ABSTRACT

Erythema is a hallmark skin response to excessive ultraviolet radiation (UVR) and is associated with cutaneous inflammation. Both are mediated by inflammatory mediators including nitric oxide (NO), prostaglandin E_2 (PGE_2) and chemoattractants such as 12-hydroxyeicosatetraenoic acid (12-HETE) leading to vasodilation and increased leukocyte infiltration. The erythematous response is more pronounced in individuals with low basal melanin levels or who fail to respond to UVR with a robust up-regulation of melanogenesis. While melanin production is a key function of melanocytes, these cells can also produce NO and PGE_2, and are located in close proximity to the dermal vasculature. It has been hypothesized that melanocytes with poor melanogenic capacity may participate in the inflammatory response to UVR.

The aim of this project was to investigate the inflammatory response in the skin of individuals with either skin phototype (SPT) 1 or 4 to UVR. Sixteen normal healthy individuals were selected for study (8 SPT-1 & 8 SPT-4). Buttock skin was investigated by immunohistochemistry for leukocyte subtypes, eicosanoid producing enzymes and NO synthases under basal and UVR-stimulated conditions. In addition primary cultures of epidermal melanocytes (EM) were established from 16 individuals (8 SPT-1 & 8 SPT-4) and assessed for the presence of eicosanoid-producing enzymes, melanogenic enzymes and NO synthases, by immunocytochemistry, Polymerase Chain Reaction and Western Blotting and for the production of the main pro-inflammatory eicosanoid PGE_2 by ELISA and Mass Spectrometry. Moreover, the fatty acid composition of cultured melanocytes was assessed by Gas Chromatography.

Results showed that individuals with SPT-1 had significantly greater neutrophil infiltration into the epidermis than those with SPT-4 at 24 hrs post-UVR. Moreover,

$CD3^+$ lymphocyte infiltration into the dermis was significantly greater in individuals with SPT-4 than those with SPT-1 at 24 and 72 hrs post-UVR. NOS-1, NOS-3, 12-LOX and COX-2 expression were significantly increased in SPT-1 skin, while NOS-2 and 15-LOX were significantly increased in SPT-4 skin. As 12-LOX and COX-2 products are chemoattractive (for neutrophils) and pro-inflammatory respectively these data could explain the greater observed neutrophil infiltration in SPT-1. The 15-LOX product (15-HETE) is anti-inflammatory and may suggest that 15-LOX up-regulation in SPT-4 skin may aid resolution of the sunburn response, which in part may be mediated by $CD3^+$ lymphocytes and a class-switch in eicosanoid production from COX to LOX products.

Melanocyte primary cultures surprisingly showed that SPT was not correlated with melanin content or melanogenic enzyme expression/activity suggesting that all melanocytes *in vitro* contained the necessary cellular machinery to produce melanin. This finding may reflect also their equal treatment under these enriched culture conditions, which may or may not be available to these cells *in situ*. Moreover, all melanocytes expressed the necessary machinery (PLA_2, COX-1, cPGES) to produce PGE_2. However, only some cultures did so at baseline and in response to UVR, and this was not correlated with SPT. A positive correlation was found however between expression level of dopachrome tautomerase (DCT) and protection against PGE_2 production in response to UVR, which may suggest a novel role for DCT unrelated to melanogenesis.

In summary this research project has generated data that highlights differences between the skin of individuals with SPT-1 and those with SPT-4, and may provide evidence that the keratinocyte partner contributes significantly to the SPT-associated response. This research may also suggest DCT as a novel therapeutic target to protect EM from participation in the UVR-associated inflammatory response in skin.

ACKNOWLEDGEMENTS

I would like to thank Prof. D. J. Tobin for providing me with guidance and encouragement for what at times felt an almost impossible task. I would also like to thank him for always making time to see me and discussing any problems that I may have been encountering. Also just as importantly I would like to thank him for stimulating me to come up with solutions for some of these problems.

