

## Panthenyl triacetate transformation, stimulation of metabolic pathways, and wound-healing properties in the human skin

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### Synopsis

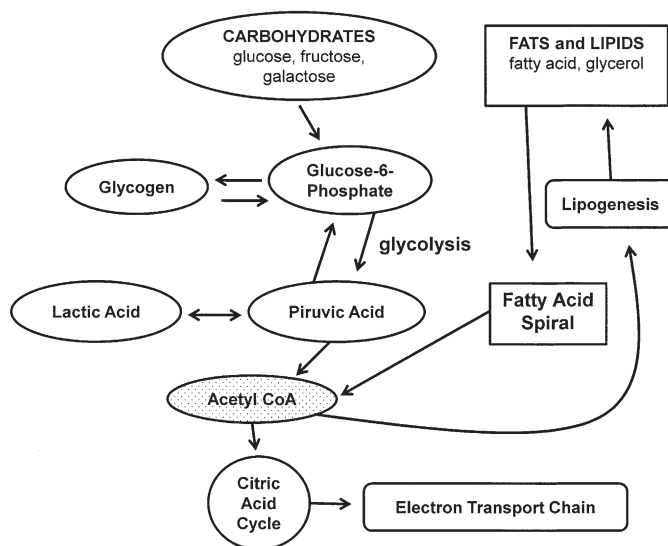
Vitamin B5 and its derivatives are well known in personal care applications and are often used in wound healing and soothing compositions. However, little is known about the biochemical pathways involved. A better knowledge of these pathways would help to understand some of the mechanisms of action and suggest further applications. We have investigated the transformation of D-panthenyl triacetate (PTA) into D-panthenol (PAN) and its skin diffusion on human volunteers by Raman spectroscopy. Additionally, we have utilized human skin biopsies and quantitative RT-PCR to demonstrate the effect of PTA compared to PAN on 27 metabolic markers when introduced at 2% in a cosmetic emulsion. Then we conducted a double-blind clinical study to measure the effect of PTA compared to PAN on wound healing, measured by transepidermal water loss (TEWL), when incorporated at 3% in a cosmetic emulsion. Results show de-acetylation of PTA into PAN and an increased activity of PTA compared to PAN over time in the skin. Metabolic marker analysis demonstrates stimulation of energetic pathways such as glycolysis and the citric acid cycle, but also of synthesis pathways such as isoprenoids and lipid synthesis, by PTA and PAN. Finally, the clinical study demonstrates a statistically significant effect by PTA on wound healing after 72 hours when compared to a saline treatment. Statistical significance was not achieved by PAN or a placebo treatment. Due to the differences between PTA and PAN action, different applications in personal care products can be suggested. Moreover, PTA seems more effective than PAN for a long-lasting wound healing action.

### INTRODUCTION

D-panthenyl triacetate (PTA) is a derivative of D-panthenol (PAN) and a precursor of pantothenic acid. Pantothenic acid is essential to normal epithelial function. It is part of coenzyme A, which serves as a cofactor for a variety of enzymatic reactions that are important in the metabolism of carbohydrates, fatty acids, and proteins (Figure 1). PAN, being the stable alcoholic analog of pantothenic acid with good skin penetration, has several properties in topical skin applications. Early literature in the 1950s demonstrated its function as a healing ingredient in cutaneous wounds (2). As dexpantenol, its use in treating various types of dermatoses, such as atopic dermatitis, and as a natural alternative to

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**Figure 1.** Carbohydrates, fats, and lipid metabolism summary. D-panthenyl triacetate is a derivative of D-panthenol and pantothenic acid, a component of acetyl CoA.

topical corticosteroids, has been extensively studied (1,3). Furthermore, its predominant role as a strong anti-irritant and its ability to protect and rebuild the skin barrier through the stimulation of barrier lipid synthesis has been suggested by several studies (4–7). The capacity of PAN to repair the skin barrier has also been postulated as a possible way for PTA to normalize sebum release by strengthening the follicular barrier in persons with oily skin (8). Although several clinical studies on the efficacy of PAN are available, very little is known on the biochemical mechanism involved. In the context of wound healing, a study on fibroblasts has shown the capacity of PAN to increase protein synthesis and release (9). Interestingly, PAN and PTA have shown the capacity to protect skin from UV-induced erythema (1; G. Dell’Acqua, unpublished data), and data on *in vitro* and on human skin explants have shown that both PAN and PTA would stimulate the proteins involved into UV-induced oxidation protection and repair (10–12). Taken together, these data suggest that an array of genes could be stimulated by PAN and PTA. Recently, research on proliferating fibroblasts in connection with wound healing has shown the activation of genes such as IL-6, IL-8, and HMOX-1 by calcium pantothenate (13), while studies on normal human keratinocytes in connection with an inflammatory profile have shown the capacity of PTA in combination with flavonoids to inhibit a series of pro-inflammatory genes (7).

