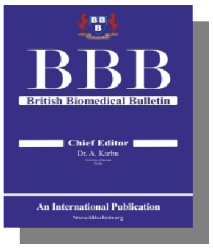
Journal Home Page www.bbbulletin.org

BRITISH BIOMEDICAL BULLETIN



Original

Cell Culture Study on the Effect of Bio Available Curcumin – "Cureit" on Elastase Inhibition Activity

Sreeraj Gopi*[1], Robin George[1] and V.T. Sriraam[2]

[1]*R&D Centre, Aurea Biolabs (P) Ltd – A Plantlipids company, Cochin, India*
[2]*Aurous HealthCare Research and Development India Private Limited, Chennai, India*

A R T I C L E I N F O

Received 20 Aug. 2014
Received in revised form 17 Sep. 2014
Accepted 18 Sep. 2014

Keywords:
Elastases,
Neutrophils,
Curcumin,
Bio availability.

Corresponding author: R&D Centre, Aurea Biolabs (P) Ltd – A Plant Lipids Company, Cochin, India.
E-mail address:
Sreeraj.gopi@plantlipids.com

A B S T R A C T

Elastases are matrix degrading enzymes involved tissue homeostasis and are mainly produced by epithelial cells in the skin, lungs and neutrophils. Neutrophil-derived elastases play a major role in the regulation of vascular injury and inflammation, such as ischemia-reperfusion injury. The potential elastase inhibitors could severely as targets in anti inflammatory therapy. The other important target organ or elastases are the matrix proteins in the skin, which impart the structural and functional integrity to it. Curcumin, isolated from popular Indian spice *Curcuma longa,* could be a potential molecule for inhibiting the activity of elastases. The poor bio availability of curcumin was addressed and arrived at a bio available formulation – "cureit". Its elastases inhibiting activity in human cell lines were described through this spectrophotometrical study and inferred that, "cureit" can inhibit elastases activity, in higher concentrations.

Introduction

Aging of the skin is a continuous process associated with increased wrinkles, deep lines and irregular pigmentation[1]. An important event in the process of aging is the production of reactive radical species by oxidative phosphorylation processes and from exogenous sources. Free Radicals are the cause of deterioration of the skin's supporting structures, leading to decreased elasticity and resilience. There are two primary skin ageing processes, intrinsic and extrinsic. Variations in individual genetic background are thought to govern intrinsic ageing, which results as time passes[2-5]. By definition, this form of ageing is inevitable and, thus, apparently not subject to manipulation through changes in human behaviour. Conversely, extrinsic ageing is engendered by factors originating externally that are introduced to the human body, such as smoking, excessive alcohol consumption, poor nutrition, and chronic exposure to the sun. Exposure to such elements, which falls within the voluntary realm, although it may sometimes occur under duress, is not inevitable and thus represents premature skin ageing. Of these external factors, sun exposure is considered to be far and away the most significantly deleterious to the skin. Indeed, 80% of facial ageing is believed to be due to chronic sun exposure[6-8]. In a study conducted in Thailand, curcuminoids loaded skin cream was found to be statistically significant in improving skin wrinkles, hydration, melanin content, biological elasticity, and viscoelasticity, compared with the cream base and the baseline from week 3 onward. The product showed no sign of skin irritation, both objectively and subjectively, throughout the study[9].

Curcumin (1, 7 bis (4-hydroxy-3-methoxy phenyl)-1, 6 heptadiene-3, 5-dione), is a principal curcuminoid of the popular Indian spice *Curcuma longa* commonly known as turmeric, a perennial herb of *the Zingiberaceae* (ginger) family, which is a 3-5 ft tall bearing oblong, pointed, short-stemmed leaves and funnel-shaped yellow flowers. Turmeric is widely cultivated in India, China and other tropical countries[10]. It is a powerful antioxidant with the ability to scavenge free radicals generated in the body as a result of various metabolic processes. It is also well known for its anti-inflammatory, anti-angionenic and immunomodulatory effects. Curcumin is used for treatment of various diseases like Arthritis, Gastrointestinal upset etc[11-14]. Curcumin is available in the state of the art in the form of dietary supplement because of its antioxidant benefits as it provides protection against cell-damaging free radicals. However, the extent to which the human body benefits from consumption of curcumin is doubtful and limited because of poor bioavailability of curcumin[16]. Aurea Biolabs (A plant lipids company), developed a novel composition of curcumin, which is more bio available – *"Cureit"* The present invention describes the bio efficacy of *Cureit* in human cell lines.

