Boosting of retinol activity using novel lecithin:retinol acyltransferase inhibitors

NT_343

Imfeld, Dominik[1]*; Schütz, Rolf[1]; Wikstroem, Peter[1]; Rawlings, Anthony[2];
*corresponding author: Imfeld Dominik; (Dominik.Imfeld@dsm.com)
[1] Research and Development PCA, DSM Nutritional Products Ltd, Kaiseraugst, Switzerland;
[2] AVR Consulting Limited, Northwhich, Cheshire, England UK

Introduction:

ROH and its esters are well accepted as efficacious skin anti-aging ingredients [1]. However, there is an ongoing desire in the cosmetic industry to boost the efficacy of either exogenous or endogenous ROH in order to be able to reduce the concentration in topical compositions to reduce its irritative effects and/or increase concentrations maximize its efficacy. In the skin, ROH is metabolized to retinoic acid using a variety of metabolic steps **(Figure 1)**. Thus, activation or inhibition of these enzymes offer routes to improve the efficacy of retinol.

ROH is mostly converted to retinyl esters in skin cells to become storage reserves and thus its esterification mechanism is the rate-limiting target to manipulate its levels [2]. It has been shown that lecithin:retinol acyltransferase (LRAT) is a main enzyme responsible for ROH esterification [3]. The enzyme transfers the sn-1 fatty acyl group, largely linoleic acid, from phosphatidyl choline to retinol [4, 5]. Thus, inhibition of LRAT would allow more ROH to be available for subsequent conversion to retinoic acid making LRAT an attractive target for the development of very effective anti-ageing skin actives.
The focus of this preliminary work was to identify novel LRAT inhibitors and to demonstrate their retinoid boosting, namely ROH, activity, on collagen III synthesis.

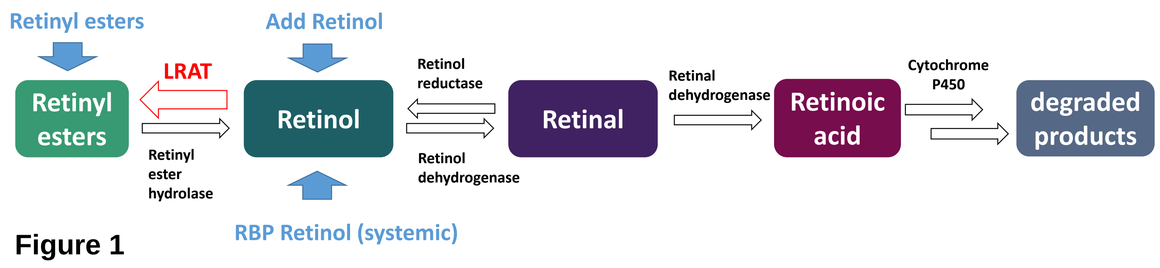

Figure 1

Materials & Methods:

1. Selected ingredients were assayed *in vitro* for their LRAT inhibitory potential in a phosphate buffer containing retinol, dilaurylphosphatidylcholine, dithioerythritol and bovine serum albumin (pH 7.0) for 60 min at 37° C. The reaction was quenched with ethanol before extraction with pentane and the resulting extracted retinoids were determined by reverse phase high performance liquid chromatography.
2. As a proof of concept study to underline the promising potential based on LRAT inhibition we performed an *ex vivo* study on human skin via topical application. The inhibitors were applied 3 times (at day 0, 1, 4, 6) and the skin was harvested for histologic analysis at day 8. The collagen III level was assessed by immunohistological staining and % stained surface of papillary dermis was quantified by image analysis using CellSense software. For each condition nine samples were stained and analysed (n=9). Data was collected in Microsoft Excel and statistics was calculated using unpaired Student's T-test.

Results (1):

Table I. *Effect of LRAT inhibitors and*

Inhibitor	Conc. [µM]	Inhibition %
DODS-(D,L)-F(3AMD)-Pzd(N-SO_2Me) x HCl *Dodecanesulphonyl (C12)*	100	94.3
DECS-(D,L)-F(3AMD)-Pzd(N-SO_2Me) x HOAc *Decanesulphonyl (C10)*	100	91.5
OCTS-(D,L)-F(3AMD)-Pzd(N-SO_2OCt) *Octanesulphonyl (but different SO2 ester) (C8)*	100	89.9
HEDS-(D,L)-F(3AMD)-Pzd(N-SO_2Me) *Hexadecaneseulphonyl (C16) (acylgroup too big)*	100	39.3
DODS-(L)-F(3AMD)-Pzd(N-SO_2Me) x HOAc *L variant as active as D,L*	100	96.7
DODS-(L)-F(3AMD)-Pzd(NH) *Importance of methyl sulphonate group*	100	44.6
Negative controls		
H-(D,L)-F(3AMD)-Pzd(N-SO_2Me) x 2 HCl *No acyl group, no activity*	100	6.8
DODS-(L)-F(4NH_2)-Pzd(N-SO_2Me) *Importance of AMD-amidino group*	100	0