I would like to thank Prof. A. Nicolaou for acting as my second supervisor. Her knowledge of lipid chemistry was invaluable. I would like to thank Prof. L. E. Rhodes, M. Brownrigg and A. Haylet for providing me with the skin specimens, without which this project would not have been possible and Prof. A. J. Thody and aforementioned for the opportunity for discussion of my data. I would like to thank Dr. Mojgan Masoodi for her help with Mass Spectrometry and Dr. Karen Massey and Juliano Miyake for their help with Gas Chromatography.

I would like to thank my Tobin lab colleagues Dr. Suman K. Singh, Dr. Judy (Man Ching) Leung, Dr. Aisha N. Meskiri, Dr. Sobia Kauser and Dr. Nik Papageorgiou for their help in and out of the lab.

I would like to thank my girlfriend Victoria Hutchinson, my parents Martin and Dee Gledhill and my sister Joanne Gledhill for their love and support.

Finally, I would like to thank the School of Life Sciences at the University of Bradford and the Wellcome Trust for funding this research project.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

12-lipoxygenase (12-LOX)

15-lipoxygenase (15-LOX)

3, 4-dihydroxy-L-phenylalanine (L-DOPA)

4, 6-diaminidino-2-phenylindole (DAPI)

5, 6-dihydroxyindole (DHI)

5, 6-duhydroxyindole-2-carboxylic acid (DHICA)

5-lipoxygenase (5-LOX)

8-lipoxygenase (8-LOX)

Acetylcholinesterase (AChE)

Adenosine Triphosphate (ATP)

Adrenocorticotropic Hormone (ACTH)

Amino Ethyl-Carbazole (AEC)

Arachidonic Acid (AA)

Basement Membrane Zone (BMZ)

Basic Fibroblast Growth Factor (bFGF)

Blank (Blk)

Bovine Serum Albumin (BSA)

Butylated Hydroxytoluene (BHT)

Calcitonin Gene Related Peptide (CGRP)

Carbon Dioxide (CO_2)

Cell Adhesion Molecules (CAMs)

Complementary Deoxyribonucleic Acid (cDNA)

Cyclic-Adenosine Monophosphate (cAMP)

Cyclooxygenase-1 (COX-1)

Cyclooxygenase-2 (COX-2)

Deoxyribonucleic Acid (DNA)

Diacylglycerol (DAG)

Diethyl Pyrocarbonate (DEPC)

Dimethylsulphoxide (DMSO)

Donkey Serum (DS)

Dopachrome Tautomerase (DCT)

Endothelin-1 (ET-1)

Enhanced Chemi-Luminescence (ECL)

Enzyme Linked Immunosorbant Assay (ELISA)

Ethylenedaiminetetraacetic acid (EDTA)

Extracellular Matrix (ECM)

Fatty Acid Methyl Ester (FAME)

Fetal Calf Serum (FCS)

Flavin Adenine Dinucleotide ($FADH_2$)

Fluorescein Isothiocyanate (FITC)

Gas Chromatography (GC)

Goat Serum (GS)

G-Protein Coupled Receptor (GCPR)

Guanine Diphosphate (GDP)

Guanine Triphosphate (GTP)

Hair Follicle Melanocytes (HFMs)

Heneicosanoic Acid (C21:0)

Hour (hr)

Hydrochloric Acid (HCl)

Hydrogen Peroxide (H_2O_2)

Hydroperoxyarachidonate (HPETE)

Hydroxy-5, 8, 10, 14-eicosatetraenoate (HETE)

Hydroxyl Radical ($^{\cdot}OH$)

Immunoglobulin E (IgE)

Inducible Nitric Oxide Synthase (iNOS)

Inositol Triphosphate (IP_3)

Interleukin (IL)

Langerhans Cells (LC)

Leukemia Inhibitory Factor (LIF)

Liquid Chromatography/Electrospray Ionization-Mass Spectrometry/Mass Spectrometry (LC/ESI-MS/MS)

Lysophosphatidylcholine (LPC)

Maximum Binding (B_0)

Melanocortin-1 Receptor (MC-1R)

Melatonin 1A Receptor (MEL-1A)

Melatonin 1B Receptor (MEL-1B)

Messenger Ribonucleic Acid (mRNA)

Methoxylamine (MOX)

Methoxylamine Hydrochloride (MOX HC)

