Toxicon 55 (2010) 1331–1337

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Comparative effects of lantadene A and its reduced metabolite on mitochondrial bioenergetics

Andréa F. Garcia [a], Hyllana C.D. Medeiros [a], Marcos A. Maioli [a], Michele C. Lima [a], Bruno A. Rocha [b], Fernando B. da Costa [b], Carlos Curti [c], Milton Groppo [d], Fábio E. Mingatto [a,*]

[a] Laboratório de Bioquímica, Faculdade de Zootecnia, UNESP–Univ Estadual Paulista, Campus Experimental de Dracena, Dracena, SP 17900-000, Brazil
[b] Departamento de Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP 14040-903, Brazil
[c] Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP 14040-903, Brazil
[d] Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP 14040-901, Brazil

ARTICLE INFO

Article history:
Received 1 September 2009
Received in revised form 23 January 2010
Accepted 3 February 2010
Available online 10 February 2010

Keywords:
Lantana camara
Lantadene A
Reduced lantadene A
Hepatotoxicity
Mitochondria
Bioenergetics

ABSTRACT

Lantana (*Lantana camara* Linn.) is a noxious weed to which certain medicinal properties have been attributed, but its ingestion has been reported to be highly toxic to animals and humans, especially in the liver. The main hepatotoxin in lantana leaves is believed to be the pentacyclic triterpenoid lantadene A (LA), but the precise mechanism by which it induces hepatotoxicity has not yet been established. This work addressed the action of LA and its reduced derivative (RLA) on mitochondrial bioenergetics. At the concentration range tested (5–25 μM), RLA stimulated state-4 respiration, inhibited state-3 respiration, circumvented oligomycin-inhibited state-3 respiration, dissipated membrane potential and depleted ATP in a concentration-dependent manner. However, LA did not stimulate state-4 respiration, nor did it affect the other mitochondrial parameters to the extent of its reduced derivative. The lantadenes didn't inhibit the CCCP-uncoupled respiration but increased the ATPase activity of intact coupled mitochondria. The ATPase activity of intact uncoupled or disrupted mitochondria was not affected by the compounds. We propose, therefore, that RLA acts as a mitochondrial uncoupler of oxidative phosphorylation, a property that arises from the biotransformation (reduction) of LA, and LA acts in other mitochondrial membrane components rather than the ATP synthase affecting the mitochondrial bioenergetics. Such effects may account for the well-documented hepatoxicity of lantana.

1. Introduction

Lantana (*Lantana camara* Linn.) is one of the most poisonous weeds in the world. The noxious properties of the plant are well documented: it causes cholestasis, hepatotoxicity, photosensitization, and even fatality in cattle, horses, sheep, dogs, and humans (Wolfson and Solomons, 1964; Tokarnia et al., 1984; Black and Carter, 1985; Fourie et al., 1987; Sharma et al., 1988; Pass, 1991; Brito et al., 2004). However, despite its toxic effects, *L. camara* is extensively used in popular medicine because of its anti-inflammatory, antipyretic, antispasmodic, and antibiotic properties (Sharma et al., 2007b).

The most well-known lantana compounds are the lantadenes, which belong to the pentacyclic triterpenoid oleanane series. The most abundant triterpene acid is lantadene A (LA); it has been implicated as the main culprit responsible for the toxic effects of the plant (Sharma et al., 1991, 2000, 2007b). Despite evidence that mitochondria are affected by compounds present in lantana (Sharma et al., 1982; Sharma, 1984), the mechanism by which it

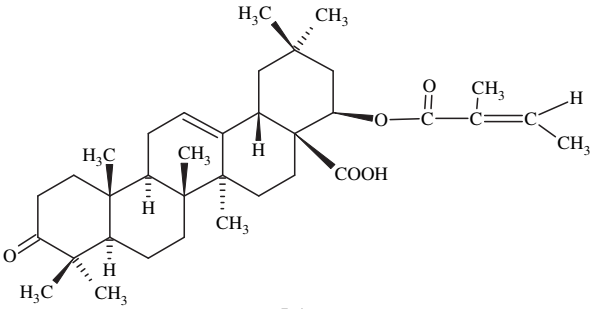
* Corresponding author. Tel.: +55 18 3821 8200; fax: +55 18 3821 8208.
E-mail address: fmingatto@dracena.unesp.br (F.E. Mingatto).

0041-0101/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2010.02.004

induces toxicity has not yet been clearly established. On the other hand, it was recently demonstrated that some lantadenes exhibit *in vitro* and *in vivo* antitumor activity (Sharma et al., 2007a, 2008; Kaur et al., 2008).