In order to better understand the underlying molecular mechanism of PTA, we have decided to investigate the activation of several biochemical markers in human skin explants when treated with PTA at 2% in an emulsion. We have compared these data with D-panthenol (PAN) in the same condition. We have verified *in vivo* that PTA penetrates the stratum corneum and that it is de-acetylated in PAN. We have, finally, analyzed the wound-healing properties of PTA compared to PAN when applied on the skin by measuring the transepidermal water loss (TEWL) of human volunteers who have been subjected to suction blisters.

## MATERIALS AND METHODS

### SUBSTANCES

D-panthenyl triacetate (Induchem AG, Volketswil, Switzerland) and D-panthenol (Dai-ichi Fine Chemical, Takaoka, Japan) were tested diluted in either a water-based gel or in different emulsions.

### RAMAN SPECTROSCOPY ON THE SKIN OF HUMAN VOLUNTEERS

Human volunteers ( $n = 3$ ) applied three different products on three different areas on the volar side of the forearm: a water-based gel control, a water-based gel containing 3% PTA, and a water-based gel containing 3% PAN. The applied volume of the product was  $2 \text{ mg/cm}^2$ . The treated areas were measured by confocal Raman microspectroscopy (3510 Skin Composition Analyzer, River Diagnostics BV, Rotterdam, The Netherlands) one hour, five hours, and 24 hours after application. The measurements were taken to a depth of approximately  $25 \mu\text{m}$  in the skin, covering the whole stratum corneum and a small layer ( $\pm 5 \mu\text{m}$ ) in the viable epidermis. Ten repeated measurements were taken to obtain an average for the heterogeneity of the skin. The results were expressed as milligrams per active/gram of keratin versus the skin depth. The results were averaged for all three volunteers.

### HUMAN SKIN EXPLANTS AND TREATMENT

Twelve skin explants were prepared from an abdominal skin biopsy removed in plastic surgery; adipose tissue was removed, and the skin was cut off ( $5 \text{ cm}^2$ ), placed in DMEM medium (10% FCS) into six-well plates, and maintained at  $37^\circ\text{C}$ , 5%  $\text{CO}_2$ , humidity controlled. The medium was then replaced by new medium, and different products were topically applied on the epidermis in the plates. The products were a placebo cream, cream containing 2% PAN, and cream containing 2% PTA. The plates were then incubated for six or 24 hours. Untreated skin explants were used as controls in parallel. After each incubation period, three punches were performed on each skin explant and washed in phosphate buffer saline (PBS). The epidermal tissue was removed from the punches and immediately frozen at  $-80^\circ\text{C}$  for further mRNA extraction.

### QUANTITATIVE RT-PCR ANALYSIS

The mRNA of each segment of epidermis was extracted using Tri-Reagent<sup>®</sup> (Ambion, Austin, TX). The contaminant DNA was removed by treatment with a "DNA-free" system kit (Ambion), and reverse transcription of mRNA was conducted in the presence of oligo(dT) and Superscript II reverse-transcriptase (Invitrogen, Carlsbad, NM). The mRNA of triplicates was pulled together. Twenty-seven markers of skin metabolism were analyzed (see Table II) by quantitative RT-PCR. A PCR (polymerase chain reaction) was performed in triplicate using the LightCycler<sup>®</sup> system (Roche Molecular Systems Inc., Pleasanton, USA).

### WOUND-HEALING DOUBLE-BLIND CLINICAL STUDY

The double-blind study was conducted on 40 subjects (female and male) with normal, healthy skin. The 37 subjects who finished the study were of ages between 40.3 and 60.8 years

(52.0 ± 5.5 years). Before starting the study there was a preconditioning period of three days. Within this period the use of skin care products on the volar forearms was not allowed. To generate small suction blisters of about 5-mm diameter on both volar forearms (two or three areas per forearm), a vacuum of 450–800 mbar was applied. Transepidermal water loss (TEWL) was detected after 30 min (T0), 48 hours (T48), and 72 hours (T72) from suction blisters. The treatments were immediately after formation of the suction blisters and consisted of an emulsion containing 3% PTA, an emulsion containing 3% PAN, an emulsion placebo, and saline. They were compared to each other and to an untreated area. Treatments were applied at 100 µl in occlusion using Finn Chambers on Scanpor® (Almirall Hermal, Reinbeck, Germany) over a period of 30 minutes, 48 hours, and 72 hours. All procedures were performed in a climate-controlled room at 21.5°C (± 1°C) and 50% (± 5%) relative humidity.

