concentrations of Zn, and in the case of digestive gland, Cu and Cd, were determined by flame atomic absorption spectrometry (Perkin Elmer AAnalyst 100) and Cu, Cd and Pb by a quadropole ICP-MS (Thermo Elemental, X-Series). The accuracy of these analytical methods was assessed by the analysis of international certificate standards (DORM-1, DORM-2—dogfish muscle; DOLT-1—fish liver and TORT-1, TORT-2—lobster hepatopancreas). The results obtained were in good agreement with the certified values ($p<0.05$). Procedural blanks always accounted for less than 1% of the total metal in the samples. All the results are given as medians and ranges in micro-gram per gram of dry mass tissue ($\mu g\, g^{-1}$; dm).

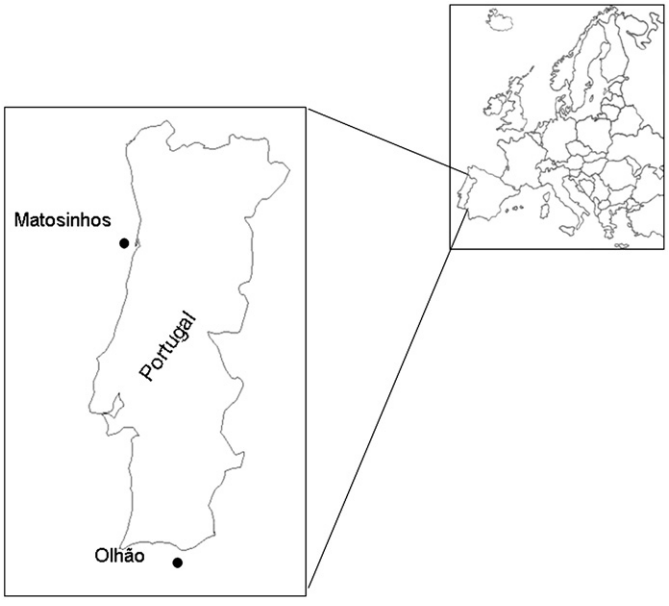

Fig. 1. Location of the two areas of capture of *Octopus vulgaris* in the Portuguese Coast: Matosinhos and Olhão.

2.2.2. DNA strand breaks

DNA total strand breakage (DNA-SB) was assessed through the alkaline single-cell gel electrophoresis (comet) assay (Singh et al., 1988) and adapted from the method described by Costa et al. (2008). Aliquots of fresh digestive gland, gill, kidney and gonad cells were resuspended in KSS (Kenny's salt solution) in the proportion 1:1 (w/v), with the aid of a micropipette with a cut tip. No collagenase, mincing or other disaggregation treatments were necessary. Samples were placed on slides pre-coated with 1% (w/v) normal melting-point agarose in TAE buffer (the slides were allowed to dry for at least 48 h) and covered with a coverslip. After agarose solidification (15 min, 4 °C) the coverslip was removed and the slides were dipped for 1 h at 4 °C in lysis solution (2.64% NaCl (w/v), 3.72% EDTA (w/v) and 5 mM Tris) to which was added 10% (v/v) DMSO and 1% (v/v) Triton-X 100 just before use. Slides were afterwards placed in cold (4 °C) electrophoresis solution (pH 13) for 40 min to allow DNA-unwinding and enhanced expression of alkali-labile sites. Electrophoresis was for 30 min at 25 V, in the cold (4 °C), using a Sub-Cell model 96 apparatus (Bio-Rad). Slides were afterwards neutralised in 0.1 M Tris–HCl buffer (pH 7.5) for 15 min. All preparatory steps were performed under controlled temperature (≈ 16 °C) to avoid gel lifting from the slides and all solutions and electrophoresis apparatus were kept in the dark and in the cold to minimise accessory DNA degradation. Approximately 100 comets were analysed per slide after staining with 0.02 mg mL^{-1} ethidium bromide (EtBr). Comets were analysed using the CometScore (TriTek). The percentage DNA in the tail was employed as a direct measure of DNA-SB (Lee and Steinert, 2003). DMLB microscope adapted for epifluorescence with an EL6000 light source for mercury short-arc reflector lamps was used, equipped with an N2.1 filter, all from Leica Microsystems. The comet assay was successfully employed in all surveyed tissues, as indicated by the retrieving well-defined nucleoids as well as damaged cells (Fig. 2).

2.3. Statistical analysis

Prior to statistical analyses, metal concentrations were tested for normality and equality of variances. Non-compliance with parametric ANOVA assumptions led to employment of the Kruskal–Wallis H (KW-H) and Mann–Whitney (U) non-parametric tests were used to evaluate the existing differences between metal concentrations and DNA fragmentation of individuals from the study areas and between tissues. The significance for statistical analyses used was always $p=0.05$. Statistical analyses were performed using Statistica (Statsoft).

3. Results

3.1. Influence of size/weight and gender

The size and weight of octopuses sampled at Matosinhos and Olhão ranged in the following intervals: 146–165 mm and 165–205 mm, 1162–1399 g and 1231–1957 g, respectively. The proportion female:male was the same in the two areas: 3:3 (Matosinhos) and 3:3 (Olhão). Concentrations of Zn, Cu, Cd and Pb in digestive gland, gills, kidney and gonads showed no significant (U, $p>0.05$) differences with the size/weight of the captured octopus in each sampling area. The DNA-SB also presented a lack of relationship with those two biological parameters. Levels of Cd and Pb showed no significant differences (U, $p>0.05$) with

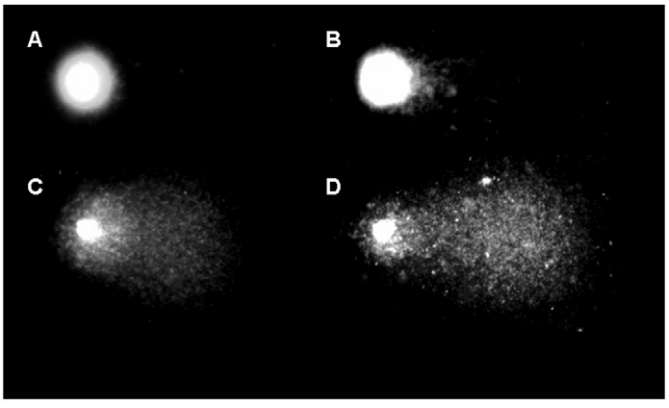

Fig. 2. Comet examples of DNA-SB from *Octopus vulgaris*: ≅0% (A, gonads), ≅27% (B, kidney), ≅68% (C, gills) and ≅74% (D, digestive gland).