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Cytotoxic effect on human keratinocytes of crude extracts from planktonic Cnidaria

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Abstract

Biotoxin production is a characterizing aspect of the physiology and ecology of several marine organisms. In this study the cytotoxic effect of crude extracts from jellyfish Pelagia noctiluca, Chrysaora hysoscella and Aurelia aurita (Cnidaria: Scyphozoa) against cultured human keratinocytes NCTC 2544 was evaluated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay and Neutral Red Uptake (NRU). Low cytotoxic effects were observed with NRU, while MTT showed a dose-dependent effect, with a 'plateau' at highest protein concentrations. Therefore, crude extracts didn't show effect on lysosomes but MTT test emphasized cell survival decrease and a 'plateau' effect at high extract doses which could be related with the inhibition of the mitochondrial dehydrogenase activity.

Introduction

Plants and animals have been used ever since to obtain extracts with pharmacological and therapeutical activity utilized to treat several pathologies or to support the functional maintaining of tissues, organs and apparatuses. The study of natural substances produced by organisms – chemistry of natural products – led to the development of biologically active compounds, several of them having application as substances of biomedical interest such as drugs, pigments, insecticides, or were utilized to synthesize biologically active molecules; in particular, several substances of marine origin have demonstrated significant activity as antiviral, anti-inflammatory and antitumoral agents (Newman *et al.*, 2000, 2003) and have shown different pharmacological application (Fenical and Jensen, 1993). Biotoxins are included among these compounds and their production is a peculiar and interesting aspect which characterizes the physiology and the ecology of several marine species.

The study of toxins from marine organisms led to important scientific results such as the discovery of a lot of prostaglandins (15R)-PGA2 in the gorgonian *Plaxaura homomalla* and the synthesis of ARA-A (D-arabinosyl-adenine) and ARA-C (D-arabinosyl-cytosine) respectively with antiviral and antitumoral activity, starting from spongotimidine and spongouridine found in the sponge *Cryptotheca crypta*. The utilisation of Tetrodotoxin (TTX) from Tetraodontidae, Diodontidae and Molidae teleosts, even though limited, as muscle relaxant and analgesic (Ghiretti and Cariello, 1984), the local anaesthetic activity of the vasoconstricting agent palytoxin (PTX), extracted from the zoanthid *Palythoa toxica* which was indicated as one of the most poisonous substance known to date and as a tumor promoter, the antitumoral and cytotoxic activity of cyclic peptides (Didemnin B) derived from the marine tunicate *Tridemnium solidum*, the antiviral, antitumoral and antiinflammatory activity of saponins from echinoderms were also reported.

In particular, some marine-derived substances such as Bryostatin 1 isolated from the bryozoan *Bugula neritina*, Ecteinascidin 743 a metabolite produced by the tunicate *Ecteinascidia turbinata*, Dolastatins produced by molluscs *Dolabella auricularia*, Alicondrin B derived by sponges (*Halicondria okadai*, *Lissodendoryx* sp.) seem promising as potential antitumoral agents (Cragg and Newman, 1999). These, and also other, substances are to date in a preclinical phase or also in clinical phase I and II studies (Nuijen *et al.*, 2000).

Cnidarians play an important role among biotoxin producers; their venoms are stored in nematocysts, peculiar capsules provided with a spiralized thread able to became everted so injecting the venom; it is thought that cnidarian toxins are also included in tissues (Allavena *et al.*, 1998). The damage caused by these organisms on human skin is well known; for this reason the study of the action of cnidarian venoms on skin cells, composed mainly by keratinocytes in various differentiation stages, is a matter of concern. On this basis, aim of this research was to verify the cytotoxic effect induced by extracts from marine jellyfish *Pelagia noctiluca*, *Chrysaora hysoscella* and *Aurelia aurita* (Cnidaria: Scyphozoa) on cultured human keratinocytes NCTC 2544.

