European Journal of Pharmacology 734 (2014) 35–41

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Review

Anti-inflammatory effects of diethylcarbamazine: A review

Christina Alves Peixoto *, Bruna Santos Silva

Laboratório de Ultraestrutura, Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz, Cidade Universitária, Av. Moraes Rego s/n, Recife CEP 50670-420, PE, Brazil

ARTICLE INFO

Article history:
Received 6 January 2014
Received in revised form
19 March 2014
Accepted 24 March 2014
Available online 12 April 2014

Keywords:
Diethylcarbamazine
Cyclooxygenase
Lipoxygenase
NF-κB

ABSTRACT

Diethylcarbamazine (DEC) interferes with cyclooxygenase and lipoxygenase pathways, reducing the production of thromboxane, prostacyclin, prostaglandin and leukotrienes. Recent studies using different experimental models of inflammation have indicated that DEC, in addition to inhibiting cyclooxygenase and lipoxygenase pathways, also inhibits nuclear transcription factor kappa B (NF-κB) activation, which is a key regulator of proinflammatory genes such as TNF-α, IL-1β, inducible nitric oxide synthase (iNOS) and even cyclooxygenase 2 (COX-2). The aim of the present study is to provide a comprehensive summary of DEC, including a description of filaricidal action, inhibition of synthesis and secretory pathways, immunomodulatory activity, and specific inhibition of lipoxygenase and cyclooxygenase pathways.

Contents

1. Introduction

The well-known drug diethylcarbamazine (DEC) is used throughout the world against lymphatic filariasis. However, in recent years many studies have described other pharmacological activities of DEC. Some preliminary clinical studies have found DEC to be reasonably effective in asthmatic conditions, and a number of experimental studies have used DEC as a potent leukotriene inhibitor. It has now been established that DEC interferes with the cyclooxygenase and lipoxygenase pathways, reducing: eicosanoid production. The aim of this review is to provide a brief perspective of research into the role of DEC in inflammatory dysfunction.

2. DEC as a filaricidal drug

Diethylcarbamazine (DEC) has been used successfully by public health authorities as a key tool in the elimination of lymphatic filariasis in several countries. Its filaricidal activity was discovered in a study by Hewitt et al. (1947) of wild cotton rats infected with *Litomosoides sigmodontis*. Afterwards, Santiago-Stevenson et al., (1948) demonstrated the effectiveness of the drug against *Wuchereria bancrofti* in human patients. Although DEC is the drug of choice for the treatment of lymphatic filariasis, its mode of action is still controversial.

One of the most frequent findings is that DEC increases microfilarial adhesion to endothelial cells and granulocyte (Johnson et al.,

* Correspondence to: Laboratório de Ultraestrutura Centro de Pesquisas Aggeu Magalhães (CPqAM-FIOCRUZ)Av. Moraes Rego s/n, CEP: 50670-420, Cidade Universitária, Recife PE, Brazil. Tel. +55 81 2101 2557; fax. +55 81 2101 2500.
E-mail address: peixoto.christina@gmail.com (C.A. Peixoto).

http://dx.doi.org/10.1016/j.ejphar.2014.03.046
0014-2999/

1988; Piessens and Beldekas, 1979; Rácz et al., 1982). Further biochemical studies found this adhesion to be the result of the inhibition of cyclooxygenase and lipoxygenase pathways (Mathews and Murphy, 1982; Ogletree, 1987; Razin et al., 1984). From these results it was suggested that DEC stimulates the innate arm of the immune system. In contrast, evidence indicates that the microfilaricidal effect of DEC is not dependent on a specific humoral response. Weiner and Soulsby (1982) found that DEC reduced microfilariae levels by 95.8%, even when microfilariae of *L. sigmodontis* released in vitro were transfused into a naive animal, suggesting that an adaptive immune response was not a sufficient condition for DEC effectiveness. Similarly, Vickery et al. (1985) showed that DEC mediates the clearance of *Brugia pahangi* microfilariae in immunodeficient nude mice.

Several other proposals have suggested that DEC has no directly effect on microfilarial surface, as exposure of the microfilariae to high concentrations of DEC left them unharmed. These results led to the idea that DEC has no direct effect on filarial parasites (Hawking, 1979; Hawking et al., 1950; Hawking and Laurie, 1949; Johnson et al., 1988). Barranco et al. (1962) described the paralysis of microfilariae treated *in vitro*, but unfortunately the methodology of this study used imprecise DEC dilutions. However, Rathaur et al. (2009) incubated adult female worms and microfilariae of *Setaria cervi*, a bovine filarial parasite, with 100 μM of DEC, aspirin or indomethacin for 4 hours and observed that aspirin irreversibly affected the motility of both microfilariae and adult worms, while indomethacin and DEC were effective only at the microfilarial stage, and had no significant effect on adult parasites, even at higher concentrations. DEC treated microfilariae were straight, immobile and had a wrinkled surface.

Ultrastructural studies developed in the laboratory of the authors of the present study showed drastic morphological damage to microfilariae of *W. bancrofti* after in vitro and in vivo treatment with DEC, indicating a possible direct mode of action. DEC caused severe damage of microfilarial cells, including the presence of large vacuoles, lysis of the cytoplasm and chromatin and bodies extruding from the plasma membrane, features indicative of an apoptotic process (Florêncio and Peixoto, 2003a, 2003b; Peixoto et al., 2004) which were confirmed using molecular testing such as ligation-mediated polymerase chain reaction and in situ terminal deoxynucleotidyl transferase mediated dUTP nick end-labeling at light and transmission electron levels (Peixoto et al., 2008).