Elastase Inhibition Assay

Elastases are matrix degrading enzymes involved tissue homeostasis and are mainly produced by epithelial cells in the skin, lungs and neutrophils. Neutrophil-derived elastases play a major role in the regulation of vascular injury and inflammation, such as ischemia-reperfusion injury. Elastases are available both as membrane bound and intracellular forms. Intracellular elastases breaks down foreign proteins, whereas the extracellular elastases released by neutrophils and mostly bound to the neutrophil plasma membrane, assists neutrophil migration to inflammatory sites by degrading various host proteins, such as extracellular matrix proteins.

Under normal conditions, these enzymes are under the tight control of endogenous inhibitors like elafins α1-antitrypsin (α1-AT), secretory leukocyte proteinase inhibitor, and α2- macroglobulin. However, large amounts of oxygen radicals and proteases released by leukocytes recruited to inflammatory sites can inactivate these endogenous inhibitors leading to severe inflammation flare-ups and injuries to the tissues[17]. Hence potential elastase inhibitors could severely as targets in anti inflammatory therapy. The other important target organ or elastases are the matrix proteins in the skin, which impart the structural and functional integrity to it. During the process of chronological ageing the metabolic events like the formation of advanced glycation end products in the skin draws inflammatory infiltrates leading to formation of wrinkles and compromised wound healing. Hence elastase inhibitors could also serve as putative anti aging therapeutics targets.

Reagents and Materials Used

- Test Compound – Bio available Curcumin – "cureit"
- Porcine pancreatic type I elastase
- Tris buffer
- Succinyl – (Ala) 3-p-nitroanilide (SANA)

Methodology

Enzyme activity was determined by monitoring the release of p-nitroaniline by measuring absorbance at 410nm. Porcine pancreatic elastase was assayed spectrophotometrically using SANA as substrate. Different concentrations of the test material (bio available curcumin – "cureit") were incubated with a mixture of 800 of 200mM Tris buffer (PH 8.0), 1 unit of enzyme for 15 minutes. The enzyme reaction was initiated by the addition of substrate and further incubated for 15 minutes at 37^{o}C. The absorbance was monitored at 410nm using UV spectrophotometer. Four batches of reaction were performed, and the average was taken and tabulated.

Results and Discussion

The elastase inhibitory property of the test material was studied in the concentration range of 10 to 100 (µg/ml). From the studies it has been inferred that the low concentrations failed to inhibit the elastase activity (Table 1).

However the higher concentrations of the test material showed considerable inhibitory activity. Much higher doses could not be studied due to the issue of precipitation. The present investigation clearly suggests the anti inflammatory and anti aging effect of bio active curcumin.

It was also observed from the co culture study of melanocytes and keratinocytes that the cell integrity, morphology and functionality were intact by cureit – the bio available curcumin. (See figure 1).

Conclusion

The bio available curcumin - "cureit" was screened for its bio efficacy for elastase inhibition activity and it was observed that it has very good activity at higher concentrations (100 µg/ml). It was also observed from the co culture study of melanocytes and keratinocytes that the cell integrity, morphology and functionality were intact by the cureit – the bio available curcumin.

