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Correlation between treatment time, photobleaching, inflammation and pain after photodynamic therapy with methyl aminolevulinate on tape-stripped skin in healthy volunteers

Catharina M. Lerche[1], Susanne Fabricius[1], Peter A. Philipsen[1], and Hans Christian Wulf[1]

[1]Department of Dermatology, Copenhagen University Hospital, Bispebjerg, Copenhagen, Denmark

Corresponding author:

Catharina M. Lerche, PhD

Copenhagen University Hospital, Bispebjerg

Department of Dermatology, D92

Bispebjerg Bakke 23

DK-2400 Copenhagen NV, Denmark

Phone: +45 35312778

Fax: +45 35316010

E-mail: catharina.margrethe.lerche@regionh.dk

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Novel aspects of the work

Shorter incubation time with methyl aminolevulinate in photodynamic therapy regimens in healthy volunteers results in decreased photobleaching and also less inflammation and pain.

Abstract

Photodynamic therapy (PDT) is an attractive treatment option for skin diseases such as actinic keratosis, since large skin areas can be treated with high response rates and good cosmetic outcome. Nevertheless inflammation and pain are still major side effects.

The aim of this study was to investigate to what extent less time-consuming PDT treatment regimens using methyl aminolevulinate (MAL) decrease protoporphyrin IX (PpIX) photobleaching, inflammation and pain.

Twenty-four healthy volunteers were treated with 4 different interventions on each forearm. All 8 fields were tape-stripped 10 times. On the right arm MAL was applied for 20, 40, 60 or 180 min., followed by further incubation after wiping off MAL until 180 min. after start and then illumination with red light 180 min. after start. On the left arm MAL or vehicle was applied for 30, 60, or 90 min. and illuminated immediately after MAL removal. PpIX fluorescence, photobleaching, objective and subjective erythema (as a measure for inflammation), pigmentation and pain were measured.

The results showed a significant correlation between incubation time, time until illumination and photobleaching. Furthermore, there was a significant correlation between photobleaching and erythema and also between photobleaching and pain.

In conclusion, shorter PDT regimens result in decreased photobleaching and also less inflammation and pain. We hypothesize that shorter incubation time is important for optimal specific subcellular distribution of PpIX and to avoid unspecific distribution. We propose a shorter

PDT regimen, “Pulse PDT”, comprising, for example 30 min. incubation with MAL and illumination after 180 min., and we have planned a study of actinic keratosis and “Pulse PDT”.

Key words: Photodynamic therapy (PDT), methyl aminolevulinate, PpIX fluorescence, incubation time, erythema.

Introduction

Photodynamic therapy (PDT) has become an established treatment for conditions like actinic keratosis, Bowen’s disease and basal cell carcinoma (1;2). Major advantages of PDT are less invasiveness and good cosmetic outcome (1;2). However, pain and inflammation are still serious side effects and the conventional PDT regimen is time-consuming for the clinic (1).

There are many variable factors in PDT treatment such as incubation time, photosensitizer, light source, treatment duration, and administration of analgesics (2). The conventional PDT regimen includes superficial curettage, application of methyl aminolevulinate 16% (MAL) and occlusion for 3 h., which results in a high accumulation of the photosensitizer protoporphyrin IX (PpIX) in the lesions (2). After this PpIX is activated by red light, resulting in damage to and destruction of the diseased cells (2). The effect of PDT is dependent on the formation of PpIX, but it is not completely clear whether a high PpIX concentration is needed for a good outcome with complete clearance of the lesion. A previous study compared incubation times with MAL 16% of 1 and 3 h. on 58 patients with actinic keratosis (AK) (3). The response rates were slightly higher with 3 h. of incubation with MAL 16%; 87% for thin AK, 84% for moderately thick AK, and 82% for thick AK compared with 78%, 69% and 88% after 1 h. of incubation time (3). Two smaller studies have also investigated shorter