We developed an enzymatic screening method for LRAT *in vitro* and analyzed the inhibitory activity of 80 compounds.
A common lead structure of a modified phenylalanine, DODS-(D,L)-F(3AMD-Pzd(N-SO2Me), was identified.
Structure-activity relationship (SAR) analysis showed that a long-chain fatty acid residue as DODS (dodecansulfonyl) or DECS (decansulfonyl) was essential for activity whereas H-(D,L)-F(3AMD)-Pzd(N-SO2Me) with a free N-terminus exerted very low activity **(Table I & Figure 2)**.

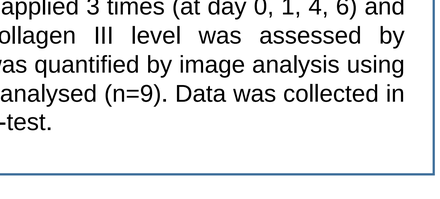

Figure 2. *Summary of essential features of LRAT inhibitors.*

Results (2) and Discussion:

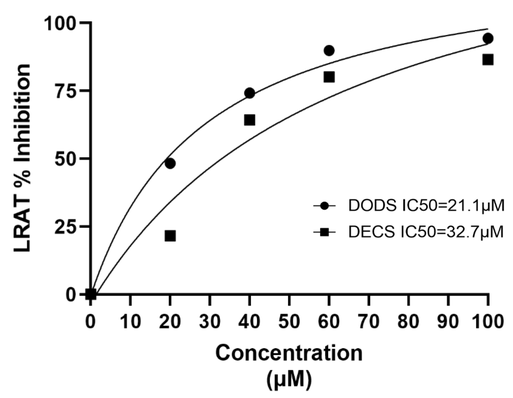

Figure 3. *Dose response effect of inhibitor A & B.*

We selected two inhibitors for further analysis: DECS-(D,L)-F(3AMD-Pzd(N-SO_2Me) = Inhibitor A and DODS-(D,L)-F(3AMD-Pzd(N-SO_2Me) = inhibitor B. Their IC_{50} for LRAT inhibition is shown (**Figure 3)**.

In the ex-vivo studies, ROH alone at 0.05% exerted as expected a moderate positive effect on collagen III synthesis. The LRAT-inhibitors were tested alone at 0.005% and 0.1% with a slight positive effect that was dose dependent, and the same inhibitors were tested in combination with 0.05% ROH. These combined tests revealed the strongest and also dose dependent increase on collagen III and the inhibitor B was even slightly better than inhibitor A consistent with their IC50's (**Figure 4**).

