J. Biol. Res. 2011 n.1 - Vol. LXXXIV - Rubbettino - Soveria Mannelli

Does zinc sulfate inhibit the *in vitro* cytotoxicity of crude toxin from *Pelagia noctiluca?*

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KEY WORDS: *Pelagia noctiluca*, jellyfish, cytotoxicity, zinc sulfate, protection

Abstract

The Scyphomedusa* Pelagia noctiluca *is known to be an harmful species able to cause contact dermatitis and also systemic symptoms in sensitive subjects. Taking into account that some compounds are known to be protective agents against jellyfish venoms, in this research the protection of non-cytotoxic zinc sulfate concentrations was evaluated on cultured L929 mouse fibroblasts exposed to nematocyst crude venom at doses ranging between $60x10^3$ and $240x10^3$ nematocysts/ml. The results indicate that the pre-treatment with 10^{-6}M zinc sulfate allowed a significant cell survival increase and protection after exposition to nematocyst doses from $60x10^3$ to $180x10^3$ nematcysts/ml. Therefore, zinc sulfate could be a valuable protective agent in barrier creams applied to bathers'skin with the purpose to protect from* Pelagia noctiluca *stings.

Introduction

Cnidarian toxicity has a remarkable influence on some human activities and on public health because jellyfish stinging capsules, the nematocysts (N), can damage human skin producing contact dematitis with erytema, oedema and vesicles, and sometimes cause stronger damage such as severe dermonecrosis, systemic symptoms, cardio- and neuro-toxicity highly harmful in sensitive subjects [1]. Furthermore, jellyfish outbreaks that occurred in the Mediterranean many times during the last decades have notably affected the safeness of bathers as well as some economic activities, such as fishing and tourism [2-4]. As a matter of fact, these outbreaks were mainly supported by the Mauve Stinger *Pelagia noctiluca* Forsskål, 1775 (Cnidaria: Scyphozoa) [2, 3, 5]; this species is known to be the most harmful Mediterranean jellyfish, so the research aimed to produce protection systems against the effects of its venom, such as barrier creams, has become a subject of concern. *Pelagia noctiluca* is a pink-mauve-violet jellyfish widely distributed in the Mediterranean [4] that carries nematocyst battieries in tentacles, in oral arms as well as in the whole upper bell surface. The cytotoxic properties of crude venom (CV) from *Pelagia noctiluca* were repeatedly reported [6-11] and it is known to cause extensive damage to cell membrane and to affect cell survival and ATP synthesis [7]. Therefore, taking into account the well-known *in vitro* cytotoxicity of CV from *Pelagia noctiluca*, aim of this research was to evaluate the cell-protection and the inactivation of CV by zinc sulfate as well as the comparison with what is known about the protection of other compounds.

Materials and methods

Sampling

Jellyfish *Pelagia noctiluca* were collected in the Ligurian Sea and subsequently frozen, transferred to the laboratory and maintained at -20°C until they were used.

CV extract

CV was prepared according to [7] with the modifications reported in [12]. The amount of nematocysts (N) into the extracts was used as a parameter to define the doses; preliminary tests were carried out to determine the range of doses that include the IC_{50}. In the experiments the following doses were considered: A) $60x10^3$ N/ml, B) $120x10^3$ N/ml; C) $240x10^3$ N/ml; D) $480x10^3$ N/ml.

Cell cultures

Mouse fibroblasts of the continuous cell line L929 (normal phenotype) maintained in DMEM medium supplemented with 5% FBS, 1% penicillin-streptomycin, 1% l-glutamine at 37°C (EuroClone, Pero, Milano, Italy) in humidified atmosphere with 5% CO_2 were used in the experiments.

Cell treatments

All treatments were carried out on 200,000 L929 cells seeded in culture plates, against untreated controls; the cytotoxicity was assessed with MTT assay (standard method ISO 10993-5 1999) through the measure of formazan formation evaluated by the absorbance of samples. All measures were carried out with a Spekol mod. 1300 spectrophotometer (Analytic Jena AG, Germany). To evaluate the cytotoxicity of CV, cells were exposed in triplicate for 20 minutes to the different doses of extract. Each

experiment was repeated five times treating a total of 3×10^6 cells. Zinc sulfate, analytical grade, was assessed as cell-protective factor; its no observed effect concentration (NOEC) was determined through cytotoxicity tests at concentrations ranging from 10^{-1}M to 10^{-10}M. The protection of zinc sulfate at NOEC concentration was evaluated after 20 min pre-treatment of cultured cells subsequently treated with CV. The IC_{50} was calculated with the Trimmed Spearman-Karber Method [13].

Results

The cytotoxicity of zinc sulfate on L929 cells is shown in Fig.1; 10^{-6}M was the concentration closest to the NOEC.