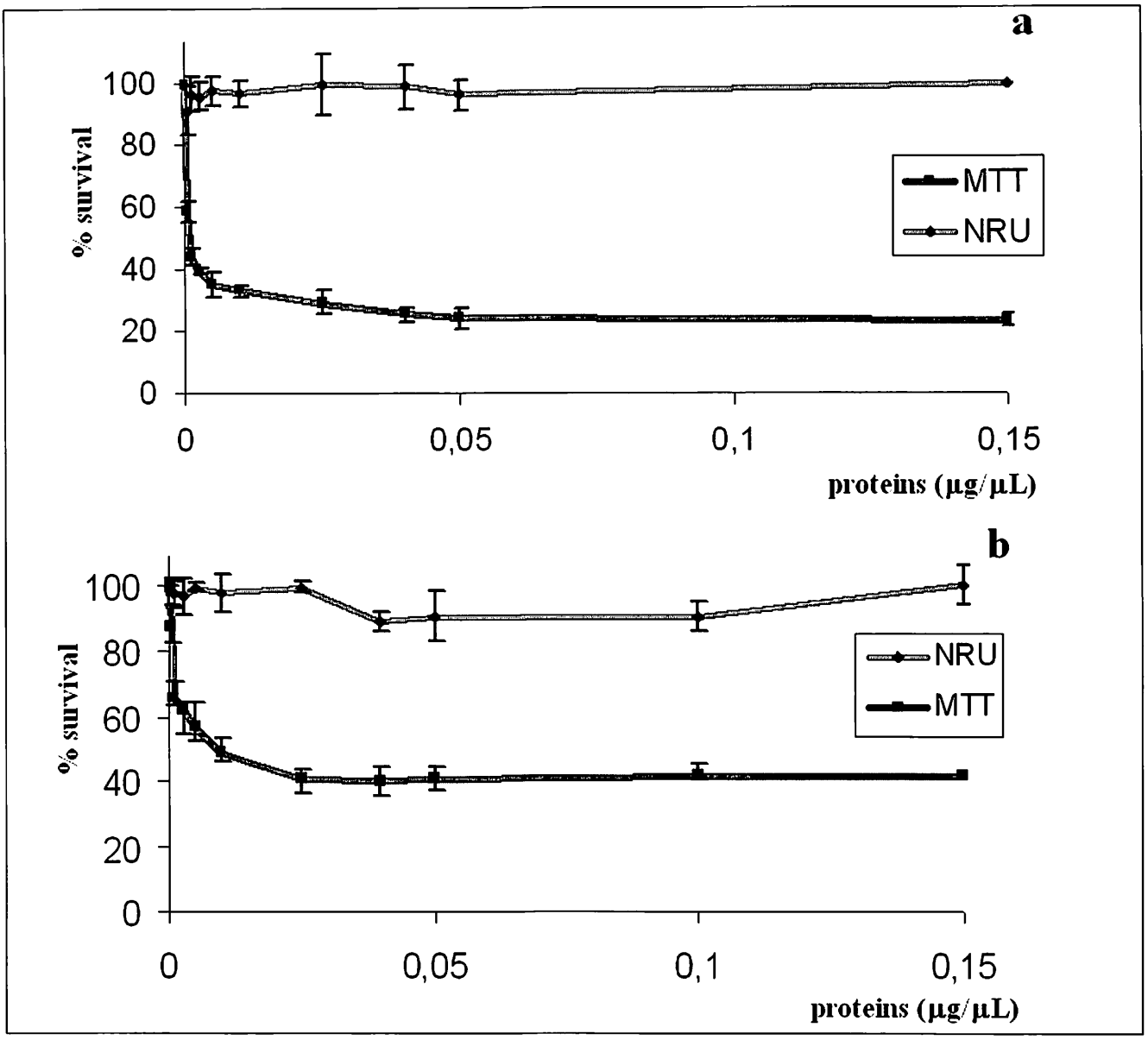
Materials and Methods

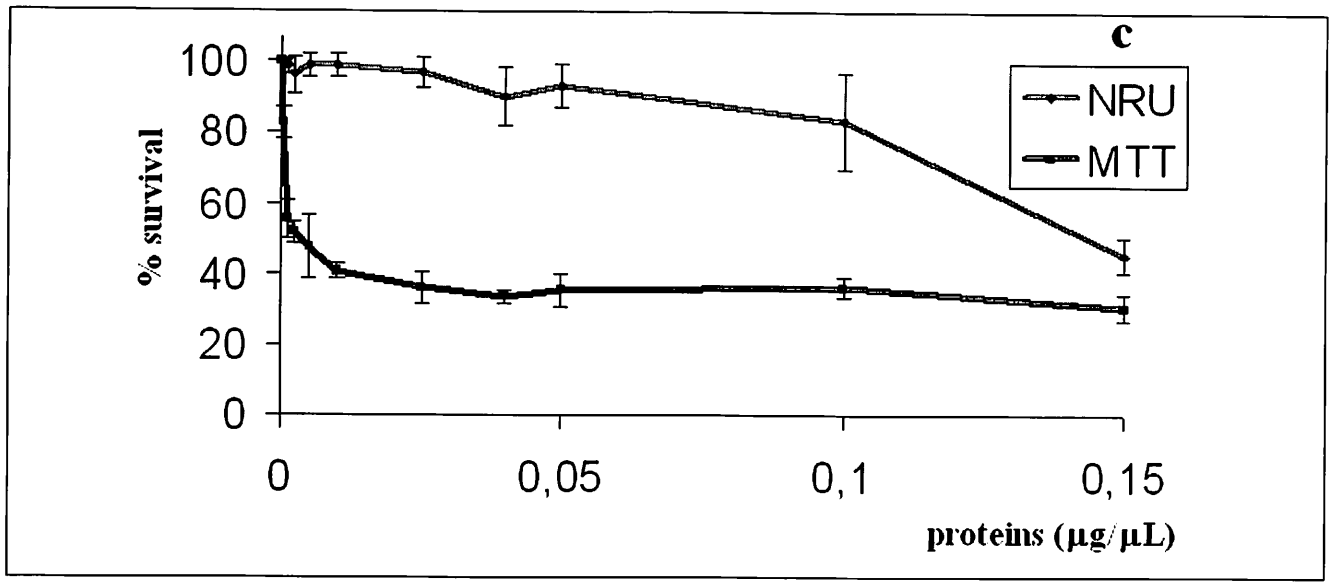
Jellyfish were collected in the Ligurian Sea and subsequently maintained at -20°C until experimental utilization. Tissues were treated as reported (Mariottini *et al.*, 1993, 2002; Carli *et al.*, 1996), to obtain a suspension of nematocysts and extranematocystic tissue. Nematocysts were subsequently induced to discharge by sonication in ice bath. The extract was analyzed for its total protein content, which was used as a parameter to determine the cytotoxic effect on cultured human keratinocytes NCTC 2544. Cells were maintained at 37°C and 5%CO_2 in DMEM medium with 5% FBS and 2% l-glutamine and exposed, in serum free medium, for two hours to extracts with protein concentrations ranging from 0 to 0.15 µg/µl; the cytotoxicity was evaluated by MTT Assay and Neutral Red Uptake (NRU).

Results

RNU showed low cytotoxicity of the considered extract; in particular, for *Aurelia aurita* and *Pelagia noctiluca* extracts cell survival was always ≥90%, therefore wasn't possible to calculate the IC50 value. The extract from *Chrysaora hysoscella* induced 55% cell survival decrease at the highest dose; so it was possible to calculate the IC50 value (0.14 µg/µl).
MTT test showed a dose-dependent cytotoxicity increase with a 'plateau' effect at the highest protein concentrations. All extracts were cytotoxic at low concentrations as well; in particular, the extract from *Aurelia aurita* showed a IC50 value of 0.00063 µg/µl. Lower effects have been observed for *Chrysaora hysoscella* and *Pelagia noctiluca* extracts (0.0019 µg/µl and 0.0086 µg/µl respectively).
Pertinent results are shown in Fig. 1 (a, b, c).

Fig. 1 - *Percent survival of NCTC 2544 keratinocytes after treatment with Aurelia aurita (a), Pelagia noctiluca (b) and Chrysaora hysoscella (c) crude venoms evaluated by MTT and NRU.*