incubation times but using ALA instead of MAL. The first showed a similar clearance rate in 18 patients with AK lesions at 5 months following incubation with ALA for 1 h. compared with 2 h. (90% vs. 89%, respectively) (4). The second study showed a clearance rate of 75% of AK lesions in 12 patients after incubation with ALA for 1 h. followed by illumination with blue light (5). PPIX fluorescence was not measured in these studies. One study describes the correlation between PPIX fluorescence, photobleaching, pain and clinical outcome of actinic keratosis but it does not describe shorter incubation time and erythema (6). Longer incubation times seem to result in an only slightly better response rate. It is known from in vitro studies that cellular localization of PpIX is an important issue (7-9). A study using hexyl aminolevulinate as the photosensitizer in two human lymphoma cell lines proposes that mitochondria and the endoplasmic reticulum are the targets for achieving apoptosis (9). If it was possible to attack only these targets, it could result in a treatment with less unspecific PpIX fluorescence and thus fewer side effects. A shorter incubation time could result in a more specific PpIX fluorescence leading to a treatment regimen with fewer side effects.

The aim of this study was to investigate the relation between incubation time, PPIX florescence and inflammation after photodynamic therapy with MAL on tape-stripped skin in healthy volunteers; more specifically to investigate how shorter incubation time and shorter time until illumination affect erythema (inflammation) and pain.

Results

Twenty-four healthy volunteers were each treated with 8 different interventions after tape-stripping the skin 10 times (Table 1). The 4 interventions on the right arm consisted of incubation with MAL for 20, 40, 60, or 180 min., further incubation after MAL removal and all treatment fields being illuminated with red light after 180 min. The 4 interventions on the left arm consisted of incubation with MAL or vehicle for 30, 60, or 90 min. with the treatment fields being illuminated with red light just after incubation.

PpIX fluorescence

The measured PpIX fluorescence and photobleaching values are given in Table 2. The PpIX fluorescence before illumination for the different treatment regimens is shown in Figure 1. Longer incubation time results in more PpIX fluorescence. Also longer time until illumination results in more PpIX fluorescence, which can be seen in the MAL60/180 group, where there is more PpIX fluorescence (1112 AU) than with MAL60/60 (446 AU). The remaining PpIX fluorescence after illumination is the same in all groups. There is a significant correlation between incubation time of MAL and photobleaching in data for illumination after 180 min., ($P=7.1 \times 10^{-11}$, $R^2=0.422$) and for data with illumination immediately after cream removal, ($P=6.4 \times 10^{-14}$, $R^2=0.549$).

Objectively measured erythema

Figure 2 and Table 3 show the erythema percentages. No significant difference was found between the erythema percentages of the fields before MAL application. The percentages just after treatment were significantly lower in nearly all treatment regimens than in the standard regimen (Table 3). On Days 1-3 after treatment the erythema percentages were significantly lower

for all treatment regimens than for the standard regimen although not significantly lower in all groups on Days 2 and 3. On Day 8 after treatment the erythema percentages were practically the same for all treatments (Table 3).

There is a significant correlation between the increase in erythema after illumination and photobleaching $P=1.6 \times 10^{-15}$, $R^2=0.385$, if data are divided into interventions with illumination after 180 min., ($P=2.2 \times 10^{-9}$, $R^2=0.370$) and interventions with illumination immediately after cream removal, ($P=4.5 \times 10^{-12}$, $R^2=0.493$) (Figures 3A and 3B).

Patient evaluation of erythema

One hour after treatment 31% of treatment fields on the arms (60 out of 192) were reported as red. The day after treatment 25% of the fields were evaluated as being red while two days after treatment only 14% of the fields were reported as red. From Day 4 and onwards 16 fields were still reported as red, 8 of these were the fields treated with the conventional PDT regimen.

Expert evaluation of erythema

The expert evaluation on Day 1 after treatment also showed less erythema after all shorter treatment regimens than after the standard treatment. Also on Days 2, 3 and 8 after treatment there was less erythema in all the groups although the difference was not significant in all groups (Table 3). The day after treatment the expert evaluated 23% of the fields as being red; this is very close to the value of patient-evaluated redness of 25%. The same tendency is seen for the other days, and there is a strong correlation between expert- and subjectively evaluated redness of these fields ($P=2.5 \times 10^{-36}$ – $P=3.8 \times 10^{-22}$, R=0.624 – R=0.753).

