Effect of the novel wound healing agent, OPAL A on leukotriene B₄ production in human neutrophils and 5-lipoxygenase activity

Russell FD, Windegger T, Hamilton KD & Cheetham NWH

Abstract

OPAL A is a papaya pulp that is heated and alkalised with bicarbonate (the OPAL process) and is undergoing clinical trials for treatment of chronic wounds. The aim of this study was to investigate possible inhibitory effects of OPAL A and a non-alkalised papaya filtrate on the 5-lipoxygenase signalling pathway. Human isolated neutrophils were incubated with or without OPAL A, non-alkalised papaya or sodium bicarbonate and then exposed to the calcium ionophore, ionomycin to stimulate leukotriene B₄ (LTB₄) production. The production of LTB₄ was inhibited in a dose-dependent manner by all three treatments. The effect of these treatments on 5-lipoxygenase activity, the enzyme involved in the production of precursors of LTB₄ was investigated using a cell-free assay. 5-Lipoxygenase activity was inhibited by OPAL A and non-alkalised papaya, but not bicarbonate. Column chromatography was used to show that the active components within OPAL A were non-proteinaceous polar compounds. The inhibitory effects of OPAL A and a non-alkalised papaya filtrate on 5-lipoxygenase activity and LTB₄ production suggest a possible anti-inflammatory mode of action.

Keywords: papaya, OPAL A, wound healing, 5-lipoxygenase activity, leukotriene B₄.

Introduction

Inflammation is a response to cellular injury and results in the killing of microbial pathogens and tissue destruction. Resolution involves a switch from a pro-inflammatory phase that produces mediators such as leukotriene (LT) B₄ to a proresolving phase where resolvins and protectins are produced. Following activation of neutrophils, [Ca²⁺]i is elevated, initiating the translocation of 5-lipoxygenase to the perinuclear envelope where it interacts with a 5-lipoxygenase activating protein and arachidonic acid to produce LTA₄, the precursor of LTB₄. LTB₄ activates BLT₁ and BLT₂ receptors to elicit recruitment and penetrative transmigration of neutrophils from the postcapillary venule, and prolongation of neutrophil survival. On the basis of such multifaceted involvement in inflammation, BLT₁ and BLT₂ receptors and the synthetic 5-lipoxygenase signalling pathway have become potential therapeutic targets in the management of conditions in which inflammation is implicated.

OPAL A is a filtrate that has been manufactured following the homogenisation, heat treatment, alkalisation and filtration of the pulp of the ripened fruit of Carica Papaya (the OPAL process), and is currently being examined in a clinical trial conducted by Phoenix Eagle Company in patients who have non-healing wounds (ClinicalTrials.gov Identifier NCT00933348). The mechanisms by which OPAL A contributes to wound healing are not known. We have previously reported a nitric oxide-dependent vasorelaxant effect of OPAL A and a non-alkalised papaya filtrate which raised the possibility that OPAL A might improve perfusion within the wound region. The aim of this study is to examine possible inhibitory effects of OPAL
A and a non-alkalised papaya filtrate on LTB₄ production and 5-lipoxygenase activity.

Materials and methods
Isolation of human neutrophils

Whole blood was obtained from the antecubital vein of five healthy men (22–55 years) and placed in EDTA tubes. Neutrophils were isolated by differential centrifugation using polymorphprep according to manufacturer’s instructions (Axis-Shield, Oslo, Norway). Briefly, whole blood was layered onto polymorphprep in a 1:1 ratio and centrifuged at 500xg for 30 minutes at 22°C. The cell pellet was resuspended in 450 μl M199 media, and spun at 450xg for 30 minutes at 22°C in an Eppendorf Centrifuge 5702 with a swing rotor. The fraction containing neutrophils was collected and diluted with 8 ml M199 culture media containing 20% fetal calf serum, 2 mM Glutamax-1, 2.5 μg/ml fungizone and 50 μg/ml penicillin/streptomycin (M199 media), and spun at 500xg for 30 minutes at 22°C in an Eppendorf Centrifuge 5702 with a swing rotor. The fraction containing neutrophils was collected and diluted with 8 ml M199 culture media containing 20% fetal calf serum, 2 mM Glutamax-1, 2.5 μg/ml fungizone and 50 μg/ml penicillin/streptomycin (M199 media), and spun at 500xg for 30 minutes at 22°C. The cell pellet was resuspended in 450 μl Hanks’ Balanced Salt Solution (HBSS), with 10 μl of sample smeared onto a microscope slide and stained using Diff Quik differential dye (A). OPAL A was more potent than non-alkalised papaya filtrate or bicarbonate and this was subtracted from all readings.

