

Medizinische Fakultät der Charité – Universitätsmedizin Berlin
Campus Benjamin Franklin
aus der Klinik für Dermatologie, Venerologie und Allergologie
(Direktor: Prof. Dr. med. W. Sterry)

**THE INVOLVEMENT OF LIPOXYGENASES IN
INFLAMMATORY SIGNALLING PATHWAYS OF
HUMAN SEBOCYTES IN VITRO**

Inaugural-Dissertation
zur Erlangung der
medizinischen Doctorwürde
der Charité – Universitätsmedizin Berlin
Campus Benjamin Franklin

vorgelegt von
Theodosios Alestas
aus Korinth, Griechenland

Referent: Herr Prof. Dr. med. Christos C. Zouboulis

Korreferent: 1. Frau Prof. Dr. med. U. Blume-Peytavi
2. Herr Prof. Dr. med. S. Goerdt

Gedrückt mit Genehmigung der Charité – Universitätsmedizin Berlin
Campus Benjamin Franklin

Promoviert am: 15.12.2006

« Τα αγαθά κόποις κτώνται »

“ No pain, no gain”

Acknowledgements

I would like to thank sincerely:

Prof. Dr. med. Prof. Dr. h.c. C.E. Orfanos for the great opportunity he gave me to work at the Department of Dermatology, University Medical Center Benjamin Franklin, The Free University of Berlin,

Prof. Dr. med. Ch.C. Zouboulis for his full support during my research at his laboratory,

Ms. C. Beutler, Dr. S. Fimmel, Mr. A.M. Hosseini and Dr. P. S. Pattabhiraman for friendly advice and help.

CONTENTS

1.	INTRODUCTION	8
1.1	The lipoxygenase family	8
1.2	The metabolism of arachidonic acid	8
1.3	The P450 mono-oxygenase	9
1.4	The cyclo-oxygenases	9
1.5	The 5-lipoxygenase	10
1.5.1.	Purification and amino-sequence	10
1.5.2.	Enzyme location	11
1.5.3.	Catalytic reactions	11
1.5.4.	Phosphorylation	12
1.5.5.	5-Lipoxygenase inactivation	12
1.6.	5-Lipoxygenase activating protein	13
1.7.	Formation of hydroxy-eicosatetraenoic acids	13
1.8.	Leukotrienes	13
1.8.1..	Peptido-leukotrienes	14
1.9.	Leukotriene A ₄ hydrolase	14
1.10.	15-Lipoxygenase	14
1.10.1.	15-Lipoxygenase-1	15
1.10.2.	15-Lipoxygenase-2	16
1.11.	12-Lipoxygenase	17
1.11.1.	Platelet 12(S)-lipoxygenase	17
1.11.2.	Leukocyte 12(S)-lipoxygenase	17
1.11.3.	Epidermal 12(S)-lipoxygenase	18
1.11.4.	12(R)-lipoxygenase	18
1.12.	Hepoxilins and lipoxins	18
1.13.	Biological role of eicosanoids	19
2.	AIM OF THE STUDY	22
3.	METHODS AND MATERIALS	23
3.1.	Cell cultures	23
3.2.	Zileuton extraction	23
3.3.	Lactate dehydrogenase cytotoxicity assay	24
3.4.	Protein detection	25
3.4.1.	Cell cultures, treatment and protein extraction	25
3.4.2.	Protein quantitation	26
3.4.3.	Western blot analysis	27
3.4.4.	Membrane stripping and immunoblotting of β-actin	31
3.5.	RNA detection	31
3.5.1.	Cell culture and treatment	31
3.5.2.	Total RNA isolation	32
3.5.3.	Quantitation of total RNA	33
3.5.4.	Reverse transcription	33
3.5.5.	RT-PCR	34
3.5.6.	Agarose gel electrophoresis	36
3.6.	Leukotriene B ₄ enzyme immunoassay	37
3.7.	Cytokine detection	38
3.8.	Lipid detection	40