Pigmentation

Comparisons of the pigmentation percentages after the different treatment regimens are presented in Table 2. There was a small difference in pigmentation percentages before treatment but there was no increase 8 days after treatment.

Pain

The mean pain score during treatment is very low in all the treatment regimens, as seen in Figure 4. The mean pain was significantly less in all the different treatment regimens compared with the standard treatment regimen except MAL40/180 (P=0.051) and MAL60/180 (P=0.115), where it was less but not significant (Table 2). There was a significant correlation between pain and photobleaching in all treatment fields, ($P=5.9 \times 10^{-5}$, $R^2=0.116$). We can also see a correlation between MAL application time and pain if data are divided into illumination at 180 min., (P=0.025, $R^2=0.229$) and illumination immediately after cream removal, ($P=7.9 \times 10^{-8}$, $R^2=0.265$).

Discussion

The present study investigates the relation between incubation time (understood as both incubation time and time until illumination), amount of PpIX (measured as PpIX fluorescence and photobleaching), inflammation (measured as erythema) and pain after photodynamic therapy with MAL on tape-stripped skin in healthy volunteers. The focus of the study was to look at optimizing regimes to reduce inflammation and pain.

As expected, we found that shorter incubation times with MAL and also shorter time until illumination resulted in development of less fluorescence and thereby also less photobleaching. If we compare the two groups MAL60/180 and MAL60/60, it is interesting to see that even though

both groups were incubated with MAL for 60 min., the difference in time to illumination had an effect on the production of PpIX fluorescence. MAL60/180 resulted in a PpIX fluorescence of 1112 and MAL60/60 resulted in fluorescence of 446 (Table 2). This can be explained by the fact that MAL in the skin was still being converted into the photosensitizer PpIX after 60 min. when the excess amount of cream was wiped off and until the illumination started at 180 min. The increase in PpIX fluorescence after cream removal indicates bioavailability of MAL in the skin. This reservoir effect has been described previously (10).

Longer incubation times and longer time until illumination resulted in more erythema. The erythema percentage was less in all shorter treatment regimens than in the standard treatment regimen. Again it was interesting to compare MAL 60/180 and MAL 60/60, which resulted in 32% and 25% erythema immediately after treatment, respectively. This can be explained by the fact that more fluorescence results in more erythema. There is a correlation between increase in erythema percentage just after illumination and photobleaching (R^2=0.385, P=1.6 x 10^{-15}). We have previously performed a study on the same healthy volunteers with application of different concentrations of MAL on their backs (11). In that study there was also a strong correlation between increase in erythema and photobleaching (11).

Pain in the present study was overall scored very low. There was less pain during illumination when incubation time and time until illumination were shorter and hence also less PpIX. There was less pain during illumination when time until illumination was 90 min. compared to 180 min. with the same incubation time of 60 min. Pain during illumination was strongly correlated to photobleaching. Pain during illumination was also strongly correlated to erythema. We know from

a previous study that also erythema and pain are correlated (11). However not all studies have shown this correlation between erythema and pain (12).

This shows that to reduce inflammation it is important to reduce incubation time as much as possible. However, do we know that one would still have similarly effective regimes or do we need the inflammation for efficacy? We know from a previous study by Wiegell SR et al. that topical corticosteroid reduced erythema 24 h. after PDT without compromising the efficacy of the treatment, with no significant difference in lesion response rate between the two treatments 3 months after PDT (13). Neither PpIX fluorescence prior to illumination nor increased erythema was correlated with the efficacy of the PDT treatment (13). We know that phototoxic effect during PDT is dependent on the amount of PpIX accumulated in the tissue. But high levels of PpIX fluorescence and inflammation are not necessary for effective treatment of AK (13). Moreover, we hypothesize that shorter incubation time is important for optimal specific subcellular distribution of PpIX and to avoid unspecific distribution.

