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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Oxidation of di- and polyamines: in vitro effect of amino aldehydes on the vitality of *Leishmania promastigotes*

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**Abstract** The aminoaldehydes 4-aminobutanal and 5-aminopentanal, derived from the oxidation of the diamines putrescine and cadaverine, and 1-(3-aminopropyl)-4-aminobutanal and aminodialdehyde, derived from the oxidation of the polyamines spermidine and spermine, were produced utilizing a copper amine oxidase (CAO) from *Euphorbia characias* latex and tested with in vitro cultivation of *Leishmania infantum* promastigotes. Whereas the aminoaldehydes derived from the oxidation of the diamines were stimulating factors for growth of *Leishmania infantum* promastigotes, the aldehydes derived from polyamines oxidation had a drastic inhibitory effect on the vitality and growth of these parasites. Thus, a double scenario arises, showing the use of aldehydes from diamines to obtain a large number of organisms of *Leishmania infantum* promastigotes to use in serological studies, whereas the aldehydes derived from polyamines could be used as a new strategy for therapeutic treatment against these parasites.

**Keywords** Leishmania · Promastigotes · Aldehydes · Amine oxidase · Diamines · Polyamines

## Introduction

Leishmaniasis is an acute systemic polymorph protozoary disease of skin, mucous membrane, and internal organs, caused by intracellular obligated parasites belonging to the *Leishmania* genus. In clinical medicine leishmaniasis is defined

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as visceral, cutaneous, and mucocutaneous. Cutaneous and mucocutaneous leishmaniasis initially appears with a papule, which evolves into an ulcer, and its cicatrization occurs within an extremely variable range of time. *Phlebotomus perfiliewi*, a hematophagous insect belonging to the *Phlebotomus* genus, is responsible for cutaneous leishmaniasis transmission, and the etiological agent is *Leishmania infantum*. Visceral leishmaniasis manifests with fever, lynphadenopaty, hepatomegaly, reduced weight, anemia, leukopenia, thrombocytopenia, and infaust effect if not promptly treated. *Phlebotomus perniciosus* is mainly responsible for visceral leishmaniasis transmission, but *P. major* or *P. ariasi* can also be responsible. The etiological agent of visceral leishmaniasis can even be *Leishmania infantum*.

Latest studies highlight that intracellular proliferation and differentiation of some species of *Leishmania* amastigotes are improved by substances as the polyamines spermine and spermidine (Cona *et al*., 1991). Many species of *Leishmania* are able to synthesize high levels of putrescine, which seems to play an important role in proliferation processes and cell differentiation.

The physiological function of aliphatic amines, such as putrescine, cadaverine, spermidine, and spermine, in living organisms, has yet not been completely established. Intracellular concentrations of polyamines are highly regulated (Cohen, 1998). If they accumulate excessively within cells, due to either very high extracellular concentrations or deregulation of the systems that control their homeostasis, polyamines can cause cytotoxic effects.

Copper amine oxidases (CAOs) [amine: oxygen oxidoreductase (deaminating) (copper containing); EC 1.4.3.6] are important enzymes in the cellular and extracellular metabolism of amines. CAOs catalyze the oxidative deamination of primary amino groups of mono-, di-, and polyamines, extracting two electrons from amines and transferring them to molecular oxygen, to form the corresponding aldehyde, ammonia, and hydrogen peroxide (Medda *et al*., 1995).

Thus CAOs are important enzymes contributing to the regulation of levels of polyamines, catalyzing their oxidative deamination. Spermidine and spermine may also become a source of toxic metabolites (Arancia *et al*., 2004).

The aim of this report is to study the rule played by aldehydes produced from di- and polyamine on *Leishmania* promastigotes in vitro and to observe the effects by monitoring cellular density and vitality.

## Materials and methods

Amine oxidase from the latex of *Euphorbia characias* (ELAO), a perennial Mediterranean shrub, was purified, following the method described by Padiglia *et al*., (1998). Highly purified enzyme preparations (0.5 μM) were incubated in 100 mM Tris-HCl buffer (pH 7.0) at 35°C in presence of 1–5 mM diamine (putrescine or cadaverine) or 1–5 mM polyamine (spermidine or spermine). After 30 min, when no more di- or polyamine oxidation occurred in the incubation mixture as observed by high-performance liquid chromatography (HPLC) analysis, the enzymatic reactions were stopped by heat inactivation (97°C for 5 min).

The incubates were centrifuged at 18,000 rpm for 15 min and the precipitate was discarded.