Microphthalmia Induced Transcription Factor (MITF)

Minute (min)

Multiple Reaction Monitoring (MRM)

Neutrophil Elastase (NE)

Nicotinamide Adenine Dinucleotide (NADH)

Nitric Oxide (NO)

Nitric Oxide Synthase-1 (NOS-1)

Nitric Oxide Synthase-2 (NOS-2)

Nitric Oxide Synthase-3 (NOS-3)

Non-Specific Binding (NSB)

Nuclear Factor-κβ (NF-κβ)

Oculocutaneous Albinism Type 1 (OCA1)

Optimal Cutting Temperature (OCT)

Oxygen (O_2)

p38 Mitogen-Activated Protein Kinase (p38)

Peroxisome Proliferator-Activated Receptor gamma (PPARγ)

Peroxy Nitrite (ONOO-)

Phosphate Buffered Saline (PBS)

Phosphatidylinositol (PI)

Phosphatidylinositol-3, 4, 5-Triphosphate (PIP_3)

Phospholipase-A_2 (PLA_2)

Polymerase Chain Reaction (PCR)

Polyoma Enhancer Activator 3 (PEA3)

Polyvinyldifluoride (PVDF)

Potassium Carbonate (K_2CO_3)

Potassium Chloride (KCl)

Proliferating Cell Nuclear Antigen (PCNA)

Pro-opiomelanocortin (POMC)

Prostaglandin $B_{2\text{-}d4}$ ($PGB_{2\text{-}d4}$)

Prostaglandin E Synthase (PGES)

Prostaglandin E_2 (PGE_2)

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)

Prostaglandin G_2 (PGG_2)

Prostaglandin H_2 (PGH_2)

Prostaglandin I_2 (PGI_2)

Protein Kinase A (PKA)

Protein Kinase C-β (PKCβ)

Protein Kinase-C (PKC)

Proteinase-Activated Receptor 2 (PAR2)

Reactive Oxygen Species (ROS)

Receptor for Activated C-Kinase-1 (RACK-1)

Ribonucleic Acid (RNA)

Second (sec)

Singlet Oxygen ($^1O_2$)

Skin Phototype (SPT)

Skin Phototype-1 (SPT-1)

Skin Phototype-4 (SPT-4)

Sodium Dodecyl Sulphate (SDS)

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Sodium Hydroxide (NaOH)

Solid Phase Extraction (SPE)

Stem Cell Factor (SCF)

Streptavidin Horseradish-Peroxidase (SHRP)

Superoxide Anion (O^{2-})

Tetramethyl Isothiocyanate (TRITC)

Tetramethylethylenediamine (TEMED)

Total Activity (TA)

Tris-acetate-EDTA (TAE)

Tris-EDTA (TE)

Tumor Necrosis Factor-alpha (TNFα)

Tyrosinase Related Protein-1 (TRP-1)

Ultrafast Internal Conversion (UIC)

Ultraviolet Radiation (UVR)

Upstream Transcription Factor-1 (USF-1)

Urocanic Acid (UCA)

UVA (Ultraviolet Radiation with wavelength 320-400 nm)

UVB (Ultraviolet Radiation with wavelength 280-320 nm)

UVC (Ultraviolet Radiation with wavelength 200-280 nm)

Water (H_2O)

α-Melanocyte Stimulating Hormone (α-MSH)

α-Tocopherol (vitamin E)

β-Endorphin (β-END)

β-Melanocyte Stimulating Hormone (β-MSH)

μ-Opiate Receptor (μ-OR)

REAGENTS

Table 1. Table of reagents used in this project.