It is a considerable limitation of this study that our results are based on normal human tape-stripped skin instead of dysplastic or malignant human skin lesions. Uptake and distribution of MAL are known to vary between normal skin and lesions about 10-fold (14). This study was not blinded but open to the assessor. However, we used predominately objective methods such as fluorescence pictures and measures erythema and pigmentation using the optimizer. However, we cannot exclude the possibility of assessor bias completely.

Experimental

Healthy Volunteers

Twenty-four healthy male volunteers of Scandinavian ancestry were included in the study (mean age 30, range 20-51). Exclusion criteria: allergy to MAL, exposure to sunlight or use of sunbed within 30 days before inclusion. The study was conducted during winter with low temperatures in Denmark and therefore there was limited risk of natural sunlight exposure as the arms are almost always covered by clothes. All volunteers received both oral and written information about the purpose, nature and possible risks and benefits of the trial. The study was performed in accordance with the Declaration of Helsinki. Written consent was obtained from all volunteers before enrolment in the trial, which was approved by the local Ethics Committee (H-D-2009-007).

Interventions

A treatment area was selected on the inside of each forearm of the volunteer. With the help of a prefabricated flexible template each treatment area was divided into four minor treatment fields 2x5 cm in size with at least 3 cm between each field. The borders of the fields were outlined with a black non-fluorescent marker (Stabilo, OHPen universal, Permanent). In order to imitate skin lesions all fields were tape stripped 10 times with occlusive dressing before treatment (Tegaderm™ Roll, 3M, Glostrup, Denmark).

On the left forearm excess of MAL 16% (Metvix®, Photocure, Oslo, Norway) cream was applied to the first three fields, and the vehicle Unguentum M was applied to the remaining field. All fields were covered with light impermeable occlusive dressing. After 30 min. (Field 1), 60 min. (Field 2)

and 90 min. (Fields 3 and 4) the dressing was removed and excess cream wiped off (Table 1). The fields were illuminated with red light immediately after removal of excess cream.

On the right forearm copious amounts of MAL 16 % (Metvix®, Photocure, Oslo, Norway) were applied to all four fields of treatment, which were then covered with light-impermeable, occlusive dressings. After 20 min. the dressing was removed from the first field and the excess cream gently wiped off. The field was covered again with a thin piece of gauze and light impermeable dressing. After 40 and 60 min. the same procedure was followed with the second and third fields. 180 min. after application of MAL the dressings were removed from all four fields, and the excess cream was gently wiped off the last field (standard treatment) (Table 1). All four fields were illuminated with red light.

Illumination was performed in both studies with red LED light 630 nm peak (Aktilite^{TM} 128; Photocure ASA, Oslo Norway) using a total light dose of 37 J/cm^2 given over 9 min. During and after illumination pain was recorded. The volunteers were equipped with a special diary for recording pain in the days after treatment. Four follow-up visits were performed at Days 1,2,3 and 8 after treatment.

Randomizing

The study was designed as an open randomized trial. A statistical adviser performed the randomization. Since the sequence of treatment duration was predefined, randomization was only determining for which of the four treatment fields should be the first.

PpIX Fluorescence

MAL-induced PpIX fluorescence was depicted non-invasively using a fluorescence camera (Medeikonos AB, Gothenburg, Sweden). The amount of PpIX fluorescence was calculated from the photographs by the programme MatLab ® (MatLab ®, MathWorks, Natic, US). The amount of fluorescence was measured after tape-stripping and before cream application (baseline) and before and after illumination.

The calculated PpIX fluorescence (Arbitrary Units, AU) is the mean pixel value minus the mean pixel value of the background image. The photobleaching is then the difference in PpIX fluorescence (AU) calculated from the pre- and post-illumination images.

Erythema and pigmentation

As an indicator of inflammation erythema was measured. The erythema was assessed by an expert evaluator and measured objectively.

The expert evaluator used a scale from 0 to 3, where 0=no erythema, 1=mild erythema (just visible, light pink skin) 2=moderate erythema (clear red skin colour) 3=severe erythema (dark red to bluish skin colour).

