Antinociceptive, anti-inflammatory and muscle relaxant activity of (+)-curcuphenol isolated from marine sponge *Didiscus oxeata*

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Summary

Anti-inflammatory activity of (+)-curcuphenol isolated from the marine sponge *Didiscus oxeata* was confirmed through the carrageenan induced rat paw edema method at the doses of 100, 150 and 200 mg/kg by oral administration, while the antinociceptive activity was verified through the method of writhings induced by acetic acid in mice at the doses of 50, 100, 150 and 200 mg/kg orally administered and the muscle relaxant activity was shown by the isolated organ technique. It was found that (+)-curcuphenol has a high antinociceptive and a moderate anti-inflammatory activity and a potent muscle relaxant activity in toad abdominal rectum muscle in concentration of 100 μg/ml possibly by an antagonistic mechanism with acetylcholine.

Key words: (+)-Curcuphenol - Anti-inflammatory activity - Antinociceptive activity - Muscle relaxant activity.

Resumen

Actividad antinociceptiva, anti-inflamatoria y relajante muscular de (+)-curcufenol aislado de la esponja marina *Didiscus oxeata*

Mediante el método de edema plantar inducido por carragenina en la pata de la rata, (+)-curcufenol aislado de la esponja marina *Didiscus oxeata* mostró una actividad antiinflamatoria moderada, en las dosis de 100, 150 y 200 mg/kg administradas por vía oral, mientras que la actividad antinociceptiva fue confirmada por el método de contorsiones inducidas por ácido acético en ratones mostrando un efecto analgésico muy alto, en las dosis de 50, 100, 150 y 200 mg/kg administradas por vía oral, en comparación con la actividad observada en las dosis evaluadas para los patrones empleados. Además mostró una actividad relajante muscular en el músculo abdominal del sapo, en una concentración de 100 μg/ml, posiblemente por un mecanismo antagonista sobre acetilcolina.

Palabras claves: (+)-Curcufenol – Actividad antiinflamatoria – Actividad antinociceptiva - Actividad relajante muscular.

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Introduction

(-)-Curcuphenol and curcuhydroquinone were isolated and identified as derivatives of the monocyclic aromatic sesquiterpenoid α-curcumene from the Gorgorian soft coral *Pseudopterogorgia rigida* (1). (+)-Curcuphenol and curcudiol (Figure 1) were isolated from the sponge *D. flavus* and their cytotoxic and antifungal activities were evaluated. (+)-Curcuphenol is cytotoxic against in vitro tumor cell lines. An IC50 of 7 μg/ml was observed against P-388 murine leukemia and minimum inhibitory concentrations (MIC) of 10, 1.0 and 0.1 μg/ml were noticed against the human tumor cell line A-549 (lung), HCT-8 (colon) and MDAMB (mammary) respectively. While (+)-Curcuphenol and (+)-Curcudiol have MIC of 8 and 250 μg/ml against *Candida albicans* respectively (2). Also (+)-curcuphenol which was isolated and identified as 2-(1,5-dimethyl-4-hexenyl)-5-methyl phenol from the marine sponge *Didiscus oxeata*, showed that it is responsible for the antimicrobial activity of the sponge against *S. aureus*, *B. anthracis*, *B. subtilis*, *S. epidermis*, *B. cereus* and the fungi *Aspergillus sp.* and *Penicillium sp.* (3).

Recently we have isolated the same compounds from the marine sponge *D. oxeata* and their toxicity in *Artemia salina* and the antitumour activity were determined (4).

This work was carried out with the aim of showing the anti-inflammatory, antinociceptive and muscles relaxant activities of (+)-curcuphenol isolated from the sponge *D. oxeata*.

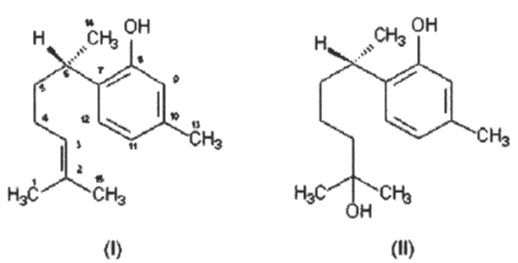

Figure 1. Chemical structure of (+)-curcuphenol (I) and curcudiol (II).

