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INTRODUCTION

Turmeric (*Curcuma longa*) is a plant belonging to the ginger family that has a long record of use in Indian Ayurvedic Medicine. Extracts of the turmeric plant contain curcumins, which have strong anti-inflammatory and anti-oxidant properties. Here we verify the anti-inflammatory and anti-oxidant activities of a colorless, stable, turmeric extract and identify potential mechanism of action for these activities.

METHODS

Skin-Equivalent Cultures

- Melanocyte- and keratinocyte-containing cultures, reconstituted from human epidermis, were purchased from SkinEthic (Nice, France).
- Turmeric extract was applied topically to the cultures for 2 days (n=3).
- Water was applied topically to the control cultures daily for 2 days (n=4).
- Cultures were harvested after the second day of treatment.
- RNA was isolated from the harvested cultures and preserved using RNAlater (Ambion, Austin, Texas).

Genomic Analysis

- RNA was isolated and GeneChip targets were generated using the Enzo BioArray™ HighYield™ labeling kit and standard procedures.
- Affymetrix HG-U133 Plus 2.0 GeneChips were hybridized, washed, stained, and scanned using standard parameters.
- Summary statistics were calculated using standard methods

OBJECTIVE

To explore the anti-inflammatory and anti-oxidant benefits of turmeric extract and to investigate potential mechanisms of action using genomic comparisons.

Glutathione Reductase

- Activation of the glutathione reductase enzyme was measured by coupling the reduction of glutathione with the oxidation of NADPH.
- The oxidation of NADPH was monitored at 340nm.

Anti-oxidant Response Element (ARE)

- ARE was assayed using the ARE-32 reporter cell line (CXR-Biosciences).
- Cells were transfected by CXR Biosciences with pGL8x-ARE, which contains 8 copies of the rat GST ARE linked to the luciferase gene.
- Cells were treated for 24 hours before the luciferase substrate was added.
- Total luminescence was measured using the Envision plate reader.

Cyclooxygenase 2 (COX2) Inhibition

- COX 2 inhibition activity was measured using the Cayman Chemical Colorimetric COX (ovine) Inhibitor Screening Assay Kit.
- The peroxidase activity of COX2 was measured colorimetrically by monitoring the appearance of oxidized N, N, N', N'-tetramethyl-p-phenylenediamine (TMPD) at 590nm.

Neutrophil Elastase

- Neutrophil elastase (Biomol) was assayed using a small peptide substrate (suc AAPV-paranitroanilide from EMD).
- A continuous assay format was developed that detects the release of para-nitroanilide at 405nm.

RESULTS

Anti-oxidant activity profile of turmeric extract:

- **In vitro assay results:**
 - Turmeric extract showed significant anti-oxidant activity in both the ARE (1060%, p<0.02) and glutathione reductase (187%, p<0.02) assays relative to control.
- **Anti-oxidant genes specifically up-regulated by the turmeric extract (Figure 1, green):**
 - **AKR1C3** and **HSDL2:** reductase/oxidoreductase responsible for electron transport
 - **ARG2:** regulates the bioavailability of nitric oxide
 - **DEGS1, DUSP1, GPX2, EGLN3:** senses and responds to oxidative stress.
 - **MOAB:** antioxidant protection
 - **LOXL2:** oxygen scavenger
 - **NR4A2:** ARE transcription factor
 - **AKR1C1:** detoxification of oxidative species
- **Anti-oxidant genes specifically up-regulated by the turmeric extract (Figure 1, blue):**
 - **DDAH1:** nitric oxide production
 - **MYH:** cellular marker of oxidative stress

Anti-inflammatory activity profile of Turmeric extract:

- **In vitro assay results:**
 - Turmeric extract showed significant anti-oxidant activity in both the COX2 (74%, p<0.01) and neutrophil elastase (IC50 0.001%) assays relative to control.
- **Anti-inflammatory genes specifically up-regulated by the turmeric extract (Figure 2, gray):**
 - **IL-13:** inhibits the production of pro-inflammatory cytokines and chemokines
 - **CXCL2, PTGER3, PTGS2:** regulators of inflammation
- **Anti-inflammatory genes specifically down-regulated by the turmeric extract (Figure 2, pink):**
 - **NFX1, ADANTS1, NFATC3:** Inflammatory transcription factors.

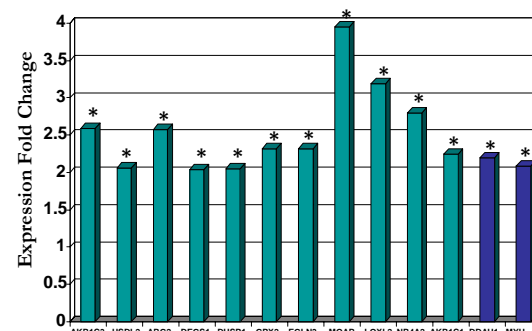


Figure 1. The up-regulation (green) and the down-regulation (blue) of thirteen antioxidant genes responsible for sensing and responding to oxidative stress or regulating oxidative species. Absolute value fold changes relative to vehicle control are shown. *For all genes p<0.05.

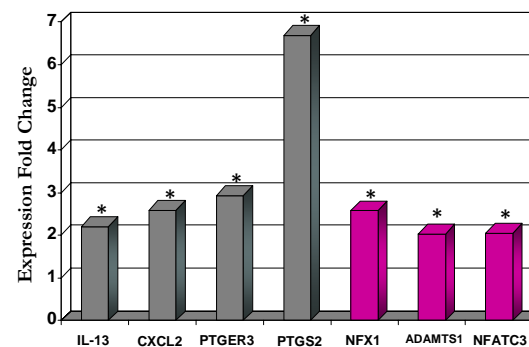


Figure 2. The up-regulation (gray) and the down-regulation (pink) of seven genes responsible for mediating inflammation. Absolute value fold changes relative to vehicle control are shown. * For all genes p<0.05.

CONCLUSIONS

Turmeric extract contains multiple anti-oxidant and anti-inflammatory activities, supporting its history of use as a cellular protector.

Genomic insights into the mechanism of action suggest that turmeric extract provides cellular protection by regulating genes in both anti-inflammatory and anti-oxidant pathways. Genes activated by turmeric extract were responsible for limiting inflammation, increasing electron transport, sensing oxidative stress, and directly limiting the production of oxidative species. Turmeric extract was also able to limit the effects of cellular stress by down-regulating genes responsible for the production of oxidative species and inflammatory transcription factors.

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