3.8.1. Nile-red staining	40
3.8.2. MUH test	41
3.9. Immunocytochemical studies	42
3.9.1. Cytocentrifugation	42
3.9.2. APAAP	42
3.10. Statistical Analysis	44
 4. RESULTS	44
4.1. Cytotoxicity assay	44
4.2. Western blot analysis	45
4.2.1. β -Actin	45
4.2.2. 5-Lipoxygenase	45
4.2.3. 15-Lipoxygenase	47
4.2.4. LTA ₄ hydrolase	48
4.3. RT-PCR analysis	48
4.3.1. 5-Lipoxygenase	48
4.3.2. 15-Lipoxygenase	49
4.3.3. LTA ₄ hydrolase	49
4.4. 5-Lipoxygenase activity	49
4.5. Cytokines release	50
4.5.1. IL-1 β	50
4.5.2. IL-6	50
4.5.3. IL-8	50
4.5.4. TNF- α	51
4.5.5. IL-10 and IL-12 _(p70)	51
4.6. Lipid synthesis	51
4.6.1. Non-polar lipids	51
4.6.2. Polar lipids	51
4.7. 5-Lipoxygenase immunostaining	52
 5. DISCUSSION	52
5.1. Restrictions and troubles	53
5.1.1. Treatment of cells	53
5.1.2. Western blot analysis	54
5.2. The skin	55
5.3. The sebaceous glands and the sebum	57
5.4. SZ95 sebocytes	58
5.5. Essential fatty acids	59
5.6. 5-Lipoxygenase in the skin	59
5.7. Leukotriene A ₄ hydrolase in the skin	61
5.8. 15-Lipoxygenase in the skin	62
5.9. Leukotriene B ₄ and the skin	65
5.10. Peptido-leukotrienes and the skin	67
5.11. 12-Lipoxygenase in the skin	67
5.12. Cyclo-oxygenase in the skin	67
5.13. P450 mono-oxygenase in the skin	68
5.14. PPARs and the skin	68
5.15. Cytokines and lipoxygenases	71
5.16. Involvement of eicosanoids in skin diseases	73
5.16.1. Psoriasis	73

5.16.2. Atopic dermatitis	75
5.16.3. Systemic sclerosis	76
5.16.4. Acne vulgaris	77
5.17. Lipoxygenase inhibitors	80
5.17.1. Zileuton	82
5.17.2. Lipoxygenase inhibitors and skin diseases	82
6. CONCLUSION	84
7. FIGURES	88
8. REFERENCES	102
CURRICULUM VITAE	113

ABBREVIATIONS

- 5-HPETE = 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid
- 5-LOX = 5-lipoxygenase (EC 1.13.11.34)
- 12-HPETE=12-hydroperoxy-5,8,10,14-eicosatetraenoic acid
- 12-LOX = 12-lipoxygenase (EC 1.13.11.31)
- 15-HPETE=15-hydroperoxy-5,8,11,13-eicosatetraenoic acid
- 15-LOX = 15-lipoxygenase (EC 1.13.11.33)
- AA = arachidonic acid
- AD = atopic dermatitis
- APAAP = alkaline phosphatase anti-alkaline phosphatase
- bp = base pair
- CaI = calcium ionophore A23187
- Caf = caffeic acid
- DEPC = diethyl pyrocarbonate
- DHA = docosahexaenoic acid
- DMSO = dimethyl sulfoxide
- EIA = enzyme immuno assay
- EPA = eicosapentanoic acid
- ETYA = 5,8,11,14-eicosatetraynoic acid
- FBS = fetal bovine serum
- FFA = free fatty acid
- FLAP = 5-lipoxygenase activating protein
- Gus = glucuronidase
- HETE = hydroxy-eicosatetraenoic acid
- IL = interleukin
- kDa = kilodalton
- LA = linoleic acid
- LDH = lactate dehydrogenase
- LTA₄ hydrolase = leukotriene A₄ hydrolase (EC 3.3.26)
- LTA₄ = 5,6-trans-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid
- LTB₄ = 5,12-dihydroxy-8,10-trans-6,14-cis-eicosatetraenoic acid
- LTC₄ = 5-hydroxy-6-glutathionyl-7,9-trans-11,14-cis-eicosatetraenoic acid
- LTD₄ = 5-hydroxy-6-cysteinylglycyl-7,9-trans-11,14-cis-eicosatetraenoic acid
- LTE₄ = 5-hydroxy-6-cysteinyl-7,9-trans-11,14-cis-eicosatetraenoic acid
- NDGA = nordihydroguaiaretic acid
- NLS = nuclear localization signal
- PBS = phosphate buffered saline
- PG = prostaglandin
- PLA = phospholipase A
- PPAR = peroxisome proriferator-activated receptor
- PVDF = polyvinylidene fluoride paper
- RT-PCR = reverse transcriptase polymerase chain reaction
- SS = systemic sclerosis
- TNF- α = tumor necrosis factor- α
- UVB = ultra-violet B
- Zil = zileuton

"Mein Lebenslauf wird aus Datenschutzgründen in der elektronischen Version meiner Arbeit nicht mit veröffentlicht."

Erklärung

„Ich, Theodosios Alestas, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema: „The Involvement of Lipoxygenases in Inflammatory Signalling Pathways of Human Sebocytes In Vitro“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

15.11.2005