

Inhibition of the Cytotoxicity of *Pelagia noctiluca* Venom by Lanthanum Sulfate

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Abstract Cnidarians exert a meaningful influence on human activities and health being able to produce several local and systemic symptoms on humans. Mediterranean jellyfish are scarcely poisonous, but during outbreaks the impact of some of them, such as the *Scyphomedusa Pelagia noctiluca*, is noteworthy. As some compounds containing lanthanum ions could act as protective agents, the protection of lanthanum sulfate was evaluated on cultured L929 cells treated with three doses (120,000 nematocysts (N)/ml, 240,000 N/ml and 480,000 N/ml) of jellyfish crude venom. The protection was effective against all considered doses of crude venom and showed the maximum efficacy after preincubation of cells with 10^{-5} M lanthanum sulfate. These results indicate that lanthanum sulfate could be an useful component in preparations devoted to struggle the cutaneous and systemic effects consequent to *Pelagia noctiluca* stings.

Introduction

Cnidarian toxicity is an important subject owing to its influence on several human activities and on public health; in particular, the proliferations of jellyfish can affect bathing, fishing and tourism with serious consequences both from the sanitary and the economic point of view [1]. The stinging capsules of jellyfish, called nematocysts, after contact with human skin are able to produce erythematous and oedematous lesions with formation of vesicles, swelling, burning and dermonecrosis and in sensitive subjects can give rise to systemic symptoms with cardiovascular and nervous involvement. In some regions, such as in tropical seas and in Australian and Asian seas, jellyfish stinging is a pressing problem because in these waters live extremely poisonous species able to cause serious damage and also to kill human beings [2]; on the

contrary, Mediterranean species do not induce lethal or highly harmful effects, excepting in particular sensitive cases. In spite of this, the mauve stinger *Pelagia noctiluca* Forsskål, 1775 (Cnidaria: Scyphozoa), one of the most common Mediterranean jellyfish, being a powerful stinging species, can cause several health problems to humans as it took place during the blooms of the last decades [3-5]. To date, the scarce poisonousness of Mediterranean jellyfish didn't stimulate a specific research aimed to develop drugs or medical aids to counter their stinging effect. Nevertheless, to counteract the unpleasant outcomes of the encounters with *Pelagia noctiluca* a research aimed to verify if some compounds could act as protective agents against jellyfish stinging was undertaken. On the basis of previous studies indicating the protective activity of lanthanum sulfate against jellyfish venoms [6], in this paper the evaluation of the protection of this compound for L929 cells treated with jellyfish crude venom has been carried out; the pertinent results are here presented and discussed.

Materials and methods

Sampling

Specimens of *Pelagia noctiluca* were collected in several zones of the Ligurian Sea and transferred to the laboratory; they were subsequently frozen and maintained at 20°C until utilization.

Preparation of extracts

Crude extracts were prepared by modifying the previously reported method [7]; briefly, specimens were thawed and tissues were disjointed by magnetic stirrer to allow the release of nematocysts. The treatment was prolonged for 24-48 hours until complete breakdown of jellyfish tissues. Afterwards the so-treated tissues were filtered in succession by 200 µm and 64 µm nets to remove the remaining tissue fragments. The nematocysts released from disjointed tissues were counted by hemocytometer Thoma, and their integrity was verified. Subsequently the samples were exposed to several ultrasound cycles (30 seconds alternated with 30 seconds interval) for a total of 30 minutes of effective sonication.

Through this procedure the discharge of approximately 80% of the nematocysts was obtained. The extracts were then centrifuged at 10,000 rpm for 30 minutes to allow the remaining fine particles of tissue and the capsules of discharged nematocysts to settle. The supernatant was then collected and was used to assess the cytotoxicity on cultured cells. The doses were chosen taking into account preliminary tests and using as parameter the number of nematocysts (N) in the crude extract; the following doses were used: A) 120,000 N/ml; B) 240,000 N/ml; C) 480,000 N/ml.

Cell cultures

The continuous cell line L929 (mouse fibroblasts, normal phenotype) was used in the experiments. Cells were maintained at 37°C, in humidified atmosphere with 5% CO₂, in DMEM medium supplemented with 5% FBS, 1% penicillin-streptomycin and 1% L-glutamine.

Evaluation of the cytoprotective activity of lanthanum sulfate

To evaluate the protective activity of lanthanum sulfate, 200,000 L929 cells/well were seeded in 12-well plates; cells were pre-incubated with this compound at three concentrations varying from 10⁻⁴M to 10⁻⁵M for 20 minutes as it was indicated in tests with the venom of *Physalia physalis* [6]; within this range falls the NOEC concentration, as it was verified by preliminary trials. The preliminary tests to evaluate the cytotoxicity of lanthanum sulfate and to find the no observed effect concentration (NOEC) were carried out by using the MTT test. Subsequently, pre-incubated L929 cells were undergone to the three doses of crude extract and cell viability was evaluated.