STATISTICAL ANALYSIS

To analyze the data from the clinical study, the intergroup comparisons were performed by Student’s *t*-test. The pair differences varied in their distribution, and so for comparisons with the normally distributed pair differences, a *t*-test for dependent samples was used. The following table summarizes the statistical data obtained for all conditions tested:

Comparison		48 hours	72 hours
3% PTA	vs 3% PAN	0.8073	0.3153
3% PTA	vs Placebo emulsion	0.6690	0.2727
3% PTA	vs Saline	<b>0.0001</b>	<b>0.0162</b>
3% PAN	vs Placebo emulsion	0.3488	0.7003
3% PAN	vs Saline	<b>0.0007</b>	0.0620
Placebo emulsion	vs Saline	<b>0.0008</b>	0.1962

Bold: statistically significant differences.

RESULTS

D-PANTHENYL TRIACETATE AND D-PANTHENOL PENETRATION IN THE HUMAN SKIN

As shown in Figure 2, the spectra profiles of the water-based gel (panel A), the PAN gel (panel B), and the PTA gel (panel C) differ when the gels are applied on the skin. Importantly, it is possible to detect a peak shift in the 1722 cm<sup>-1</sup> region of the panel C spectrum that allows differential detection between PTA and PAN. This shift corresponds to the acetylated groups present in PTA but not in PAN.

In Figure 3 the depth penetration profile of PAN is shown. PAN penetrates to a maximum depth of 20 µm, which covers the whole stratum corneum. Five hours after application a rise is seen in the content in the skin, and deeper in the stratum corneum. Twenty-four hours after application, PAN is still present in the stratum corneum.

In Figure 4 the depth penetration profile of PTA is shown. Compared to PAN, PTA is detected mainly in the upper layer of the stratum corneum. After five hours the product is diffused through the upper layers and the content is decreased. After 24 hours almost no active is visible in the skin. The reason, principally, is the conversion of PTA into PAN,

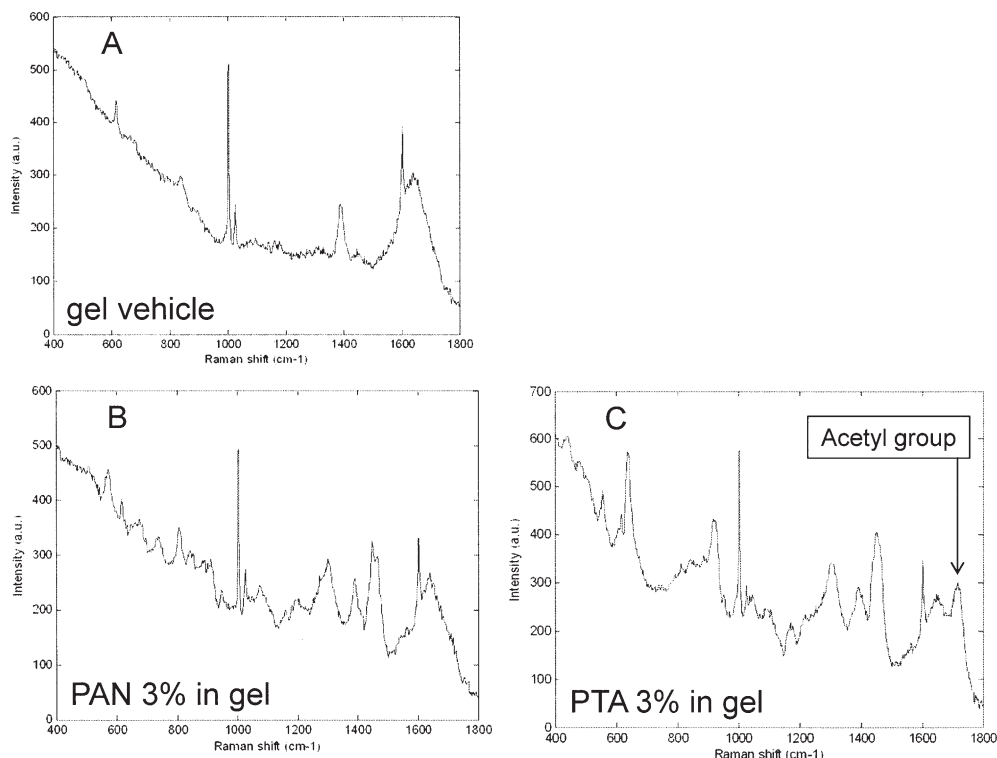


Figure 2. Raman spectra profiles for tested D-panthenol (PAN) and D-panthenyl triacetate (PTA). Note the shift at  $1722\text{ cm}^{-1}$  (panel C) that identifies the acetylated groups of PTA and allows differential detection of the two molecules when absorbed in skin.