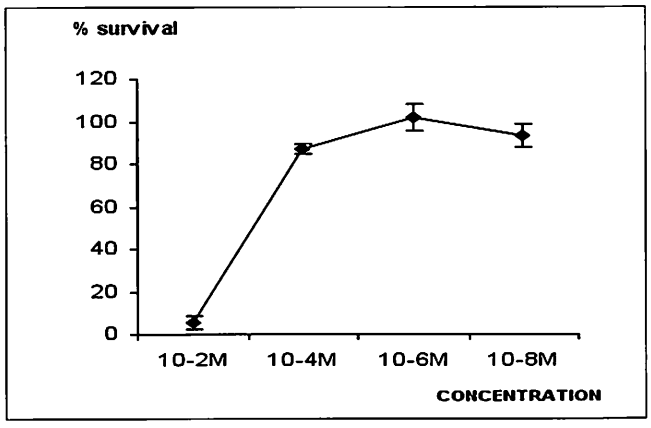

Figure 1. Percent survival of L979 cells after 20 min treatment with different concentrations of zinc sulfate evaluated through MTT assay

The CV from *Pelagia noctiluca* showed remarkable cytotoxic properties when assessed with MTT assay. Increasing the dose from 60×10^3 to 240×10^3 N/ml the cytotoxicity showed a moderate decrease but it stabilized at survival lower than 60%; this moderate absorbance increase was supposed to be due partly to the colorimetric interferences occurring in the formazan production caused by the CV itself. Survival of L929 cells pre-incubated with zinc sulfate and treated with 60×10^3 to 180×10^3 N/ml remarkably improved; the pre-treatment did not influence cell damage caused by 240×10^3 N/ml (Fig. 2).

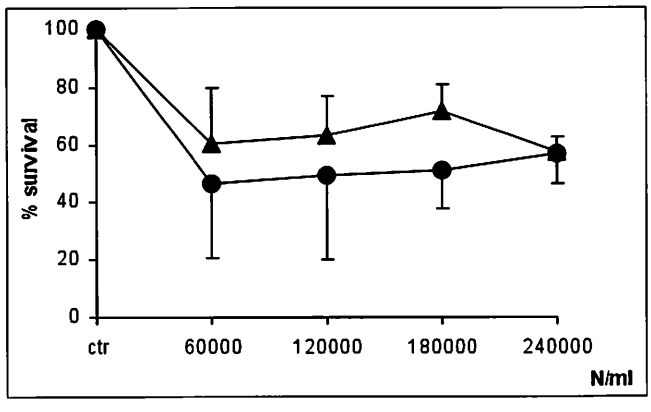

Figure 2. Percent survival of L929 cells exposed for 20 min to CV of Pelagia noctiluca, assessed with MTT assay. N/ml = Nematocysts/ml. ● = cells treated with crude venom alone; ▲ = cells pre-treated for 20 min with 10^{-6}M zinc sulfate and subsequently treated for 20 min with CV

Discussion

Jellyfish stinging is a pressing current topic in several coastal areas around the world and it is a matter of concern also in the Mediterranean region where several injuries presumptively mainly due to *Pelagia noctiluca* [4, 5, 13, 14] and to other species [15, 16] have been reported. In the Mediterranean Sea *Pelagia noctiluca* occurs manly in spring-summer; its venom is of protein nature, antigenic, and has dermonecrotic and hemolytic properties. It is widely accepted that the reduction of tetrazolium salts [17] and the changes in MTT activity reflect changes in both cell number and metabolic activity and are consistent with the differences between overall cell metabolism [18], being a reliable way to evaluate cell survival and proliferation. In this work the cytotoxicity of CV from *Pelagia noctiluca* assessed with MTT assay has been first demonstrated. After 20 minutes exposition the doses ranging from 60×10^3 N/ml to 240×10^3 N/ml caused increased cytotoxicity on L929 cells up to reach near 60-70% survival; on the whole, contrary to what expected, as well as to that is known using other methods such as Trypan blue [7], a moderate increase of formazan production was recorded; this could be due to colorimetric interferences caused by the CV itself or to cell metabolic disorders; in this connection, an uncoupling action accelerating the oxidative chain enhancing MTT reduction was emphasized for some natural compounds [19] that could anyhow interfere with MTT assay [20]. In spite of this, the pre-treatment with zinc sulfate allowed a remarkable increase of cell survival at the lower tested doses, but it was ineffective after treatment with 240×10^3 N/ml. It is known that the inflammation caused by *P. noctiluca* venom can be reduced by some substances, such as melatonin [21] and some studies emphasized the cell-protective activity of pre-incubation with lanthanum sulfate at concentrations included from 10^{-4}M to 10^{-5}M to inhibit the cytotoxicity of *Pelagia noctiluca* crude venom [12], as well as the cytolytic activity of the venom from *Physalia physalis* [22]; similarly, zinc sulfate seems to have a quite evident protective power against *Pelagia noctiluca* stinging. Therefore, the improvement of knowledge about the properties of this compound could be of concern to counteract the recurrent injuries on bathers and on sea-workers occurring particularly during summers. In conclusion, considering that the poisonousness of Mediterranean jellyfish is an increasing health problem in several coastal areas, a specific research aimed to develop drugs or medical aids to counter their stinging effect should be stimulated. Further studies, to date in progress, should also evaluate the efficacy of other chemical compounds and also of natural substances to protect human skin from jellyfish injury.

Acknowledgments

This research was carried out with grants from Pietrasanta Pharma S.p.A., Viareggio (Italy).

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