Discussion

Evident cytotoxic effect of tested extracts were not observed with NRU, while MTT showed a dose-dependent increase of cytotoxicity with a 'plateau' effect at highest protein concentrations. At the tested concentrations cnidarian extracts seem to affect scantily the intactness of lysosome membrane and the result of *C.hysoscella* extract could be a relative exception because a certain toxicity was observed only at the highest protein concentration. On the contrary, crude extracts seem to affect the mitochondrial activity already at low concentrations. The cytotoxic properties of venom from *Aurelia aurita* and *Chrysaora hysoscella* are unknown and these results are a first contribution to the knowledge in this field. Otherwise, the cytotoxicity of *Pelagia noctiluca* crude venom, as well as the hemolytic properties towards chicken and rabbit erythrocytes (Marino *et al.*, 2007), were assessed by short-term (Mariottini *et al.*, 2002) and long-term (Carli *et al.*, 1995) tests on V79 cells, observing that despite its irritating properties *in vivo*, extracts from *Pelagia noctiluca* show lower effects than those from other jellyfish and anemones (Mariottini *et al.*, 1993, 1998, 2008; Carli *et al.*, 1996; Allavena *et al.*, 1988).

In conclusion, the crude extracts didn't show evident effect on lysosomes because NRU indicated absence of cytotoxicity; on the contrary, MTT test emphasized cell survival decrease, and the observed 'plateau' effect with high extract doses could be related with the inhibition of the mitochondrial dehydrogenase activity. Further studies could explain the mechanisms of interference of crude venom with mitochondrial enzymes and identify the responsible molecules.

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