Reagent	Company	City	Country
Acetic Acid	Sigma	Poole	UK
Acetone	Fisher Scientific	Loughborough	UK
Acetonitrile	Fisher Scientific	Loughborough	UK
Acrylagel	Flowgen Bioscience	Nottingham	UK
Amino Ethyl-Carbazole (AEC) Chromagen	Vector	Peterborough	UK
Agarose	Sigma	Poole	UK
Ammonium Persulphate	Sigma	Poole	UK
Arachidonic Acid	Cayman Chemical	Tyne and Wear	UK
Basic Fibroblast Growth Factor	TEBU-bio	Peterborough	UK
Boron Trifluoride	Sigma	Poole	UK
Butylated Hydroxytoluene	Sigma	Poole	UK
Bis-acrylagel	Flowgen Bioscience	Nottingham	UK
Bovine Serum Albumin	Sigma	Poole	UK
C21 Internal Standard (Heneicsanoic Acid)	Sigma	Poole	UK
Chloroform	Fisher Scientific	Loughborough	UK
Ciprofloxacin	Bayer	Berkshire	UK
4', 6-diaminidino-2-phenylindole (DAPI)	Vector	Peterborough	UK
Developer	Sigma	Poole	UK
Di Sodium Hydrogen Orthophosphate	Sigma	Poole	UK
Dichloromethane	Fisher Scientific	Loughborough	UK
Dimethylsulphoxide	Sigma	Poole	UK
Donkey Serum	Invitrogen	Paisley	UK
Ethylenedaiminetetraacetic acid **(**EDTA)	Sigma	Poole	UK
Endothelin-1	Sigma	Poole	UK
Ethanol	Fisher Scientific	Loughborough	UK
Ethidium Bromide	Sigma	Poole	UK
Fatty Acid Methyl Ester (FAME) Cocktail	Supelco	Belleforte, PA	USA
Fast Cycling Polymerase Chain Reaction (PCR) Kit	Qiagen	Sussex	UK
Fast Green	Sigma	Poole	UK
Fetal Calf Serum	Invitrogen	Paisley	UK
Fixer	Sigma	Poole	UK
Fungizone	Gibco (Invtrogen)	Paisley	UK
Geneticin	Gibco (Invtrogen)	Paisley	UK
Glacial Acetic Acid	BDH (VWR)	Lutterworth	UK
Glycergel	DAKO	Cambridgeshire	UK

Glycerol	Fisher Scientific	Loughborough	UK
Glycine	Sigma	Poole	UK
Goat Serum	Invitrogen	Paisley	UK
Hexane	Fisher Scientific	Loughborough	UK
Hydrochloric Acid	Sigma	Poole	UK
Hydrogen Peroxide	Sigma	Poole	UK
Isopropanol	Fisher Scientific	Loughborough	UK
3, 4-dihydroxy-L-phenylalanine (L-DOPA)	Sigma	Poole	UK
L-Glutamine	Gibco (Invtrogen)	Paisley	UK
Luminol	Biochemika (FLUKA)	Buchs	Switzerland
Luzindole	Sigma	Poole	UK
Magnesium Sulphate	Sigma	Poole	UK
Methanol	Fisher Scientific	Loughborough	UK
Methyl Formate	Acros Organics (Fisher Scientific)	Loughborough	UK
Meyers Haematoxylin	Sigma	Poole	UK
Minimum Essential Amino Acids	Gibco (Invtrogen)	Paisley	UK
Minimum Essential Medium	Gibco (Invtrogen)	Paisley	UK
Nitrogen	BOC Gases	Guildford	UK
Optimal Cutting Temperature Compound	Tissue-Tek (Sakura Finetek)	Illinois	USA
P-coumaric	Sigma	Poole	UK
Penicillin/Streptomycin	Gibco (Invtrogen)	Paisley	UK
Prostaglandin B_2-*d4* (PGB_2-*d4*)	Cayman Chemical	Tyne and Wear	UK
Prostaglandin D_2 (PGD_2) Methoxylamine (MOX) Enzyme Linked Immunosorbant Assay (ELISA)	Cayman Chemical	Tyne and Wear	UK
Prostaglandin E_2 (PGE_2) ELISA	Cayman Chemical	Tyne and Wear	UK
Poly-L-lysine	Sigma	Poole	UK
Potassium Carbonate	Sigma	Poole	UK
Potassium Chloride	Sigma	Poole	UK
Potassium Dihydrogen Orthophosphate	Sigma	Poole	UK
Protease Inhibitor Cocktail	Sigma	Poole	UK
Reagent A	Bio-Rad Laboratories	Bath	UK
Reagent B	Bio-Rad Laboratories	Bath	UK
Reagent S	Bio-Rad Laboratories	Bath	UK
RNeasy Isolation Kit	Qiagen	Sussex	UK
RPMI 1640	Gibco (Invtrogen)	Paisley	UK
Serum Free Keratinocyte	PromoCell	Heidelberg	Germany