Experimental

Materials and methods

Extraction and isolation

The sample of (+)-curcuphenol employed in this study was isolated from the marine sponge *D. oxeata* in our previous research (4)

Anti-inflammatory activity

This activity was verified against standard sample of indomethacine and the vehicle as a control using the carrageenan induced rat paw edema method described by Winter et al., (5). Female Wistar rats weighing between 130 and 170 g and which were conditioned for 5 days were employed.

The rats were divided in 5 groups of 6 animals each one. By oral administration the (+)-curcuphenol in doses of 100, 150 and 200 mg/kg was administered to group 1, 2, and 3, while the standard indomethacine in dose of 6 mg/kg was administered to group 4 and the vehicle tween 80: ethanol: water (1:1:22) was administered as a control to group 5.

One hour after the substances were administered, the rat paw volumes were measured using a plethysmometer. Simultaneously the inflammatory state was induced by the injection of 0.2 ml of carrageenan 1% in saline solution, in the plantar surface of the rat's paw. At one and 3 hours of the carrageenan injection, the rat's paw

displaced volumes were measured again and the average of the experiments was obtained.

The data on anti-inflammatory activity was analyzed using analysis of variance (ANOVA) and the group means were considered statistically significant if $p < 0.05$.

Antinociceptive activity

The anti-writhing activity was evaluated using the method described by Rahola (6), employing OF1 fasted female mice weighing 20 to 25 g. (+)-Curcuphenol was evaluated by oral administration in doses of 50, 100, 150 and 200 mg/kg. Three groups of 6 mice each were employed for each dose of (+)-curcuphenol, the vehicle [tween 80: ethanol: water, (1:1:22)] and the standard acetylsalicylic acid in dose of 200 mg/kg.

One hour after the administration of the substances, the mice received 0.02 ml/Kg of 1% acetic acid intraperitoneally. For the next 20 minutes, the number of writhings shown in each mouse was recorded.

The data on antinociceptive activity was analyzed using analysis of variance (ANOVA) and the group means were considered statistically significant if $p < 0.05$.

Muscle relaxant activity

According to the observed symptoms in the mice during the determination of the antinociceptive activity (loss of postural tone, abdominal flaccidity and difficulty to walk), it was decided to determinate the muscle relaxant activity following the method of abdominal rectal muscle of the toad (7-9).

The toad abdominal rectum muscle was tied at the pelvic and scapular insertions then was placed in an organ bath at 37 ± 0.5 °C, with "Ringer Rana" Solution. The muscular tension was adjusted and the normal activity was recorded during 5 minutes in a physiograph, Narco Bio0systems DMP- 4A with amplitude 6.0 mV and paper velocity of 0.1 cm/sec, then the pharmacological responses were recorded in the same order in the following solutions: acetylcholine chloride (5 μg/ml), quinidine sulphate (100 μg/ml), acetylcholine chloride (5 μg/ml), (+)-curcuphenol (100 μg/ml), acetylcholine chloride (5 μg/ml), potassium chloride (5000 μg/ml), (+)-curcuphenol (100 μg/ml), acetylcholine chloride (5 μg/ml), (+)-curcuphenol (100 μg/ml), acetylcholine chloride (5 μg/ml) and acetylcholine chloride (100 μg/ml).

Results and Discussion

Antinociceptive activity

According to the statistic analysis ANOVA, (+)-curcuphenol at the evaluated doses decreased the writhing in mice induced by 1% acetic acid in the following percentage of inhibition: 50 mg/kg (36.3%), 100 mg/kg (68.9%), 150 mg/kg (76.5%) and 200 mg/kg (83.5%) compared with the standard acetylsalicylic acid in dose of 200 mg/kg, which produced 53.3% of inhibition. The dose of 50 mg/kg did not show significant activity compared with the

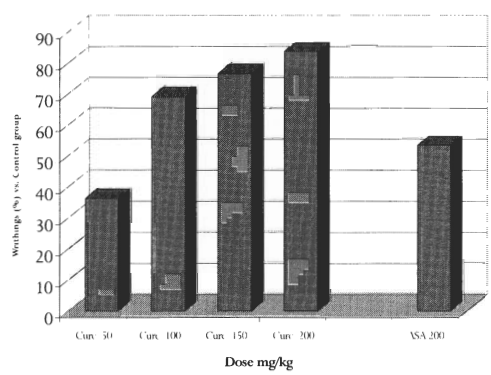

Figure 2. Antinociceptive activity of (+)-curcuphenol.

acetylsalicylic acid. Mean while the dose of 100 mg/kg showed higher activity (68.9%) than that showed by the standard acetylsalicylic acid (53.3%) in 200 mg/kg. In spite of the higher activity of the doses of 150 and 200 mg/kg of (+)-curcuphenol (76.5 and 83.5% respectively) they produced adverse effects as abdominal flaccidity and difficulty to walk (Figure 2).