Treatment of cells

To evaluate the cytotoxicity of crude extracts 200,000 L929 cells/well were seeded in 12-well plates; after 24 hours a set of plates was assigned to controls and another set was preincubated with lanthanum sulfate for 20 minutes; then all cells (both preincubated and non preincubated), excepting those assigned to controls, were exposed for 20 minutes to the different doses of crude extract.

In each experiment five wells with three replicates were considered for a total of 15 wells and 3×10⁶ treated cells. Each experiment was repeated five times. The cytotoxicity of extracts was evaluated both by MTT test and by Trypan Blue dye exclusion.

Results

The integrity of nematocysts released from disjointed tissues resulted always near to 100%. The results of the treatment of L929 cell with lanthanum sulfate evaluated by MTT assay are shown in Fig. 1. On the whole, lanthanum sulfate showed scarce cytotoxicity up to 10⁻⁴M after 20

minutes exposition, while at higher doses cell survival decreased fast. The NOEC for L929 cells was 10⁻⁵M La₂(SO₄)₃. As concerns the evaluation of the cytotoxicity of crude extract from *Pelagia noctiluca* MTT assay do not produce reproducible outcomes, perhaps owing to colorimetric interferences; on the contrary, TB showed always reproducible and accurate results, thus only TB results will be considered and reported. The results of treatments with crude venom from *Pelagia noctiluca* and the results concerning the protection by lanthanum sulfate are shown in Figs. 2-4.

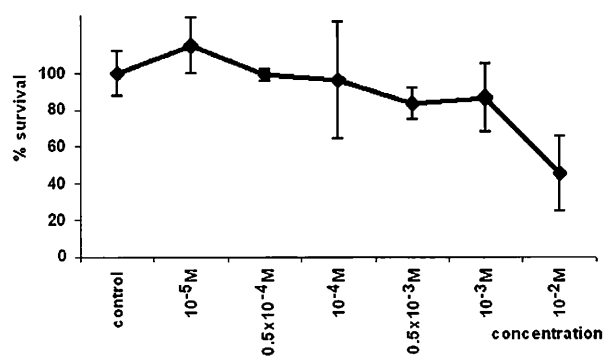


Fig. 1 – Cytotoxicity of lanthanum sulfate on L929 cells after a treatment of 20 minutes, verified by MTT assay.

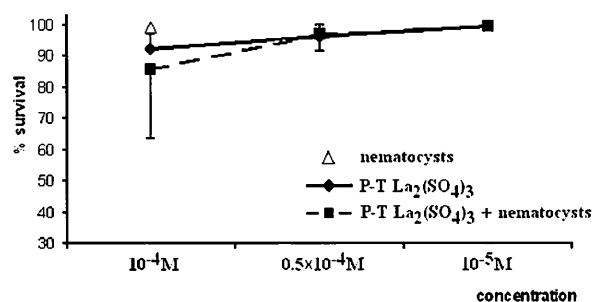


Fig. 2 – Protection of lanthanum sulfate 10⁻⁴M, 0,5×10⁻⁴M and 10⁻⁵M towards cytotoxicity of 120,000 N/ml of *Pelagia noctiluca* after 20 minutes treatment, verified by Trypan blue. The upper error bar refers to cells preincubated with lanthanum sulfate; the lower error bar refers to cells preincubated with lanthanum sulfate and then treated with nematocysts venom. PT: pretreatment.

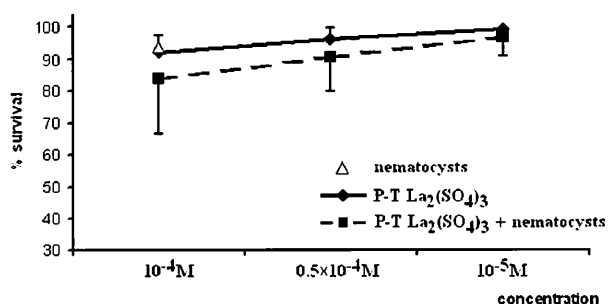


Fig. 3 – Protection of lanthanum sulfate 10⁻⁴M, 0,5×10⁻⁴M and 10⁻⁵M towards cytotoxicity of 240,000 N/ml of *Pelagia noctiluca* after 20 minutes treatment, verified by Trypan blue. The upper error bar refers to cells preincubated with lanthanum sulfate; the lower error bar refers to cells preincubated with lanthanum sulfate and then treated with nematocysts venom. PT: pretreatment.

