Report 1287COLI-COLIV-PGE2-ELISA

Effect of K-AdviColl on the Output of Prostaglandin E2, Type I and IV Collagen by the Human Dermal Fibroblasts

Prepared for

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Introduction

The objective of this project was to explore the effect of the test material listed in Table I on the output of prostaglandin E2 (PGE2), type I and type IV collagen by human dermal fibroblasts.

Table I. Test material assayed in this project.

Test Material	Date Received	Form
K-AdviColl	April 04, 2021	Liquid

Materials and Methods

Test materials (Table I) were dissolved in sterile distilled water. Samples of non-cytotoxic concentrations of K-AdviColl selected in Project 1287PCR were added in tree repeats to exponentially growing neonatal human dermal fibroblasts (nHDFp.2 Cell Applications, San Diego, CA, cat.# 106K-05n, cultured in DMEM/10%FBS) and cultures were pursued overnight. The following day, half of each experimental condition was UVB-irradiated according to Ochiai and coll., 2006, while the other half was kept in the dark. At the end of the experiment (24h post UVB irradiation), the target antigens were quantified in formalin-fixed cultures (type IV collagen) or in the cell culture-conditioned medium (type I collagen and PGE2) by ELISA assays using reagents listed in Table II. MAP was the positive control for type I collagen.

The negative control was sterile distilled water.

All colorimetric measurements were performed using Molecular Devices microplate reader MAX190 and SoftMax3.1.2PRO software.

Statistical significance was assessed with paired Student test. Deviations of >20% as compared to water control with p values below 0.05 were considered statistically significant.

Results and Discussion

Type I collagen output data are reported in Table III, type IV collagen – in Table IV and PGE2 – in Table V. Compared to water, K-AdviColl had no statistically significant modulatory effect on type I collagen and prostaglandin E2 secretion by human dermal fibroblasts, however, it did significantly increase type IV collagen output by these cells.

Table II. Assays performed in this project.

Target Quantified	Type I Collagen	Type IV Collagen	PGE2
Materials and Methods	Reagents from Southern Biotechnology, (Birmingham, AL), sandwich ELISA protocol per Dobak et al., 1994; Zhao et al., 2005).	Southern Biotechnology (cat.# 1340-08; Birmingham, AL) biotinylated anti-type IV collagen antibody/streptavidin-HRP	Competitive anti-PGE2 monoclonal antibody assay from Cayman (Ann Arbor, MI; cat.# 514010)

Test Material	Type I Collagen (% Water Control)	p value
H2O	**100**	**1**
K-AdviColl 1%	97	0.650
K-AdviColl 0.2%	105	0.524
K-AdviColl 0.04%	96	0.628
MAP 50µg/ml	301	0.000
MAP 100µg/ml	268	0.000
H2O+UVB	**100**	**1**
K-AdviColl 1%+UVB	107	0.441
K-AdviColl 0.2%+UVB	89	0.267
K-AdviColl 0.04%+UVB	88	0.227
MAP 50µg/ml+UVB	285	0.000
MAP 100µg/ml+UVB	310	0.000

Table III. Effect of different experimental conditions on type I collagen output with or without UVB irradiation, expressed as % of water control.

Test Material	Type IV Collagen (% Water Control)	p value
H2O	**100**	**1**
K-AdviColl 1%	131	0.027
K-AdviColl 0.2%	151	0.046
K-AdviColl 0.04%	99	0.974
H2O+UVB	**100**	**1**
K-AdviColl 1%+UVB	100	0.995
K-AdviColl 0.2%+UVB	116	0.619
K-AdviColl 0.04%+UVB	85	0.712

Table IV. Effect of different experimental conditions on type IV collagen output with or without UVB irradiation, expressed as % of water control.

Test Material	Type IV Collagen (% Water Control)	p value
H2O	**100**	**1**
K-AdviColl 1%	117	0.190
K-AdviColl 0.2%	123	0.148
K-AdviColl 0.04%	96	0.631
H2O+UVB	**100**	**1**
K-AdviColl 1%+UVB	86	0.017
K-AdviColl 0.2%+UVB	93	0.197
K-AdviColl 0.04%+UVB	102	1.000

Table V. Effect of different experimental conditions on PGE2 output with or without UVB irradiation, expressed as % of water control.

Note

These results are valid for only for the submitted sample.

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