SHORT COMMUNICATION

α-zingiberene, a sesquiterpene from essential oil from leaves of *Casearia sylvestris*, suppresses inflammatory angiogenesis and stimulates collagen deposition in subcutaneous implants in mice

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ABSTRACT
α-zingiberene is a phytochemical of the sesquiterpenes class, the major constituent of the essential oil from the leaves of *Casearia sylvestris*, a plant widely used in traditional medicine for the treatment of inflammatory diseases, tumours, and bacterial infections. In the present study, we evaluated the effects of daily administration of α-zingiberene (0.01, 0.1 and 1 \(\mu\)g diluted in 10 \(\mu\)l of 0.5% DMSO) on the inflammatory, angiogenic, and fibrogenic components, induced by subcutaneous sponge implants in an animal model. Treatment with sesquiterpene resulted in a reduction in macrophage activation, as well as in mean blood vessels and in the activity of metalloproteinases 2 and 9. Furthermore, it resulted in an increase in collagen deposition near the implants. These results show the therapeutic potential of α-zingiberene in the treatment of pathologies, in which processes such as inflammation and angiogenesis are exacerbated, or even for the treatment of chronic wounds.

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1. Introduction

*Casearia sylvestris* is a plant found in the entire Brazilian territory and in several countries on the American continent. Its leaves, roots and bark are used in traditional medicine to treat fever, infections, skin diseases, ulcers, and in cases of snakebite accidents, mainly due to their anti-inflammatory and healing effects (Ferreira et al. 2011). α-zingiberene is one of the main phytochemical constituents present in the extract of *C. sylvestris* leaves (Bou et al. 2013). It is a sesquiterpene, also found as a major compound in other plant specimens used in the treatment of inflammatory diseases. The anti-inflammatory potential of this and other terpenoids are associated with an inhibition of edema formation, leukocyte recruitment, and pro-inflammatory cytokine release (Bou et al. 2013; Jeena et al. 2013; Ferreira et al. 2020; Santos et al. 2021). In some pathologies, the chronicity of the inflammatory response, associated with unregulated angiogenesis and the deposition of extracellular matrix (ECM) constituents comprise key events in the development of numerous diseases (e.g. cancer, rheumatoid arthritis, or chronic wounds) (Diegelmann and Evans 2004; Gupta et al. 2018; MacDonald et al. 2018). Besides that, there are no reports on the effects of treatment with α-zingiberene on processes such as angiogenesis and fibrosis. Therefore, in this work, we sought to evaluate the effects of daily administration of α-zingiberene on the inflammatory, angiogenic, and fibrotic components induced by subcutaneous sponge implants in C57BL/6 mice. Our results demonstrate the ability of this sesquiterpene to reduce inflammatory angiogenesis and to favour implant-induced fibrogenesis.

2. Results and discussion

2.1. Chemical characterisation of α-zingiberene

Considering that α-zingiberene represents about 50% of crude oil from *C. sylvestris*, this material was fractionated over a SiO2/AgNO3 column to afford 200 mg of α-zingiberene (Figure 1), which was identified by comparison of NMR and LREIMS spectral data with those reported in the literature (Bou et al. 2013).

2.2. Daily administration of α-zingiberene reduced macrophage activity in sponge implants

Chronic inflammation is a process characterised by the persistent accumulation of inflammatory cells, such as macrophages. Although these cells can contribute to the

![Figure 1. Chemical structure of α-zingiberene.](image-url)
elimination of pathogens and tissue debridement, their persistence and intense activation can contribute to damage to neighbouring tissues (Shapouri-Moghaddam et al. 2018). In our work, the content of these cells was indirectly evaluated by measuring the activity of the enzyme N-acetyl-β-D-glycosaminidase (NAG), produced in large quantities by activated macrophages. Daily and intra-implant administration of α-zingiberene, at all evaluated concentrations, was able to reduce NAG activity in treated implants, when compared to the control group (Figure S1).

2.3. Anti-angiogenic effect of α-zingiberene

During inflammation, the formation of new blood vessels towards the inflammatory site is responsible for the local supply of O₂ and energy substrates, in addition to allowing the arrival of more leukocytes to the region (Noonan et al. 2008). Angiogenesis begins with the activation of pre-existing capillary endothelial cells in response to tissue hypoxia or to pro-angiogenic factors, such as cytokines and growth factors. Once activated, endothelial cells start to secrete proteases, such as metalloproteinase 2 and 9 (MMP-2 and MMP-9), which degrade their basement membrane and the other constituents of the ECM, allowing their migration towards the concentrated chemotactic stimulus next to the inflammatory site (Carmeliet and Jain 2011). Treatment with α-zingiberene was able to reduce the mean number of blood vessels observed in the fibrovascular tissue induced by sponge implants (Figure S2), attenuate MMP-2 activation, and reduce the synthesis of Pro-MMP9 and its activation (MMP-9) (Figure S3). In addition to their part during angiogenesis, metalloproteinases, which are highly expressed in the tumour microenvironment, end up favouring the occurrence of metastasis. Thus, the inhibition of these enzymes has gained prominence as an anti-tumour strategy (Kessenbrock et al. 2010).

2.4. Increased fibrogenesis in sponge implants treated with α-zingiberene

The fibrogenesis process comprises the mechanisms involved in the synthesis and deposition of collagen in the ECM. Such event is essential for the re-establishment of tissue architecture and comprises a fundamental step to repair (Wang et al. 2018). In implants treated with α-zingiberene, we found an increase in collagen deposition in histological sections stained with picrosirius red (Figure S4 A–B). In this same material, the deposition of still immature and thinner collagen fibers (visualized in green and generally corresponding to type III fibers) was greater than the deposition of thick fibers (observed in red/orange and corresponding to type I fibers), compared to the control group (Figure S4 C-E)). Often, greater deposition of type III collagen is associated with repair with less scar formation, for example, during embryonic healing (Merkel et al. 1988; Moore et al. 2018). In fact, diminished type III collagen deposition during cutaneous wound healing in adult mice increased scar tissue formation (Volk et al. 2011). Aberrant type I collagen deposition, on the other hand, can lead to hypertrophic wound healing or fibrosis of internal organs, with consequent loss of function (Trojanowska et al. 1998). During ventricular or vascular ECM remodelling, for example,
higher levels of type I collagen fibers provide more stiffness, with consequent dysfunction in these organs (Uchinaka et al. 2018).

3. Conclusion
In summary, daily administration of α-zingiberene sesquiterpene was able to reduce inflammatory angiogenesis induced by subcutaneous sponge implants. In addition, it promoted fibrogenesis in implants, with increased deposition of immature collagen fibers. Such results may be associated with inhibition of the activity of MMP-2 and MMP-9, proteolytic enzymes capable of helping both extracellular matrix remodeling and cell migration, favouring processes such as inflammation, angiogenesis, and tumour metastasis.

Disclosure statement
The authors declare no conflict of interest.

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