i.e., its de-acetylation, in the deeper skin layers. This is clearly visible in Figure 5, where after application of a gel containing PTA, the signal corresponding to PAN is followed. After 24 hours a rise compared to the baseline is seen at a depth of 10 – 24  $\mu\text{m}$ . This indicates that PTA over a longer period of time is transformed into PAN. It is possible,

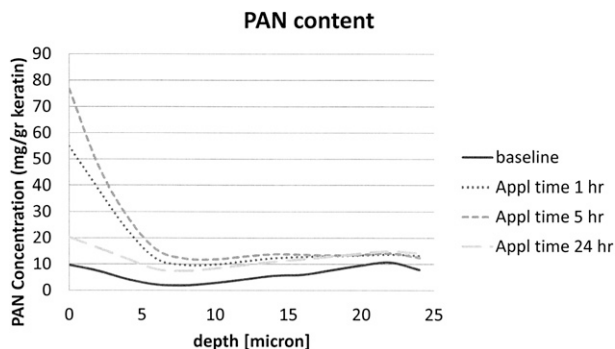


Figure 3. A water-based gel containing 3% D-panthenol (PAN) was applied on the skin of human volunteers ( $n=3$ ). The PAN signal was then followed through the skin layer by Raman spectroscopy. At 24 hours PAN was still present in the stratum corneum.

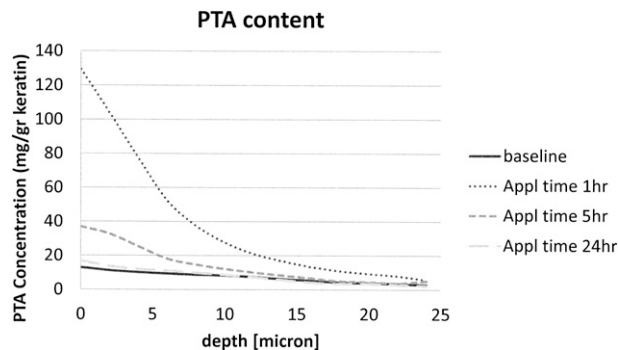


Figure 4. A water-based gel containing 3% D-panthenyl triacetate (PTA) was applied on the skin of human volunteers (n=3). The PTA signal was then followed through the skin layer by Raman spectroscopy. Almost no signal was detected at a 25-micron depth after 24 hours.

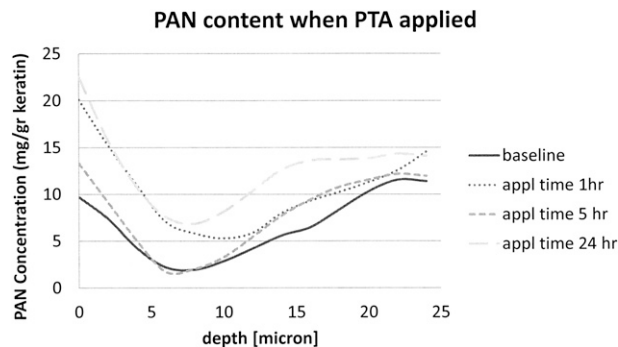


Figure 5. A water-based gel containing 3% D-panthenyl triacetate (PTA) was applied on the skin of human volunteers (n=3). Transformation of PTA into D-panthenol (PAN) was followed through the skin layer by Raman spectroscopy. The maximum PAN increase vs baseline was seen at 24 hours.

considering the de-acetylating time, that after 48 hours even a further increase in PAN might be seen; certainly it takes at least 24 hours to appreciate the transformation.

ACTIVATION OF METABOLIC MARKERS BY D-PANTHENYL TRIACETATE AND D-PANTHENOL IN THE HUMAN SKIN

In Table II, data are summarized regarding the capacity of PTA and PAN, when incorporated in an emulsion at 2%, to stimulate markers of metabolism in human skin explants (metabolism pathways are described in Figure 1 and marker analyses are summarized in Table I).

Although a general mRNA modulation is observed among all markers studied, only the differences superior or inferior at 50% have been considered. In this context, we can observe that PTA affects mainly 11 markers, while PAN affects seven markers (markers affected are in bold in Table II). Globally, these markers belong mainly to the citric acid cycle, the mevalonate pathway, glycolysis, and lipid synthesis. Graphs summarizing the data for PTA and PAN are represented in Figure 6 and Figure 7, respectively. When examining Figure 6, we can detect 11 main activities (indicated by arrows). PTA decreases,