The objective measurements of erythema and pigmentation were performed using a skin reflectance meter (Optimize Scientific 558, Chromo-Light, Espergaerde, Denmark).

This instrument measures skin remittance at 558 nm and 660 nm and calculates the content of melanin and haemoglobin in the skin independently of each other (15;16). These measurements are given in erythema and pigmentation percentages, and measurements are referred to as such. Three measurements were performed and the median value was used in the statistical calculations. Erythema and pigmentation percentages were measured before MAL application but

after tape-stripping, immediately before illumination, immediately after illumination, and at the four follow-up visits.

Pain score

The pain was scored as in to previous publications (17;18). The volunteers scored their pain every minute during illumination, and recorded their pain in the diary every hour after illumination on the treatment day, twice per day the next three days and once a day on the following five days (only results for pain during illumination are shown). Since PDT was performed at different times of the day, the number of evaluations varied from 3 to 11 the first day. Pain was assessed using a numerical scale ranging from 0 to 10, where 0 is no pain and 10 is worst imaginable pain. To make it easier for the patients to identify the differently treated fields, the diary was supplied with numbered drawings of the fields.

Statistics

The sample size was calculated on the basis of data from the literature. We set the minimal clinically relevant difference to 8.8 % (50 % of the earlier found 17.6 %) and choose a power of 0.80 and a significance level of 0.05; 22 volunteers were needed to complete the study (19).

To identify differences in florescence, photobleaching, pain score, erythema percentages and pigmentation percentages between the treatment fields we used Wilcoxon Signed Ranked Test, since all results were paired.

To assess correlations we used Spearman's rank correlation.

To determine which parameters were of importance for the development of erythema and pain, multiple regression analysis with backward elimination was performed.

For all calculations a p-value < 0.05 was considered statistically significant. All analyses were performed with PASW Statistics 19.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Due to technical difficulties erythema and pigmentation measurements are missing for one volunteer on Day 1, three volunteers on Day 2 and one volunteer on Day 3. In addition, fluorescence images are missing for four volunteers.

Conclusion

We have investigated different treatment regimens with fewer side effects such as less inflammation and less pain compared with the standard treatment regimen, which is beneficial for both patients and the clinic. We conclude that photobleaching is dependent on the incubation time. A shorter incubation time results in reduced photobleaching and also reduced inflammation. Photobleaching is also dependent on the time until illumination, and a shorter time until illumination results in reduced photobleaching and reduced inflammation. There is a strong correlation between photobleaching and inflammation. Shorter incubation time and shorter time until illumination also result in less pain. We consider the time to be crucial for optimal cellular distribution of PpIX. We believe a "Pulse PDT", of for example, 30 min. of MAL incubation and 180 min. until illumination will give a sufficient amount of PpIX but without all the inflammation and pain from unspecifically located PpIX. However, we need more knowledge regarding the minimum amount of PpIX required for a treatment still to be effective, and PpIX distribution in tissue under the different conditions that might be the most important in choice of regimen. In this study we have used tape-stripped skin on healthy volunteers, while in future studies we will use patients with actinic keratosis to investigate whether a "Pulse PDT" has the same response rate as standard PDT regimen.

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Table 1: Overview of interventions

	Topical treatment	Incubation time (min.)	Time until illumination with red light (min.)	Shortening of intervention
Right arm				
Field 1	MAL	20	180	MAL20/180
Field 2	MAL	40	180	MAL40/180
Field 3	MAL	60	180	MAL60/180
Field 4	MAL	180	180	MAL180/180
Left arm				
Field 1	MAL	30	30	MAL30/30
Field 2	MAL	60	60	MAL60/60
Field 3	MAL	90	90	MAL90/90
Field 4	Vehicle	90	90	Vehicle90/90

Table 2. Comparisons of PpIX fluorescence, photobleaching, pigmentation, and pain between the standard treatment and 7 shorter interventions. The P-values are the comparisons of each of the shorter interventions with the standard treatment.