References

1. Ammon HP, Wahl MA: *Pharmacology of Curcuma longa. Planta Med.* 57(1):1–7, 1991.

2. Chang HM, But BPH: *Pharmacology and Applications of Chinese Materia Medica, Vol 2. Philadelphia*, PA, World Scientific, 1986, pp 936–939.
3. Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, Ko JY, Lin JT, Lin BR, Ming-Shiang W, Yu HS, Jee SH, Chen GS, Chen TM, Chen CA, LaiMK, Pu YS, PanMH,Wang YJ, Tsai CC, Hsieh CY: *Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre- malignant lesions. Anticancer Res.* 21(4B):2895–2900, 2001.
4. Dobelis Hamper IN (ed): *Magic and Medicine of Plants.* Pleasantville, NY, Reader's Digest Association, 1986.
5. Jain SK, DeFilipps RA: *Medicinal Plants of India.* Algonac, MI, Reference, 1991, p 120.
6. Lampe V, Milobedeska J, Kostanecki V: *Ber Dtsch Chem Ges.* 43:2163, 1910.
7. Lampe V, Milobedeska J: *Ber Dtsch Chem Ges.* 46:2235, 1913.
8. L Baumann: *Skin ageing and its treatment. J Pathol.* 2007; 211: 241–251.
9. Leung A: *Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics.* New York, Wiley, 1980, pp 313–314.
10. Nadkarni AK: *Indian Materia Medica, Vol 1.* Bombay, India, Popular Book Depot, 1954.
11. Novoseltsev *et al*, 2001*: A homeostatic model of oxidative damage explains paradoxes observed in earlier aging experiments: a fusion and extension of older theories of aging. Biogerontology*. 2001, 2(2):127-138.
12. Paolo U. Giacomoni & Glen Rein: *Factors of skin ageing share common mechanisms.* Biogerontology 2001 ;(2): 219-229.
13. Pinyuoa Plianbangchang *et al*: *Efficacy and Safety of Curcuminoids Loased Solid Lipid Nanoparticles Facial Cream as an Anti Aging Agent. Naresuan University Journal*. 2007; 15(2): 73-81.
14. Srimal RC, Dhawan BN: *Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti- inflammatory agent. J Pharm Pharmacol*. 25(6):447–452, 1973.
15. Tu G, Fang Q, Guo J, Yuan S, Chen C, Chen J, Chen Z, Cheng S, Jin R, Li M, *et al.*: *Pharmacopoeia of the People's Republic of China.* Guangzhou, P.R. China, Guangdong Science and Technology Press, 1992, pp 202–203.
16. Uitto J. *Understanding premature skin aging. N Engl J Med*. 1997; 337(20):1463–1465.
17. Nahida Tabassum, Mariya Hamdani and Iqbal Hussain Najar: *Natural treatment for inflammatory bowl disease.*2014, *bbbulletin*.

Table 1. Elastase inhibitory activity- consolidated table

	% Inhibition					
Sample (µg/ml)	Batch I	Batch II	Batch III	Batch IV	Avg	SD
10	8.96	-8.29	1.40	5.61	1.92	7.48
25	-1.13	5.41	15.37	12.12	7.94	7.33
50	26.99	16.73	20.28	22.09	21.52	4.27
100	41.25	43.71	39.45	42.07	41.62	1.77

 ISSN-2347-5447

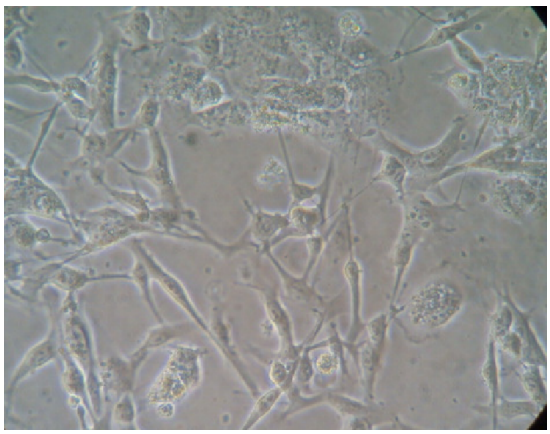


Figure 1. Co-culture of melanocytes and keratinocytes – the cell integrity, morphology and functionality are unaltered by the test compound