**Table I**  
Metabolism Markers Analyzed by Quantitative RT-PCR in Human Skin Explants

<b>Acyl-CoA and Acetyl-CoA synthesis</b> <ul style="list-style-type: none"> <li>• ATP citrate lyase, <b>ACLY</b></li> <li>• acyl-CoA synthetase, <b>FACL1</b></li> <li>• acetyl-coenzyme A acetyltransferase 1, <b>ACAT1</b></li> </ul>	<b>Glycoysis</b> <ul style="list-style-type: none"> <li>• glucose phosphate isomerase, <b>GPI</b></li> <li>• glucose-6-phosphate dehydrogenase, <b>G6PD</b></li> </ul>
<b>Citric acid cycle</b> <ul style="list-style-type: none"> <li>• pyruvate dehydrogenase (lipoamide) alpha 1, <b>PDHA1</b></li> <li>• dihydrolipoamide S-acetyltransferase, <b>DLAT</b></li> <li>• citrate synthase, <b>CS</b></li> <li>• aconitase 1, soluble, <b>ACO1</b></li> <li>• aconitase 2 mitochondrial, aconitate hydratase, <b>ACO2</b></li> <li>• malate dehydrogenase 1, NAD (cytosolic soluble), <b>MDH1</b></li> <li>• malate dehydrogenase 2, NAD (mitochondrial), <b>MDH2</b></li> </ul>	<b>Fatty acid beta oxidation</b> <ul style="list-style-type: none"> <li>• carnitine acetyltransferase, <b>CRAT</b></li> <li>• acetyl-coenzyme A carboxylase alpha, <b>ACACA</b></li> </ul>
<b>Mevalonate pathway</b> <ul style="list-style-type: none"> <li>• 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (soluble), <b>HMGCS1</b></li> <li>• 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2 (mitochondrial), <b>HMGCS2</b></li> <li>• 3-hydroxy-3-methylglutaryl-coenzyme A reductase, <b>HMGCoAred</b></li> </ul>	<b>Lipid synthesis</b> <ul style="list-style-type: none"> <li>• fatty acid synthase, <b>FAS</b></li> <li>• cholesterol sulfotransferase, <b>SULT2B1</b></li> <li>• glucosidase, beta; acid (includes glucosylceramidase), <b>GBA</b></li> <li>• glucosylceramide synthase (ceramide glucosyltransferase), <b>UGCG</b></li> <li>• sphingomyelin phosphodiesterase 1, acid lysosomal, <b>SMPD1</b></li> <li>• arachidonate lipoxygenase 3, <b>ALOXE3</b></li> <li>• serine palmitoyltransferase, long chain base subunit 1, <b>SPTLC1</b></li> </ul>
	<b>Lipid transport</b> <ul style="list-style-type: none"> <li>• ATP-binding cassette, sub-family A (ABC1), member 12, <b>ABCA12</b></li> <li>• epidermal fatty acid-binding protein 5 <b>FABP5</b></li> <li>• fatty acid transport protein (FATP) <b>SLC27A4</b></li> </ul>

six hours after application, the activities of ATP citrate lyase (-56%), dihydrolipoamide S-acetyltransferase (-52%), and the ATP-binding cassette, sub-family A (ABC1), member 12 (-62%), while after 24 hours there is a decrease in the activity of epidermal fatty acid-binding protein 5 (-65%). PTA stimulates, six hours after application, the activities of aconitase 2 mitochondrial, aconitate hydratase (+72%) and 3-hydroxy-3-methylglutaryl coenzyme A synthase 2 (mitochondrial) (+218%), while after 24 hours there is an increase in malate dehydrogenase 2, NAD (mitochondrial) (+62%), 3-hydroxy-3-methylglutaryl coenzyme A synthase 2 (mitochondrial) (+201%), glucose phosphate isomerase (+55%), glucose-6-phosphate dehydrogenase (+68%), and cholesterol sulfotransferase (+60%). Globally it appears that PTA is pushing the citric acid cycle, glycolysis, and the mevalonate pathway, but also regulating the metabolism of lipids, interestingly pushing the synthesis of cholesterol sulfate with its implications on keratinocyte differentiation, while inhibiting lipid transport.

When PAN was examined as depicted in Figure 7, seven main activities were detected (indicated by arrows). PAN decreases, six hours after application, the activities of 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (soluble) (-61%). PAN stimulates, six hours after application, aconitase 2 mitochondrial, aconitate hydratase (+191%), 3-hydroxy-3-methylglutaryl coenzyme A synthase 2 (mitochondrial) (+137%), and sphingomyelin phosphodiesterase 1, acid lysosomal (+83%), while after 24 hours there is an increase in 3-hydroxy-3-methylglutaryl coenzyme A synthase 2 (mitochondrial) (+220%), cholesterol sulfotransferase (+51%), and fatty acid transport protein (FATP)