Medium			
Sodium Bicarbonate	Sigma	Poole	UK
Sodium Chloride	Sigma	Poole	UK
Sodium Dodecyl Sulphate	Sigma	Poole	UK
Sodium Hydroxide	Sigma	Poole	UK
Sodium Sulphate	Sigma	Poole	UK
Superscript III First Strand Synthesis Super Mix	Invitrogen	Paisley	UK
Synthetic Melanin	Sigma	Poole	UK
Tetramethylethylenediamine (TEMED)	Sigma	Poole	UK
Thymol	Sigma	Poole	UK
Toluene	Acros Organics (Fisher Scientific)	Loughborough	UK
Trimethylpentane	Acros Organics (Fisher Scientific)	Loughborough	UK
Trizma Base	Sigma	Poole	UK
Tris-Acetate	Sigma	Poole	UK
Tris-Base	Sigma	Poole	UK
Trypsin/EDTA	Gibco (Invtrogen)	Paisley	UK
Tween-20	Sigma	Poole	UK
Ultrapure Water	Cayman Chemical	Tyne and Wear	UK
β-Mercaptoethanol	Sigma	Poole	UK

EQUIPMENT AND CONSUMABLES

Table 2. Table of equipment and consumables used in this project.

Item	Company	City	Country
8-Well Chamber Slides	NUNC (Fisher Scientific)	Loughborough	UK
96-Well Culture Plates	Corning Life Sciences	Schiphol-Rijk	The Netherlands
Autosampler	Agilent Technologies	California	USA
Blot Module-XCell II	Invitrogen	Paisley	UK
Counting Chamber-Depth 0.1 mm 1/400 mm^2	Hawksley	Lancing	UK
Coverslips	VWR	Lutterworth	UK
Cryostat	Leica	Wetzlar	Germany
Cryovials (1 ml)	NUNC (Fisher Scientific)	Loughborough	UK
Culture Dish	Invitrogen	Paisley	UK
Culture Flasks (225 cm^2)	Corning Life Sciences	Schiphol-Rijk	The Netherlands
Culture Flasks (25 cm^2)	Corning Life Sciences	Schiphol-Rijk	The Netherlands
Culture Flasks (75 cm^2)	Corning Life Sciences	Schiphol-Rijk	The Netherlands
Dewar (Arpege 70)	Air Liquide	Maine Laville	France
Electrophoresis Chamber-NOVEX Mini-Cell	Invitrogen	Paisley	UK
Electrospray Ionization Triple Quadruple Ultima Mass Spectrometer	Micromass	Manchester	UK
Eppendorf Tubes (1.5 ml)	Invitrogen	Paisley	UK
Gas Chromatography Column	Phenomenex	Macclesfield	UK
Gel Cassettes (1.0 mm)	Invitrogen	Paisley	UK
Gel Comb (15 well)	Invitrogen	Paisley	UK
Glass Pipettes	Fisher	Loughborough	UK
Heat Block	Thermoscientific	Cranlington	UK
Heraeus Incubator (Hera Cell)	DJB Labcare	Newport Pagnell	UK
High Performance Liquid Chromatography (HPLC) Pump (Alliance)	Waters	Elstree	UK
Hydrogen Generator (HG200)	CLAIND	Lenno	Italy
Inverted Microscope (CKX41)	Olympus	Watford	UK
Isopropanol Bath	Nalgene (Fisher	Loughborough	UK