The disappearance of di- and polyamines was monitored using Agilent 1100 series HPLC using the method described by Lozanov *et al*., (2007).

*Leishmania infantum* promastigote strain (MHOM-TN-80-IPT1), under cultivation in the Serology Laboratory of the Zooprofilatic Institute of Cagliari was provided by the Istituto Superiore di Sanità.

*Leishmanias*, withdrawn from either popliteal and axillary lymph node or rasping ulcerous lesions of ill dogs, were grown in a specific Tobie medium modified by Evans, composed of solid (containing beef extract and peptone) and liquid phase (containing a saline solution and glucose). To this medium gentamycin (0.1 mg /ml) was added, followed by incubation at 22°C.

After 1 week the cultures were observed using microscopy to verify presence or absence of parasites. Aliquots of *Leishmania infantum* promastigotes ($10^6$) were added to five flasks containing culture medium and aminoaldehydes (1–5 mM). As a control, aliquots of *Leishmania infantum* promastigotes were inoculated into five flasks containing culture medium alone or culture medium containing 1–5 mM putrescine, cadaverine, spermidine or spermine. Analyses of the cultures were done twice a week, on the fourth and seventh day. Microscopic analyses were carried out by density estimation determined by Bürker counting chamber. We considered as health index both the mobility and the form of parasites. On the contrary, in joined cells, low mobility and globular form were considered a slight illness state of the cultures.

## Results

The oxidation of mono-, di-, and polyamines by CAOs is reported in Fig. 1. The oxidation of putrescine gives hydrogen peroxide and 4-aminobutanal, which yields 1-pyrroline by spontaneous cyclization (Fig. 1a). In similar manner oxidation of cadaverine gives hydrogen peroxide and 5-aminopentanal, which yields 1-piperei-dine (Fig. 1b). Oxidation of spermidine gives hydrogen peroxide and 1-(3-aminopropyl)-4-aminobutanal. The latter spontaneously cyclizes to 1-(3-aminopro-pyl)pyrrolinium, which undergoes further spontaneous rearrangements to 1,5-diazobicyclo[4.3.0]nonane (Fig. 1c) (Smith *et al*., 1986).

The polyamine spermine is oxidized at both the terminal amino groups, giving two moles of hydrogen peroxide and a dialdehyde that can react with primary amino groups of free spermine, leading to the formation of aromatic pyrimidinic ring (Fig. 1d) (Padiglia *et al*., 1997).

Testing different concentrations of 4-aminobutanal, the aldehyde formed from putrescine oxidation (1–5 mM), the *Leishmania* promastigotes showed a negative drop in density and vitality at the fourth day of treatment (Fig. 2). Unexpectedly, a considerable increase in both density and vitality of *Leishmania* promastigotes was observed at the seventh day of treatment (Fig. 2) for all the tested aldehyde concentrations. Similar results, if not identical, were obtained using 5-aminopentanal, the aldehyde formed from the cadaverine oxidation. Neither inhibition nor activation were seen in the cultures containing 1–5 mM putrescine or cadaverine.