Table II  
Metabolism Marker mRNAs from Human Skin Explants Treated with an Emulsion Containing D-panthenyl triacetate (PTA) or D-panthenol (PAN) at 2%

mRNA	Marker	PTA 6 h	PAN 6 h	PTA 24 h	PAN 24 h
ATP citrate lyase	ACLY	-56	2	20	11
Acyl-coenzyme A synthetase	FACL1	12	-7	4	-1
Acetyl-coenzyme A acetyltransferase 1	ACAT1	-29	-4	3	1
Pyruvate dehydrogenase (lipoamide) alpha 1	PDHA1	-40	-10	-1	-14
Dihydrolipoamide S-acetyltransferase	DLAT	-52	6	11	-12
Citrate synthase	CS	-23	15	49	23
Aconitase 1, soluble	ACO1	-16	14	25	-23
Aconitase 2 mitochondrial, aconitate hydratase	ACO2	72	191	7	26
Malate dehydrogenase 1, NAD (cytosolic soluble)	MDH1	34	48	-6	-6
Malate dehydrogenase 2, NAD (mitochondrial)	MDH2	11	8	62	-5
3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (soluble)	HMGCS1	43	-61	0	-35
3-hydroxy-3-methylglutaryl-coenzyme A synthase 2 (metochondrial)	HMGCS2	218	137	201	220
3-hydroxy-3-methylglutaryl-coenzyme A reductase	HMG-CoAred	-36	-15	8	7
Glucose phosphate isomerase	GPI	14	-2	55	2
Glucose-6-phosphate dehydrogenase	G6PD	-25	-3	68	-14
Carnitine acetyltransferase	CRAT	-15	21	30	47
Acetyl-coenzyme A carboxylase alpha	ACACA	-50	32	4	36
Fatty acid synthase	FAS	-42	18	39	11
Cholesterol sulfotransferase	SULT2B1	-9	7	60	51
Glucosidase, beta; acid (includes glucosylceramidase)	GBA	-14	34	-3	6
Glucosylceramide synthase (ceramide glucosyltransferase)	UGCG	-20	12	33	36
Sphingomyelin phosphodiesterase 1, acid lysosomal	SMPD1	-10	83	-37	-20
Arachidonate lipoxygenase 3	ALOXE3	-7	37	42	50
Serine palmitoyltransferase, long chain base subunit 1	SPTLC1	-32	-9	-4	-2
ATP-binding cassette, sub-family A (ABC1), member 12	ABCA12	-62	17	-36	18
Epidermal fatty acid-binding protein 5	FABP5	-34	-19	-65	13
Fatty acid transport protein (FATP)	SLC27A4	-15	32	38	59

Differences (expressed as %) are versus a control emulsion. Treatment was for six hours or 24 hours. In bold: significant differences (see text).

(+59%). When these data are examined, it appears, as expected, that some of the activities observed for PTA are confirmed with PAN, in particular the push for the citric acid cycle, the mevalonate pathway, and the synthesis of cholesterol sulfate. However, we don't detect activities regarding glycolysis, and the effect on lipid transport is mainly positive (while PTA was negative). The results from the metabolic markers indicate that PTA and PAN have common mechanisms but also differ.



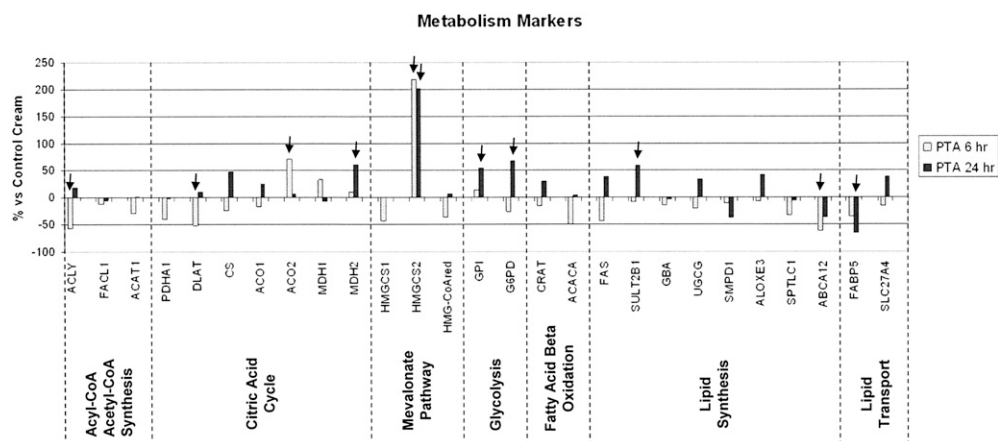


Figure 6. Metabolism marker mRNAs from human skin explants treated with an emulsion containing D-panthenyl triacetate (PTA) at 2%. Treatment was for six hours and 24 hours. Arrows indicate significant changes.