	Standard treatment	Illumination after 180 min.						Illumination after 30, 60, or 90 min.							
Treatment regime	MAL180/180	MAL20/180		MAL40/180		MAL60/180		MAL30/30		MAL60/60		MAL90/90		Vehicle90/90	
	Mean (SD)	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**
Fluorescence before illumination (AU)	1983 (1001)	403 (178)	<.001	642 (495)	<.001	1112 (748)	<.001	223 (346)	<.001	446 (288)	<.001	665 (453)	<.001	41 (249)	<.001
Fluorescence after illumination (AU)	236 (289)	203 (207)	.911	213 (275)	.881	203 (278)	.433	102 (231)	.332	149 (226)	.528	45 (262)	.078	4 (247)	.102
Photobleaching (AU)	1747 (968)	200 (232)	<.001	429 (499)	<.001	909 (781)	<.001	116 (334)	<.001	321 (277)	<.001	471 (407)	<.001	28 (263)	<.001
Pigmentation before treatment (%)	19.6 (4.7)	20.8 (5.1)	.219	21.8 (5.5)	.013	20.2 (6.4)	.331	21.0 (4.2)	.046	20.4 (4.7)	.376	21.1 (3.8)	.179	21.6 (4.2)	.013
Pigmentation 8 days after treatment (%)	19.5 (5.8)	20.5 (4.1)	.236	19.5 (5.2)	.976	20.2 (5.6)	.316	20.8 (4.8)	.168	20.4 (4.7)	.236	21.1 (4.0)	.306	20.7 (4.4)	.263
Pain during treatment (0-10)	0.8 (0.8)	0.3 (0.5)	.003	0.5 (0.8)	.051	0.6 (0.7)	.115	0.1 (0.3)	<.001	0.1 (0.3)	<.001	0.2 (0.3)	.001	0.1 (0.2)	<.001

Table 3. Comparisons of objectively measured erythema percentages and expert evaluated erythema clinical scores between standard treatment and 7 shorter interventions.

The expert-evaluated erythema clinical scores were 0 for all the volunteers before treatment. The P-values are the comparisons of each of the shorter interventions with the standard treatment.

	Stan-dard treat-ment	Illumination after 180 min.						Illumination after 30, 60, or 90 min.							
Treat-ment regime	MAL180/180	MAL20/180		MAL40/180		MAL60/180		MAL30/30		MAL60/60		MAL90/90		Vehicle90/90	
Erythema %	**Mean (SD)**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**
After tape-stripping Before treatment	22.9 (4.6)	21.3 (4.6)	.346	20.9 (4.4)	.081	22.4 (5.4)	.549	22.4 (3.8)	.587	23.3 (4.3)	.932	22.7 (4.1)	.710	22.4 (4.5)	.710
Just after illumination	36.3 (7.8)	24.3 (5.6)	<.001	29.5 (8.1)	.006	32.2 (9.0)	.086	24.0 (3.4)	<.001	24.6 (4.8)	<.001	24.5 (5.9)	<.001	23.2 (4.5)	<.001
1 day after	32.6 (5.4)	24.6 (6.5)	.001	25.3 (5.6)	.001	26.0 (5.6)	<.001	23.3 (5.8)	<.001	24.5 (5.7)	<.001	26.6 (6.7)	.003	24.7 (4.1)	<.001
2 days after	27.7 (4.2)	22.7 (4.4)	.001	23.9 (4.5)	.002	25.4 (5.7)	.050	24.6 (5.2)	.006	25.4 (5.6)	.079	25.4 (4.4)	.050	23.7 (3.9)	.002
3 days after	27.8 (4.3)	22.5 (4.5)	.001	24.2 (5.4)	.042	24.3 (5.5)	.014	23.6 (4.1)	.004	24.8 (5.2)	.136	25.0 (5.7)	.064	24.5 (4.7)	.042
8 days after	24.8 (5.4)	21.8 (4.3)	.014	23.1 (4.8)	.110	23.7 (5.2)	.290	23.2 (5.8)	.179	22.8 (4.7)	.092	23.1 (5.1)	.241	22.5 (4.8)	.067
Expert Erythema clinical scores (0-3)	**Mean (SD)**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**	**Mean (SD)**	**P-value**
1 day after treatment	0.9 (0.6)	0.0 (0.2)	<.001	0.4 (0.5)	<.001	0.5 (0.5)	.002	0.0 (0.0)	<.001	0.0 (0.0)	<.001	0.2 (0.4)	<.001	0.0 (0.0)	<.001
2 days after	0.7 (0.5)	0.0 (0.0)	<.001	0.1 (0.3)	<.001	0.3 (0.4)	.002	0.0 (0.2)	<.001	0.0 (0.0)	<.001	0.1 (0.3)	.001	0.0 (0.0)	<.001
3 days after	0.6 (0.5)	0.0 (0.0)	<.001	0.1 (0.3)	.001	0.1 (0.3)	.001	0.0 (0.0)	<.001	0.0 (0.0)	<.001	0.1 (0.3)	.001	0.0 (0.0)	<.001
8 days after	0.3 (0.4)	0.0 (0.0)	.014	0.1 (0.3)	.046	0.1 (0.3)	.083	0.0 (0.0)	.014	0.0 (0.0)	.014	0.0 (0.2)	.059	0.0 (0.0)	.014