Mitochondria carry out a variety of biochemical processes, but their main function is to produce a majority (>90%) of cellular ATP. The proton motive force, whose major impetus is the membrane potential ($\Delta\psi$) generated by electron transport along the respiratory chain in the inner mitochondrial membrane, drives ATP synthesis via oxidative phosphorylation (Mitchell, 1961). Uncouplers of oxidative phosphorylation in mitochondria inhibit the coupling between electron transport and phosphorylation reactions and thus inhibit ATP synthesis (Terada, 1990). They increase the permeability of the inner mitochondrial membrane to protons along a gradient running from intermembrane to matrix spaces; under this condition, the organelle is no longer capable of sustaining ATP synthesis (Kadenbach, 2003). It is believed that mitochondrial uncoupling is a relevant mechanism for xenobiotic-induced toxicity, particularly in the liver, which is the major site of xenobiotic uptake and metabolism (Wu et al., 1990). In addition, results from our research group suggest that mitochondria are the target organelle of toxic compounds isolated from plants (Mingatto et al., 2007; Santos et al., 2009).

To date, no antidote to lantana toxicity is available, and treatment of its symptoms has met with limited success (Sharma et al., 2007b). Thus, an understanding of the mechanism of lantana toxicity at the cellular and molecular levels would help in the development of antidotes and more rational therapies for lantana poisoning. In the present work, we addressed the actions of LA isolated from *L. camara* and its reduced derivative, known as RLA (Fig. 1), on mitochondrial bioenergetics by assessing their effects on respiration, membrane potential, ATP levels and ATPase activity in rat liver mitochondria. The influences of lantadene biotransformation on mitochondrial function and liver toxicity are considered.



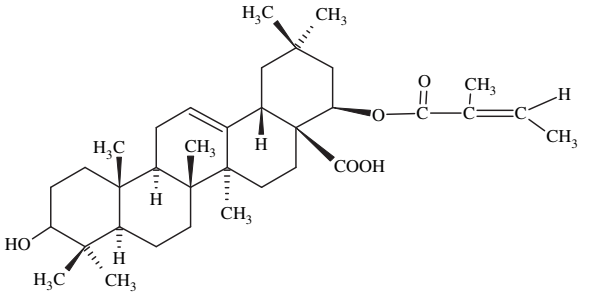


Fig. 1. Chemical structures of lantadenes used in this study.

2. Materials and methods

2.1. Plant material

L. camara Linn. (Lantana, family Verbenaceae) leaf samples were collected from a rural area in Monte Castelo (21°18′S, 51°34′10″W), São Paulo, Brazil. A voucher specimen was identified (number SPFR 10364) by Prof. Milton Groppo and deposited in the herbarium of the Departamento de Biologia da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil.

2.2. Extraction and isolation of lantadenes

The lantana leaf samples were dried in the shade at 37 °C and ground into powder in an electric grinder. To 400 g of the powder, 2000 mL of methanol were added and the material was macerated for 24 h at room temperature with intermittent shaking. The extract was filtered through a muslin cloth and decolorized with 70 g of activated charcoal, which yielded a golden yellow extract. The solvent was removed under reduced pressure, and the residue (15 g) was suspended in a methanol-H_2O mixture (1:7, v/v) and extracted with chloroform ($CHCl_3$, 2 × 40 mL). The organic layer was dried over anhydrous Na_2SO_4 and the final dried residue (6 g) was chromatographically purified over a silica gel column (180 g, 60–120 mesh Merck, 7736) using $CHCl_3$ and $CHCl_3$-methanol (99.5:0.5, v/v) as the mobile phase. The lantadene-rich fraction was further purified and isolated by thin layer chromatography (silica gel PF254, 1 mm thickness, Merck 7730) using $CHCl_3$-methanol (99.5:0.5, v/v) as the eluting solvent (Sharma and Dawra, 1991) to afford the known lantadene A and reduced lantadene A. The compounds were identified by nuclear magnetic resonance (NMR) of ^1H and comparison with data from the literature (Sharma et al., 1987, 1990). The purity of the compounds was estimated by thin layer chromatography using different solvent systems, as well as ^1H NMR analysis.

2.3. Animals

Male Wistar rats weighing approximately 200 g were housed in plastic cages under regulated temperature (20 °C) and the light/dark cycle (12 h:12 h). They were fed commercially pelleted rat food (Purina, Brazil). The experimental protocols were approved by the Ethical Committee for the Use of Laboratory Animals of the Faculdade de Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Dracena.