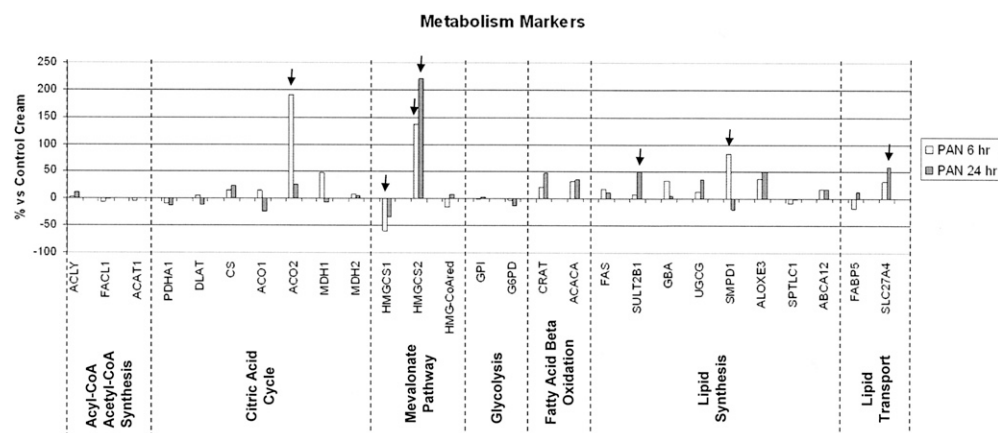
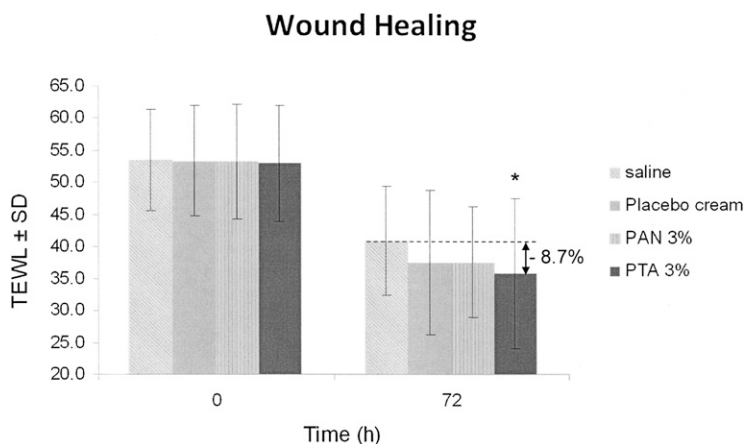


Figure 7. Metabolism marker mRNAs from human skin explants treated with an emulsion containing D-panthenol (PAN) at 2%. Treatment was for six hours and 24 hours. Arrows indicate significant changes.

#### STIMULATION OF WOUND HEALING BY D-PANTHENYL TRIACETATE AND D-PANTHENOL

The results of clinical study on wound healing in a group of human volunteers are described in Figure 8. As can be seen, transepidermal water loss (TEWL) dramatically increases 30 minutes after wound induction, and treatment with the different products (placebo, PTA 3%, PAN 3%) does not modify the TEWL when compared to a saline treatment. After 48 hours of treatment, all treatment groups produced a statistically significant effect compared to saline (see Statistical Analysis, p. 4). However, after 72 hours of treatment with the products, only PTA was statistically significant when compared to saline ( $p < 0.05$ , Student's  $t$ -test), with a difference of -8.7%, while placebo and PAN treatments were not.



**Figure 8.** Wound healing measured by transepidermal water loss (TEWL) on the skin of human volunteers ( $n = 37$ ) after treatment with an emulsion containing 3% D-panthenyl acetate (PTA) or D-panthenol (PAN). T0 is 30-minute treatment after wound induction.  $*p < 0.05$  vs saline, Student's  $t$ -test.

## DISCUSSION

This paper examines some activities of vitamin B5 derivatives such as D-panthenol (PAN) and D-panthenyl triacetate (PTA), with particular attention to their effect on metabolism markers and their capacity for wound-healing stimulation. In this paper, we have shown that both PAN and PTA penetrate the human skin when carried by a gel or by an emulsion and that both elicit metabolic changes. In particular, utilizing Raman spectroscopy, we demonstrated that both PTA and PAN can travel through the stratum corneum, while PTA is slowly de-acetylated in PAN with time. Interestingly, in human volunteers this effect peaks at around 24 hours. When a human skin biopsy is observed, the metabolic changes induced by PTA start happening more rapidly (six hours). This could be due to the faster penetration of the actives in human skin explants compared to *in vivo* skin (14) and possibly also to the vehicle used to carry the actives (emulsion vs gel), allowing an earlier bioavailability of PTA for de-acetylation in the human skin explants. Furthermore, Raman studies show that, indeed, most PTA is converted in PAN, since almost no PTA residue is seen after 24 hours (Figure 4). If most, if not all, PTA is converted in PAN over time, it is possible that the metabolic effect observed in human skin explants (see Table II) are mostly due to PAN. Analysis of the data from the metabolic marker study indicates, in fact, strong analogies between PTA and PAN treatment. Both ingredients push the citric acid cycle by stimulating mitochondrial enzymes such as aconitase 2 mitochondrial, aconitate hydratase (ACO2), while PTA also stimulates malate dehydrogenase 2 (MDH2). The effect on ACO2 and MDH2 could be explained, considering that panthothenic acid is a key component of coenzyme A (CoA) and that acetylCoA is upstream and feeding the citric acid cycle (see Figure 1).