	Scientific)		
Light Meter	Waldmann		UK
Mass Spec Column	Phenomenex	Macclesfield	UK
Microscope	Nikon		UK
Microscope slides	BDH (VWR)	Lutterworth	UK
MSE Centrifuge (Harrier 15/80)	DJB Labcare	Newport Pagnell	UK
Needles (0.6 x 25 mm)	NUNC (Fisher Scientific)	Loughborough	UK
Orbital Shaker	Grant Instruments	Cambridge	UK
PAP Pen	Invitrogen	Paisley	UK
Parafilm	Pechiney	Illinois	USA
PCR Electrophoresis Tank	Invitrogen	Paisley	UK
Photographic Film	Sigma	Poole	UK
Photographic Film Wallet	Sigma	Poole	UK
Plate Reader	GE Healthcare	Chalfont St Giles	UK
Polymerase Chain Reaction (PCR) Tubes	Geneflow	Fradley	UK
Polyvinyldifluoride (PVDF) Transfer Membrane	GE Healthcare	Chalfont St Giles	UK
Power Pack	Bio-Rad Laboratories	Bath	UK
Refrigerated Centrifuge	Dupont	Stevenage	UK
Solid Phase Extraction (SPE) Cartridges	Phenomenex	Macclesfield	UK
Spectrophotometer	Agilent Technologies	California	USA
Square Plastic Dishes	Invitrogen	Paisley	UK
Syringes (1 ml)	Invitrogen	Paisley	UK
Techne TC-512 Thermocycler	Witec		Germany
Ultraviolet (UV) Lamp	Philips		UK
Universal Tubes (25 ml)	Invitrogen	Paisley	UK
Vacuum Manifold	Phenomenex	Macclesfield	UK
Vortexer	Grant Instruments	Cambridge	UK
Water Bath	Grant Instruments	Cambridge	UK

PRIMARY ANTIBODIES

Table 3. Details of primary antibodies used in this project.

Antibody Specificity	Type	Company	Dilution			
			ICC	IHC	WB	IF
Neutrophil Elastase	Monoclonal	NovoCastra		1:70		
CD3	Monoclonal	NovoCastra		1:40		
CD4	Monoclonal	NovoCastra		1:40		
CD8	Monoclonal	NovoCastra		1:20		
Nitric Oxide Synthase-1	Polyclonal	Santa Cruz	1:100	1:100		1:50
Nitric Oxide Synthase-2	Polyclonal	Santa Cruz	1:200	1:100		1:50
Nitric Oxide Synthase-3	Polyclonal	Santa Cruz	1:100	1:100		1:50
12-Lipoxygenase	Polyclonal	Abcam	1:200		1:2000	
15-Lipoxygenase-2	Polyclonal	Abcam	1:100			
Cyclooxygenase-1	Polyclonal	Santa Cruz			1:100	
Cyclooxygenase-1	Polyclonal	Alexis	1:100			
Cyclooxygenase-2	Polyclonal	Alexis	1:200			1:50
Cyclooxygenase-2	Polyclonal	Cayman			1:50	
Malondialdehyde	Polyclonal	Santa Cruz			1:300	
Tyrosinase	Polyclonal	Santa Cruz			1:75	
Dopachrome Tautomerase	Polyclonal	Santa Cruz			1:100	
Tyrosinase Related Protein-1	Polyclonal	Santa Cruz			1:1000	
Actin	Polyclonal	Santa Cruz			1:500	
Phosphorylated Cytoplasmic Phospholipase A_2	Polyclonal	Santa Cruz	1:100		1:300	1:50
Prostaglandin E Synthase	Polyclonal	Cayman	1:100		1:100	1:50
Mel-1A	Polyclonal	Santa Cruz	1:100		1:100	
Mel-1B	Polyclonal	Santa Cruz	1:100		1:100	
NKI/beteb (gp-100)	Polyclonal	Monosan	1:50			1:50

ICC (immunocytochemistry), IHC (immunohistochemistry), WB (Western Blotting), IF (immunofluorescence).

SECONDARY ANTIBODIES

Table 4. Details of secondary antibodies used in this project.

Antibody Specificity	Conjugation	Company	Dilution			
			ICC	IHC	WB	IF
Anti-Mouse	Horseradish-peroxidase	Sigma			1:1000	
Anti-Rabbit	Horseradish-peroxidase	GE Healthcare			1:1000	
Anti-Goat	Horseradish-peroxidase	Serotec			1:600	
LSAB Kit (Anti-Mouse/Rabbit)	Biotin	Dako	X	X		
Anti-Rabbit	Fluorescein	Invitrogen				1:50
Anti-Goat	Fluorescein	Invitrogen				1:50
Anti-Mouse	Tetramethylrhodamine	Invitrogen				1:50

ICC (immunocytochemistry), IHC (immunohistochemistry), WB (Western Blotting), IF (immunofluorescence).