Column fractionation of OPAL A

To further characterise the inhibitory activity of OPAL A, a 1.5 ml aliquot of OPAL A was passed through a 0.45 μm syringe filter and the filtrate (0.6 ml) was loaded onto an OASIS column that was pre-activated by application of methanol then distilled water. The column was eluted using a mixture of methanol and water (1:1), and the eluant was collected and analysed using the 5-lipoxygenase activity assay described above. In a second series of experiments, 7.0 ml of OPAL A was mixed with 5 g of silica gel. The sample was frozen at −80°C, freeze-dried, then added to the top of a column containing 5 g silica gel that was moistened with ethyl acetate. The column was eluted with successive rinses with 40 ml of ethyl acetate, acetone, methanol and water. The ethyl acetate, acetone and methanol fractions were recovered by evaporation of solvent at 22°C under a stream of nitrogen. The aqueous fraction was freeze-dried. Samples were reconstituted in 0.5 ml phosphate buffer solution (pH 9) and analysed using the lipoxygenase activity assay as described above.
knowledge, this is the first report identifying the inhibitory action of papaya-based filtrates on the 5-lipoxygenase – LTB₄ signalling pathway.

OPAL A, non-alkalised papaya and bicarbonate alone inhibited production of LTB₄ by human neutrophils that were exposed to ionomycin. Significant inhibition of LTB₄ production occurred at a lower concentration of OPAL A than for either the non-alkalised papaya or the equivalent amount of bicarbonate. Since OPAL A contains both papaya and sodium bicarbonate, this finding suggests a possible additive inhibitory action on LTB₄ production. LTB₄ is an integral eicosanoid in the inflammatory response, with roles in recruitment and activation of leukocytes, and prevention of leukocyte apoptosis⁷. Thus, inhibition of LTB₄ production by OPAL A suggests a possible mode of action for this filtrate in the treatment of inflammatory conditions.

Discussion

Papaya latex harvested from unripe papaya fruit stimulates a pro-inflammatory response when injected into rat paw¹⁵. In this study we examined the possible anti-inflammatory effects of non-alkalised papaya and papaya that was prepared by homogenisation, heat treatment, alkalisation and filtration of the pulp of ripened papaya (the OPAL process). To our
The endogenous pathway for the synthesis of LTB₄ from arachidonic acid is well described. 5-Lipoxgenase activity is crucial to the production of LTB₄, first catalysing the oxidation of arachidonic acid to generate 5-hydroperoxycisatetraenoic acid, then the subsequent production of LTA₄. LTA₄ is in turn converted to LTB₄ in the presence of LTA₄ hydrolase. In the present study, we showed that OPAL A and non-alkalised papaya inhibited 5-lipoxygenase activity with similar potency. Interestingly, sodium bicarbonate was without effect, contrasting with the ability of bicarbonate ions to inhibit LTB₄ production in the activated neutrophils. The findings indicate that bicarbonate, or the elevation of pH resulting from the addition of bicarbonate, may have a direct suppressive effect on the neutrophils.

OPAL A was eluted from an OASIS column or silica gel column as a preliminary analysis of the chemical properties of the filtrate for inhibition of 5-lipoxygenase activity. The eluant obtained from the methanol/water rinse of the OASIS column retained inhibitory activity that was detected in the non-fractionated OPAL A. The polar solvent mixture (methanol/water) was used to selectively elute polar molecules. When OPAL A was eluted on a silica gel column using non-polar solvents (ethyl acetate or acetone), inhibitory activity was lost. However, elution with polar solvents (methanol or water) retained activity that was detected in the non-fractionated OPAL A. Proteins efficiently adsorb to silica gel, so we conclude that the active components within OPAL A are non-proteinaceous, polar molecules. Bioactivity-guided fractionation experiments could be carried out in the future to elucidate the identity of the active compound(s) present in OPAL A. Phenolic compounds such as quercetin and caffeic acid are candidates as they are polar and are expressed in OPAL A. Phenolic compounds such as quercetin and caffeic acid are candidates as they are polar and are expressed in OPAL A. The 2nd Meeting of the Australasian Wound Practice and Research Volume 19 Number 4 – December 2011

Conflict of interest

This study received financial and in-kind support from Phoenix Eagle Company, Australia.

Acknowledgements

The authors thank participants who assisted us with the collection of neutrophils. We also thank A/Prof Geoffrey Mitchell for discussions regarding this study, and Dr Denis Podger and his colleagues for provision of OPAL A and variant filtrates.

References