The same observation can be made when markers involved in the metabolism of isoprenoids or lipids, which are stimulated by PTA and PAN, are involved, with particular interest in cholesterol synthesis and modification. Cholesterol synthesis is essential for homeostasis of the epidermis, being required for both cell division and for differentiation, as well

as for maintenance of the epidermal permeability barrier. Cholesterol synthesis in the epidermis is correlated with changes in mRNA levels for key enzymes, such as the HMG-CoA synthase family and HMG-CoA reductase. Mitochondrial 3-hydroxy-3-methylglutaryl coenzyme A synthase (HMGCS2), an enzyme part of the HMG-CoA synthase family, catalyzes the first step in isoprenoid/mevalonate synthesis and under some conditions controls the flux into the pathway (15,16). The capacity of both PTA and PAN to stimulate HMGCS2 (+201% and +220% respectively, after 24 hours) is impressive. In the context of lipid synthesis and cholesterol it is also interesting to highlight the capacity of both PTA and PAN to stimulate cholesterol sulfotransferase (SULT2B1), thus influencing cholesterol modification. Also, in this case this enzyme has been linked to skin differentiation by influencing the amount of cholesterol sulfate and its binding to differentiation receptors such as PPAR and LXR (17-19). SULT2B1 has also been associated with the sulfonation of cytotoxic oxysterols and therefore could have a role in skin detoxification (20). The activation of SULT2B1 by both PTA and PAN suggests a new role for these vitamins as prodifferentiation and detoxification agents.

The increase in enzymes such as glucose phosphate isomerase (GPI) and glucose-6-phosphate dehydrogenase (G6PD), representatives of glycolysis activity, was only observed when PTA was used and only after 24 hours. It is possible that activation of glycolysis to produce more pyruvic acid is necessary to "feed" the energetic and metabolic pathways downstream (citric acid cycle and lipogenesis). It is also possible that PTA, providing a longer-term bioavailability of PAN, sustains a later activation of the glycolysis pathway only detectable at 24 hours and possibly lasting even longer, while the available PAN is consumed earlier in the skin metabolism.

The data suggest PTA as a possible long-lasting reservoir for feeding PAN to the skin. We have, indeed, some previous evidence that treatment with PTA from persons with oily skin provided a long-lasting effect even when treatment was discontinued (8), suggesting a longer bioavailability of PTA-originated PAN.

Finally, in order to correlate the metabolic activity of PTA and PAN with their capacity to heal and reduce TEWL after skin injury, we have conducted a clinical study comparing the two ingredients. PAN's capacity as a skin-healing agent (2,21) has been mainly related to its activity on fibroblasts (1) and on keratinocytes (5,6). In effect, we have confirmed that enzymes involved in skin differentiation and the building of the skin lipid barrier, such as HMGCS2 and SULT2B1, are strongly stimulated by both PTA and PAN, although we don't know whether there would also be an effect by PTA and PAN on fibroblasts in the healing process. The results of our clinical study (Figure 8) identified PTA as the only significant treatment when compared to saline at 72 hours. It is intriguing to detect this effect at a late time point, i.e., after 72 hours of treatment from wound induction. Also, in this case it can be postulated as a long-term effect from PTA. It is possible that a sustained action would be necessary to stimulate an added value of PTA when compared to a placebo or a PAN treatment in the context of wound healing.

## CONCLUSIONS

We have demonstrated *in vivo* that D-panthenyl triacetate (PTA) can penetrate the human skin deeply and be transformed into D-panthenol. We have further demonstrated, in human skin explants, the activation by PTA of the enzymes involved into metabolic

pathways such as glycolysis, the citric acid cycle, isoprenoids, and lipid synthesis. Finally, we have shown in a clinical study for wound healing and by transepidermal water loss (TEWL) that PTA can significantly decrease TEWL when compared to a saline treatment, further showing its biological activity *in vivo*.

We believe that D-panthenyl triacetate can be a valid active to incorporate in modern cosmetic formulations to target sensitive and atopic dry skin, to rebalance excessive skin sebum release, to reinforce the skin barrier, and to repair skin damage.

## ACKNOWLEDGMENTS

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