BLOCKING PEPTIDES

Table 5. Details of blocking peptides used in this project.

Partial Peptide Sequence From	Company	Dilution			
		ICC	IHC	WB	IF
Cyclooxygenase-1	Santa Cruz			1:100	
15-Lipoxygenase-2	Abcam	1:100			
Mel-1A	Santa Cruz	1:100			
Mel-1B	Santa Cruz	1:100			

ICC (immunocytochemistry), IHC (immunohistochemistry), WB (Western Blotting), IF (immunofluorescence).

PRIMERS

Table 6. Table of primers used in the Polymerase Chain Reaction in this project.

Target	Primer Sequence 5'-3'	Company	City	Country
Cyclooxygenase-1 Forward	TGCCCAGCTCCTGGCCCGCCGCTT	Sigma	Poole	UK
Cyclooxygenase-1 Reverse	GTGCATCAACACAGGCGCCTCTTC	Sigma	Poole	UK
Cyclooxygenase-1 Forward	TGCCCAGCTCCTGGCCCGCCGC	Sigma	Poole	UK
Cyclooxygenase-1 Reverse	GTGCATCAACACAGGCGCCTCTT	Sigma	Poole	UK
Cyclooxygenase-2 Forward	TTCAAATGAGATTGTGGGAAAATTGCT	Sigma	Poole	UK
Cyclooxygenase-2 Reverse	AGATCATCTCTGCCTGAGTATCTTT	Sigma	Poole	UK
Cyclooxygenase-2 Forward	TTCAAATGAGATTGTGGAAAAAATTGCT	Sigma	Poole	UK
Cyclooxygenase-2 Reverse	AGATCATCTCTGCCTGAGTATCTT	Sigma	Poole	UK
Actin Forward	CGCTGCGCTGGTCGTCGACA	Sigma	Poole	UK
Actin Reverse	GTCACGCACGATTTCCCGCT	Sigma	Poole	UK

BIOLOGICAL MATERIAL

Table 7. Table of biological material used in this project.

Subject Code	Skin Phototype	Age (yrs)	Gender	Sample Origin
23	1	32	F	Blister Roof
24	1	39	M	Blister Roof
25	1	36	F	Punch Biopsy
26	4	37	F	Punch Biopsy
27	1	44	F	Punch Biopsy
28	4	35	F	Punch Biopsy
29	1	29	F	Punch Biopsy
30	1	33	M	Blister Roof
31	4	22	M	Punch Biopsy
32	4	58	M	Punch Biopsy
33	4	52	F	Punch Biopsy
34	1	53	F	Blister Roof
35	1	36	F	Blister Roof
36	4	60	F	Blister Roof
37	1	27	F	Blister Roof
38	4	49	F	Punch Biopsy
39	4	42	M	Punch Biopsy
40	4	49	F	Blister Roof
41	1	31	M	Blister Roof
42	4	26	F	Blister Roof
43	4	51	F	Blister Roof
44	1	60	M	Punch Biopsy
45	4	26	M	Blister Roof
46	1	19	F	Punch Biopsy
47	1	31	F	Blister Roof
48	4	24	F	Blister Roof
49	4	39	M	Blister Roof
50	4	33	F	Blister Roof
51	1	32	M	Punch Biopsy
52	4	36	M	Blister Roof
53	1	50	F	Punch Biopsy
54	1	44	M	Punch Biopsy
55	1	34	M	Punch Biopsy
56	4	19	F	Punch Biopsy
F39	2/3	39	F	Breast Reduction
FM55	N/A	N/A	N/A	N/A
FM94	N/A	N/A	N/A	N/A
FM3	N/A	N/A	N/A	N/A

M (male), F (female), FM55, FM94 (Human melanoma cells), FM3 (Hamster melanoma cells), N/A (not applicable).