FATTY ACID PROFILING: A SENSITIVE TOOL TO ASSESS MERCURY CONTAMINATION IN THE SEA CUCUMBER *HOLOTHURIA FORSKALI*

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Abstract
This study assessed the Hg impact on the fatty acid (FA) composition in the sea cucumber *Holothuria forskali* body wall. Specimens were exposed to $HgCl_2$ graded doses (40, 80 and 160 µg L^{-1}) for 96 h. At the end of the trial, the body wall Hg-burden exhibited significant dose dependent increases reflecting the bio-accumulative ability of this tissue. A decrease in linoleic, arachidonic and eicosapentaenoic acid levels and an increase of docosahexaenoic acid were mainly observed at the nominal tested dose. Our findings highlighted the usefulness of the FA composition as an early sensitive bioindicators of Hg intoxication in holothurians.

Keywords: Bio-indicators, Echinodermata, Ecotoxicology, Mediterranean Sea

Mercury pollution is featuring as one of the major threat for marine ecosystem, biota and human health. Despite their great ecological and economic importance, little is known about the impact of Hg contamination on Holothurians. In a previous study [1], we have studied the responsiveness of sea cucumber to Hg exposure through aset of standard biomarkers as oxidative stress parameters. Here we investigated, for the first time, the influence of Hg intoxication on the fatty acid composition of *Holothuria forskali* in order to verify their usefulness as a biomarker of mercury intoxication in holothurians. To do this, we exposed specimens of *H. forskali* to 40, 80 and 160 µgL^{-1} of mercuric chloride ($HgCl_2$) for 96 hours. At the end of the trial, total Hg was analyzed using Direct Mercury Analyzer (DMA 80). Lipids were extracted according to the method of Folch et al. [3] and then trans-esterified using the method of Cecchi et al. [4]. Fatty acid methyl esters (FAMEs) were analyzed in agaschromatograph.As given in figure1, body wall Hg-burden exhibited significant dosedependentincreases reflecting the bio-accumulative ability of this tissue.

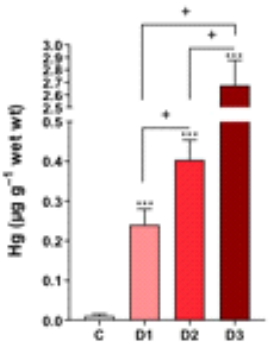

Fig. 1. Total mean mercury concentrations in the body wall of unexposed (C) and exposed *Holothuria forskali* to different concentrations of $HgCl_2$ (D1=40 µg L^{-1}, D2=80 µg L^{-1} and D3=160 µg L^{-1}) for 96 h. *** $p < 0.001$: treated groups vs. control group; + $p < 0.001$: treated groups vs. each other.

An evident toxic effect of Hg on the *H. forskali* body wall's lipid fraction was detected in all treated groups and prominently at the nominal tested dose (Table 1). The simultaneous increase of saturated fatty acids (SFA) and decrease of polyunsaturated fatty acids (PUFA) can be attributed to the lipid peroxidation mechanism and/or to cellular adaptive response by the triggering of defense and reparation mechanisms to alleviate membrane damage as suggested by Rocchetta et al. [5]. Indeed, therecorded drastic diminution of the PUFA (n-6) group in particular arachidonic acid (C20:4n-6) and its precursor linoleic acid (LA, C18:2n-6) reflects an increased metabolic demand for the regulation of cell membrane fluidity and/or for the activation of eicosanoid synthesis through arachidonic cascade pathway. Our assumption was further confirmed by the similar trend observed for the eicosapentaenoic acid (C20:5n-3) which is another eicosanoids precursor also known as an excellent energy source. The ability of *H. forskali* to cope with Hg insults through the modulation and the adjustment of its lipid metabolism was further reflectedby the increment of the docosahexaenoic acid (C22:6n-3) level. This FA, whichhas been proven to be potent antioxidant agent and to have primordial role in membrane architecture, seems to be selectively retained or biosynthesized in treated *H. forskali*.

Tab. 1. Fatty acid profile of *Holothuria forskali* body wall in response to different HgCl2 exposures: D1 (40µg L^{-1}), D2 (80 µg L^{-1}) and D3 (160 µg L^{-1}) plus control (C). Data are expressed as % of total fatty acids (mean ± S.D.). Means followed by different letters in same line are significantly different (p<0.05). SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid.

FA	C	D1	D2	D3
C18:2n-6	14.68 ±2.74^{a}	0.72 ±0.07^{b}	8.64 ±0.08^{c}	7.77 ±0.90^{c}
C20:4n-6	2.06 ±0.78^{a}	0.71 ±0.13^{b}	0.81 ±0.04^{b}	2.62 ±0.55^{a}
C20:5n-3	1.17 ±0.15^{a}	0.24 ±0.05^{b}	0.36 ±0.14^{b}	1.67 ±0.28^{a}
C22:6n-3	1.13 ±0.34^{a}	4.78 ±0.53^{b}	3.43 ±0.33^{b}	3.34 ±0.60^{b}
∑SFA	26.61 ±5.27^{a}	59.24 ±5.07^{b}	40.50 ±2.35^{c}	35.45 ±3.08^{c}
∑MUFA	15.02 ±3.36^{a}	18.14 ±2.38^{ab}	20.97 ±1.88^{b}	29.02 ±2.85^{c}
∑PUFA	58.37 ±17.63^{a}	22.62 ±2.69^{b}	38.53 ±1.53^{c}	35.53 ±1.43^{c}
∑PUFA (n-3)	8.84 ±0.64^{a}	8.04 ±1.09^{a}	13.26 ±1.48^{b}	10.96 ±1.23^{b}
∑PUFA (n-6)	44.18 ±6.08^{a}	9.64 ±2.67^{b}	19.25 ±0.35^{c}	18.02 ±0.55^{c}

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