Pharmacological studies showed that DEC interferes with arachidonic acid metabolism, acting as an anti-inflammatory drug. It has been found that DEC blocks a number of steps in both the cyclooxygenase (COX) and lipoxygenase pathways, including the inhibition of leucocyte chemotaxis, granulocyte degranulation, and peripheral vasodilation (Maizels and Denham, 1992).

Filarial parasites also synthesize and release prostanoids, particularly prostacyclin and PGE2, which are vasodilators and potent platelet anti-aggregatory factors (Brattig et al., 2006; Liu et al., 1990; Liu and Weller, 1992, 1990; Sommer et al., 2003). Furthermore, it has also been reported that DEC is a potent inhibitor of parasitic prostaglandin H synthase (PGHS) (cyclooxygenase), which is the first and rate-limiting enzyme in the transformation of polyunsatured fatty acids into prostaglandins in filarial parasites (Kanesa-thasan et al., 1991). Accordingly to Rathaur et al. (2009), *Setaria cervi* contains significant amounts of prostaglandin H synthase-like enzyme (Sc-like-PGHS). The same authors also provided experimental evidence that DEC, indomethacin and aspirin inhibit Sc-like-PGHS at very lower concentrations, and hypothesized that the microfilaricidal activity of DEC may partially depend on the inhibition of the parasitic PGHS.

Recently, Sankari et al. (2013) observed a significant reduction in PGE2 and 6-keto-PGF1α concentrations in microfilaraemic sera after 12 h of DEC treatment, suggesting that the mechanism by which DEC lowers the level of microfilariae in the circulation may in part involve its effects on host endothelial and parasite eicosanoid production.

Some studies demonstrated that nitric oxide (NO) plays an important role in host defense against filarial parasites *in vitro* (Rajan et al., 1996; Taylor et al., 1996), but no evidence was found that DEC itself induces NO synthesis in murine macrophages and rat endothelial cells *in vitro* (Rajan et al., 1998). However, McGarry et al. (2005) confirmed a lack of activity of DEC in mice deficient in iNOS infected with *B. malayi*, in addition to a lower loss of COX-1 protein in peritoneal exudate cells. According to these authors, it seems that inducible NO is essential for rapid sequestration of microfilariae, and DEC probably stimulates its secretion via interaction with cyclooxygenase pathways.

Recently, Singh and Rathaur (2010) demonstrated that exposure in vitro of the filarial parasite *Setaria cervi* to a combination of DEC plus aspirin (100 μM) decreased PGHS activity leading to an increase in NO level. NO inhibits tyrosine phosphatases, increasing mitochondrial permeability through Bax, which allows the release of cytochrome c into cytosol and activate caspases. Some apoptosis markers such as DNA fragmentation and ladder formation, upregulation of Bax and decrease of Bcl-2 suggested that adult *Setaria cervi* worms were killed due to apoptosis. However, these effects were not observed when the worms were incubated with DEC alone or aspirin. In conclusion, taking into account existing data, DEC may directly affect filarial worms mediated by the inhibition of cyclooxygenase pathways (PGHS) leading to an increase in NO levels, which in high concentrations, are known inductors of mitochondrial-mediated apoptosis.

3. DEC acting as an inhibitor of synthesis and secretory pathways

DEC appears to inhibit synthesis and excretory activities in some cells. Ridge et al. (1980) showed that macrophages in culture synthesize and secrete a soluble factor(s) that induces the synthesis of collagenase in primary cultures of rabbit chondrocytes. Since macrophages are often present in inflammatory sites this would provide a possible mechanism of local connective tissue destruction. Other studies have indicated that a lipoxygenase pathway of arachidonic metabolism is critical in activating various types of cells. The incubation of chondrocytes with MCM (Macrophage Conditioned Medium) and low doses of indomethacin (1–10 μM) had no effect on collagenase synthesis. However, the use of lipoxygenase inhibitors such as NDGA (a nonspecific inhibitor) and DEC inhibited the synthesis of collagenase in chondrocytes. These inhibitors did not affect collagenase activity nor did they interfere with the activation of latent collagenase. This data indicated that although cyclooxygenase plays no role in the MCM dependent induction of collagenase in chondrocytes, lipoxygenase activity may be essential (Nolan and Pickett, 1985).

Similarly, in Stevens et al., (1985) performed biochemical and morphological studies and demonstrated that DEC inhibited the synthesis and exocytosis of proteoglycan in rat chondrocytes. Treatment of chondrosarcoma chondrocytes with DEC altered the transport of molecules from the reticulum to the Golgi apparatus and the transport of molecules from the Golgi to the cell surface. After treatment with DEC, chondrocytes presented large vacuoles and a distended Golgi apparatus. Upon removal of DEC, the vacuoles disappeared and distended organelles returned to their normal appearance, which was coincident with the start of exocytosis of S-proteoglycan and β-D-xyloside-bound ^{35}S-glycosaminoglycan. Other studies confirmed that DEC disturbs the traffic of vesicles to and from the Golgi apparatus. Spiro et al. (1986) demonstrated that DEC altered vesicular transport from the endoplasmic reticulum to the Golgi, and from the Golgi to the plasma membrane, inhibiting the surface expression of