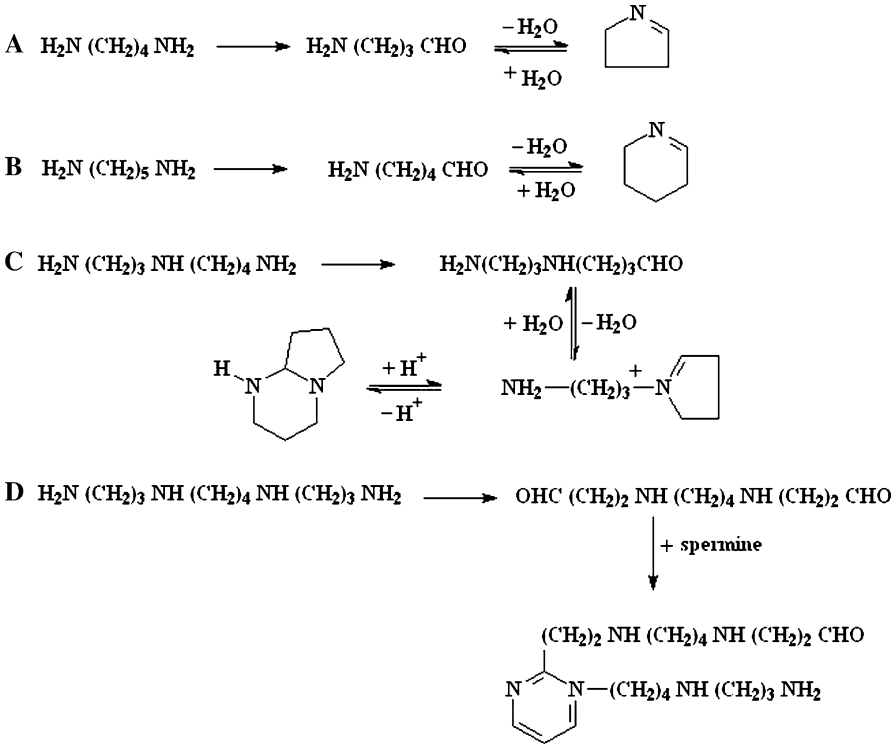


**Fig. 1** Oxidation of di- and polyamines by copper amine oxidase: **a** putrescine, **b** cadaverine, **c** spermidine, and **d** spermine

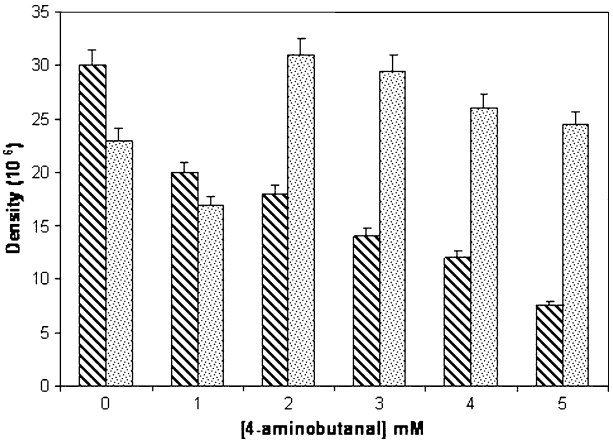


**Fig. 2** Density of *Leishmania* promastigotes grown in the presence of 1–5 mM 4-aminobutanal. Observation was performed on the fourth (▨) and seventh (⁘) day. 4-Aminobutanal is a product formed from the oxidation of putrescine by ELAO

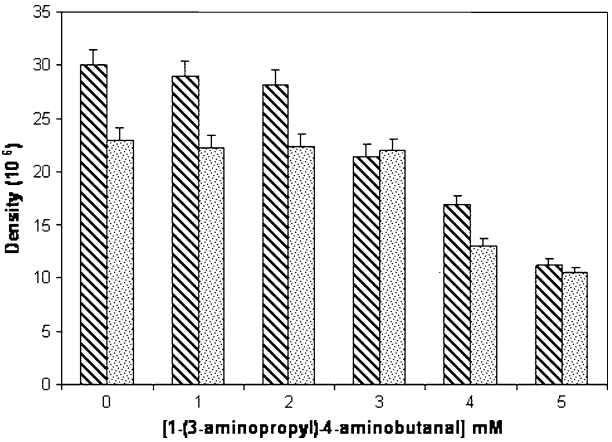


**Fig. 3** Density of *Leishmania* promastigotes grown in the presence of 1–5 mM 1-(3-aminopropyl)-4-aminobutanal. Observation was performed on the fourth (▨) and seventh (⁘) day. 1-(3-Aminopropyl)-4-aminobutanal was derived from spermidine oxidation by ELAO

In the presence of 1-(3-aminopropyl)-4-aminobutanal, the aldehyde derived from the spermidine oxidation, the tested *Leishmania* cultures were shown to be sensitive up to 3 mM aldehyde concentration, for which promastigotes density was halved (Fig. 3).

Finally, in the presence of the dialdehyde derived from the spermine oxidation, promastigotes showed a gradual decrease in density from 1 to 3 mM dialdehyde concentration, followed by a dramatic decrease at 4–5 mM dialdehyde concentration. Moreover at 5 mM dialdehyde concentration we observed a totally absence of promastigote vitality with respect to the control cultures (Figs. 4 and 5).