Therefore, we consider that the best anti-nociceptive activity with respect to the acetylsalicylic acid was produced by (+)-curcuphenol in the dose of 100 mg/kg.

Anti-inflammatory activity

All the evaluated doses of (+)-curcuphenol showed an anti-inflammatory properties on the carrageenan induced edema. The anti-inflammatory inhibition percentage was 44.2%, 46.7% and 51.7% at 3 hours for the doses of 100, 150, 200 mg/kg respectively compared with 55.9% for the indomethacine in a dose of 6.0 mg/kg.

The results showed an important anti-inflammatory effect, which inhibited almost 50% of the induced edema (Figure 3).

Muscle relaxant activity

Muscular relaxation of (+)-curcuphenol in the toad abdominal muscle was observed in the presence of acetylcholine. (+)-Curcuphenol in concentration of 100 μg/ml showed a significant decrease (70%) in the contraction induced by the acetylcholine in concentration of 5 μg/ml.

A decrease in the muscle contraction was produced by the addition of 100 μg/ml of (+)-curcuphenol similar to the effect produced by quinidine in concentration of 100 μg/ml, but it was not recovered by the addition of acetylcholine in concentration of 5 μg/ml, however to verify a possible antagonist effect of the (+)-curcuphenol and not the death of the muscle by the addition of potassium chloride in concentration of 5 μg/ml the muscle was contracted again to the normal state. But when (+)-curcuphenol in the concentration of 100 μg/ml was added to the organ bath the contraction was antagonized and did not reproduced to the initial state even by the addition of acetylcholine in concentration of 100 μg/ml (Figure 4).

According to the results (+)-curcuphenol in concentration of 100 μg/ml presents a potent muscle relaxant activity in toad abdominal rectum muscle possibly by an antagonistic

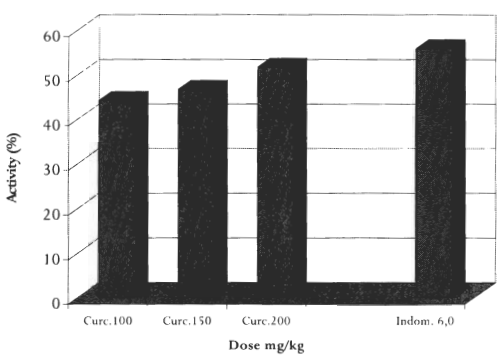

Figure 3. Anti-inflammatory activity of (+)-curcuphenol.

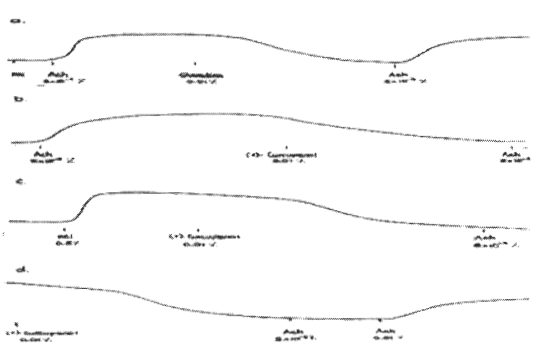

Figure 4. Muscle relaxant activity of (+)-curcuphenol.

mechanism with acetylcholine, by the comparison of the effect of quinidine and (+)-curcuphenol which inhibited the contraction induced by the acetylcholine.

Conclusions

(+)-Curcuphenol has a high antinociceptive and a moderate anti-inflammatory activities in the dose of 200 mg/kg and a potent muscle relaxant activity in toad abdominal rectum muscle in concentration of 100 μg/ml possibly by an antagonistic mechanism with acetylcholine.

The result of the present work shows these interesting pharmacological activities of (+)-curcuphenol, which are of great importance in the search for new structure models from natural source that can lead to the discovery of novel drugs in the future to improve our health. This encourage the continuation of more study of its therapeutic potentiality.

Acknowledgments

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