The crude venom of *Pelagia noctiluca* showed an increasing cytotoxicity scaling up the nematocyst number with cell survival of 99%, 94% and 71% after exposition to 120,000 N/ml, 240,000 N/ml and 480,000 N/ml respectively. The results concerning the evaluation of the protective activity of lanthanum sulfate towards cultured cells show that the efficacy of the protection treatment is high with all considered doses of crude venom. As a matter of fact, in all cases results of treatment with 10^{-5} M lanthanum sulfate get near to those obtained with the NOEC concentration of $\text{La}_2(\text{SO}_4)_3$.

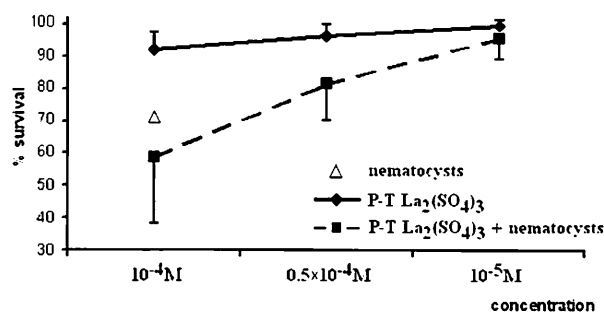


Fig. 4. Protection of lanthanum sulfate 10^{-4} M, 0.5×10^{-4} M and 10^{-5} M towards cytotoxicity of 480,000 N/ml of *Pelagia noctiluca* after 20 minutes treatment, verified by Trypan blue. The upper error bar refers to cells preincubated with lanthanum sulfate; the lower error bar refers to cells preincubated with lanthanum sulfate and then treated with nematocysts venom. PT: pretreatment.

Discussion

Despite the scarce harmfulness of Mediterranean jellyfish, as from the last decades of the last Century several troubles were caused by abnormal proliferations of these organisms, supported mainly by *Pelagia noctiluca* which is known to be a dangerous autochthonous Mediterranean jellyfish [3-5]. On the other hand, as the damage caused by jellyfish venoms on human skin is well known, the study of the cell-damaging activity of poisonous substances produced by jellyfish is of particular concern, also considering that cnidarian venoms do not come only from nematocysts, but are abundant also in tissues [8]. The crude venom of *Pelagia noctiluca* was evaluated for cytotoxicity towards cultured cells [9, 10] observing that it produced severe survival decrease even if its venom resulted less cytotoxic than that of other in nature apparently less venomous jellyfish and anemones [7, 8, 12]. The venom of *Pelagia noctiluca* is of protein nature and has an influence on ATP balance of treated cells [10, 13]. Furthermore, it produces significant hemolysis of chicken, rabbit and human erythrocytes also after freezing and lyophilization [14-16]. Cations of transition metals, such as La^{3+} , were indicated to block many cell Ca^{2+} channels through a competitive antagonism for binding sites [17] and can also inhibit cytolytic toxins such as α -latrotoxin [18] and melittin [19]. As a matter of fact, it was reported that metals, such as lanthanum, can act on membrane phospholipids decreasing their fluidity; in turn, the alteration of fluidity [20] could prevent the formation of pores or channels within cell membrane by cytolytic venoms [21]. This phenomenon could be at the basis of the protection exerted by lanthanum, which was seen to be a

powerful inhibitor of cytolytic toxins [22, 23] and to inhibit the cytolytic action of cnidarian venoms at the level of the cell plasma membrane of cultured cells blocking also, at defined concentrations, venom-induced Ca^{2+} influx into L929 cells [6]. On the basis of these results, it was hypothesized that La^{3+} inhibits Cnidarian venom "by preventing oligomerization of venom proteins in the target membrane by blocking expressed anionic sites needed for oligomerization" [6]. It was also observed that preincubation with lanthanum prevents the increase of cytosolic Ca^{2+} induced in rat myocytes by the venom of the cubomedusa *Chironex fleckeri* and produced a significant attenuation of the response to the venom of *Chironex fleckeri* and *Carybdea xaymacana*. Thus, on the whole lanthanum was indicated to be highly protective for cells and a powerful inhibitor of the cytolysis induced by cnidarian venoms, particularly when cells are pre-incubated with this substance [6] even though other statements indicate that "lanthanum blocked Ca^{2+} influx but not the cytolytic activity of the venom" [25].

As a matter of fact, also the data presented in this paper show that lanthanum sulfate at the concentration 10^{-5} M seems to be effective to protect cells by cytotoxic effects induced by crude extracts from *Pelagia noctiluca*. These results could be of interest for the utilization of lanthanum sulfate in preparations useful to struggle cutaneous and systemic effects consequent to dermatitis induced by *Pelagia noctiluca* stings. Further studies will be essential to define the mechanism of action of lanthanum sulfate to counteract the cell-damaging activity of jellyfish venom.

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