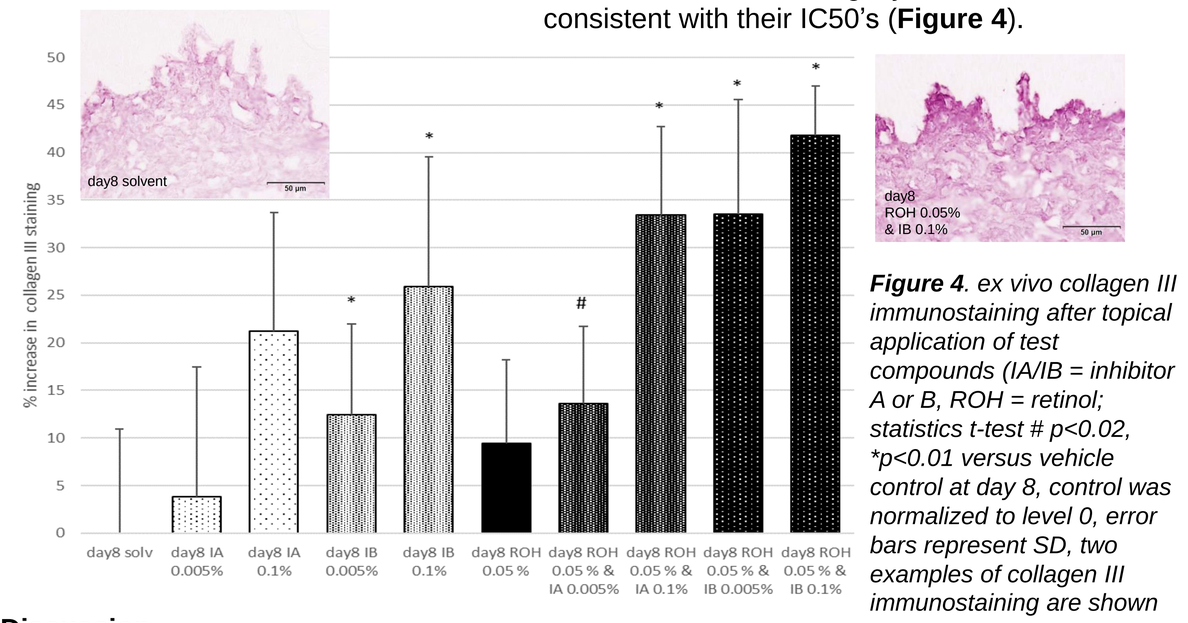

Figure 4*. ex vivo collagen III immunostaining after topical application of test compounds (IA/IB = inhibitor A or B, ROH = retinol; statistics t-test # p<0.02, *p<0.01 versus vehicle control at day 8, control was normalized to level 0, error bars represent SD, two examples of collagen III immunostaining are shown*

Discussion

ROH and its esters are well established antiaging ingredients [1]. However, their optimal efficacy is limited by the skin's capacity to storage them in an ester form, even when the retinyl esters are hydrolysed in the stratum corneum. Although several enzymes are involved in the conversion of ROH to RA which then interacts with the retinoid A & X receptors to trigger gene expression [6], we propose that the ROH esterification step is key in limiting the flux of ROH to RA.

As LRAT (EC 2.3.1.135) is inhibitable by phenylmethylsulphonyl fluoride, a serine protease inhibitor, we proposed that inhibitors of this class may be useful starting scaffolds for an LRAT inhibitors [7]. We found that amidino substituted amino acid derivatives are highly effective LRAT inhibitors and are in particular suitable to enhance the beneficial skin-ageing effects of ROH,

When combined with ROH these inhibitors increased Type III collagen levels effectively. In the *ex vivo* experiment this finally led to a synergistic stimulation of Type III collagen production **(Figure 4)** (*one example: 0.1% of IB +25.9% , 0.05% ROH +9.4%, the addition is 25.9+9.4 = +35.3%, the combination 0.1% IB & 0.05% ROH was however +41.8%*).

Further work is needed to establish if RA is effectively increased and in which cell types and/or whether a paracrine response has occurring following the ingredient application leading to neocollagenesis. Moreover, molecular modelling will be used to describe the interactions of the inhibitors with LRAT.

Conclusions:

We confirm that the LRAT inhibitors reduced the formation of retinyl esters from ROH and that the anticipated increased flux to retinoic acid in human skin resulted in increased retinoid responsive collagen III. LRAT inhibition using the acyl-(D,L)-F(3AMD-Pzd(N-SO2Me) scaffold is therefore a very promising approach to further boost the efficacy of ROH in future products to deliver superior anti-aging results.

References:

1. Tucker-Samaras S, Zedayko T, Cole C, Miller D, Wallo W, Leyden JJ. A stabilized 0.1% retinol facial moisturizer improves the appearance of photodamaged skin in an eight-week, double-blind, vehicle-controlled study. J Drugs Dermatol. 2009;8(10):932-6.
2. Chatellard-Gruaz D, Randolph RK, Hagens G, Saurat JH, Siegenthaler G. Differentiation of human epidermal keratinocytes is accompanied by increased expression of CRABP-II and increased cellular concentration of retinoic acids: retention of newly synthesized retinoic acids by CRABP-II. J Lipid Res. 1998;39(7):1421-9.
3. Kurlandsky SB, Duell EA, Kang S, Voorhees JJ, Fisher GJ. Auto-regulation of retinoic acid biosynthesis through regulation of retinol esterification in human keratinocytes. J Biol Chem. 1996;271(26):15346-52.
4. Sears AE, Palczewski K. Lecithin:Retinol Acyltransferase: A Key Enzyme Involved in the Retinoid (visual) Cycle. Biochemistry. 2016;55(22):3082-91.
5. Kang S, Duell EA, Fisher GJ, Datta SC, Wang ZQ, Reddy AP, et al. Application of retinol to human skin in vivo induces epidermal hyperplasia and cellular retinoid binding proteins characteristic of retinoic acid but without measurable retinoic acid levels or irritation. J Invest Dermatol. 1995;105(4):549-56.
6. Kurlandsky SB, Xiao JH, Duell EA, Voorhees JJ, Fisher GJ. Biological activity of all-trans retinol requires metabolic conversion to all-trans retinoic acid and is mediated through activation of nuclear retinoid receptors in human keratinocytes. J Biol Chem. 1994;269(52):32821-7.
7. Mangas I, Vilanova E, Estevez J. Kinetic interactions of a neuropathy potentiator (phenylmethylsulfonyl fluoride) with the neuropathy target esterase and other membrane bound esterases. Arch Toxicol. 2014;88(2):355-66..