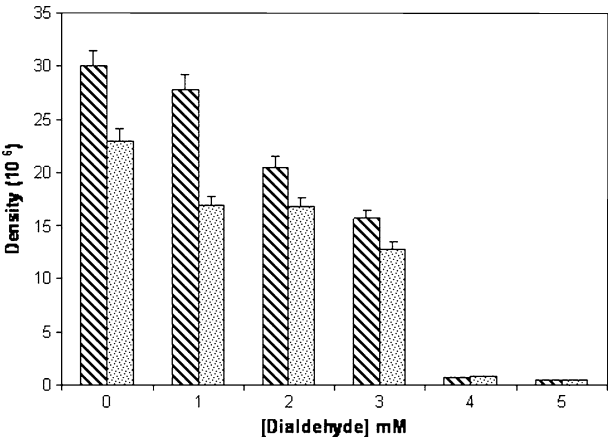


**Fig. 4** Density of *Leishmania* promastigotes grown in the presence of 1–5 mM dialdehyde. Observation was performed on the fourth (▨) and seventh (⁘) day. The dialdehyde was derived from spermine oxidation by ELAO

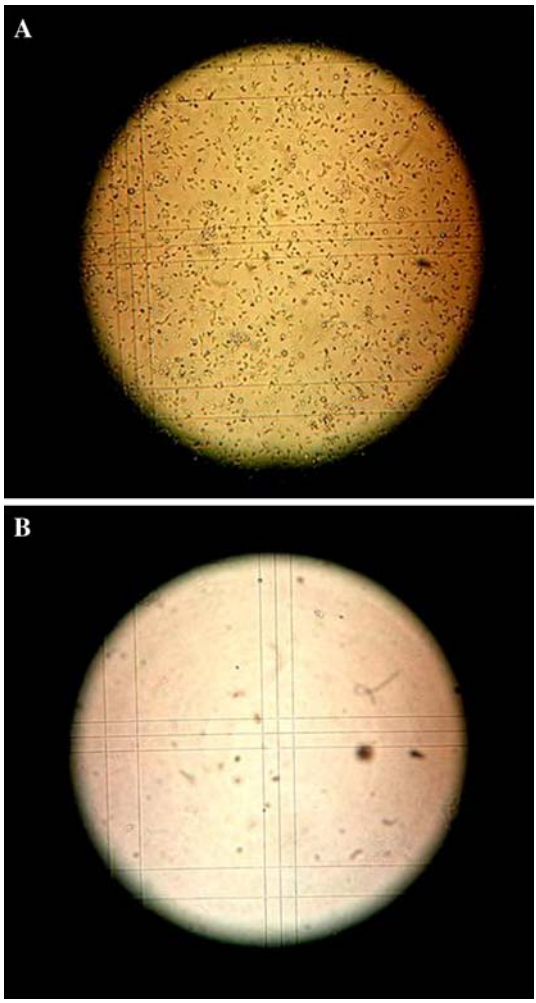


**Fig. 5** Microscopic analysis performed by Bürker counting chamber. *Leishmania* promastigotes growth in the absence **a** and presence **b** of 5 mM dialdehyde derived from spermine oxidation

Our purpose is to study the rule played by di- and polyamines in the metabolism of *Leishmania infantum*, to establish a probable correlation between parasite proliferation and availability of biogenic amines. Moreover, research on these protozoans reported to date has not evaluated the activity and vitality of promastigotes in presence of the aldehyde reaction products of di- and polyamine oxidation. The results presented herein, in combination with current knowledge of intracellular proliferation and differentiation of some species of *Leishmania,* allows us to establish two main conclusions. First, 4-aminobutanal and 5-aminopentanal seem to exert a protective effect on growth of promastigotes. This was expected since many species of *Leishmania* are able to synthesize high levels of putrescine, which seems to play an important role in proliferation processes and cell differentiation. Thus, the aminoaldehydes derived from the oxidation of the diamines putrescine and cadaverine are stimulating factors on growth of *Leishmania*

*infantum* promastigotes in vitro, and can be used to obtain a large number of organisms to use in serological studies.

Second, the aldehydes derived from the oxidation of spermidine and spermine seem to inhibit by the same mechanism, with the dialdehyde showing higher negative effect on *Leishmania* promastigote proliferation. It is probably that spermidine and spermine could become a source of toxic metabolites, or, most intriguingly, that these aldehydes could interfere with DNA duplication. Thus, the aldehydes derived from the polyamines spermidine and spermine have a drastic inhibitory effect on vitality and growth of the *Leishmania* promastigotes and could be used as a new strategy for therapeutic treatment against these parasites.

It is difficult to establish a definite role for these aldehydes in the metabolism of *Leishmania* promastigotes. Since the normally used therapeutic treatment for leishmaniasis does not lead to total healing, the discovery of new molecules and strategies to overcome leishmaniasis remains a target for the future. Thus, we think that inhibition of *Leishmania* growth and vitality by aminoaldehydes warrants further in vivo and ex vivo investigation.

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