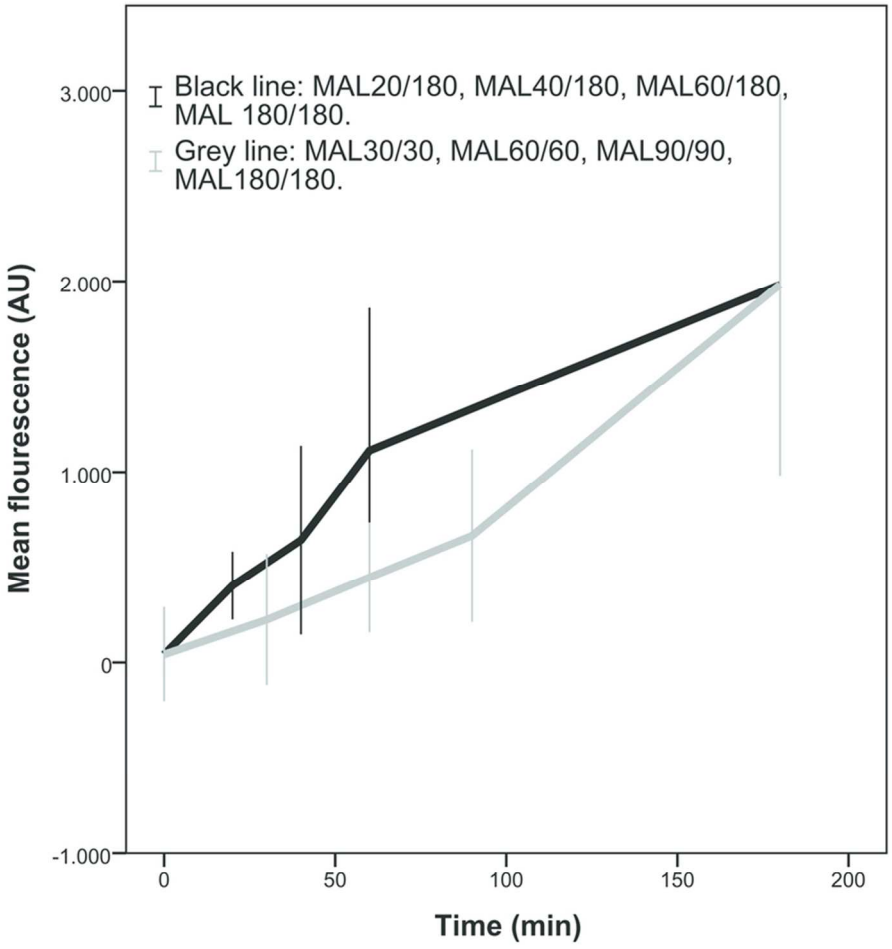
Figure legends

Figure 1. Mean fluorescence over time after incubation with MAL. Error bars indicate standard deviation.

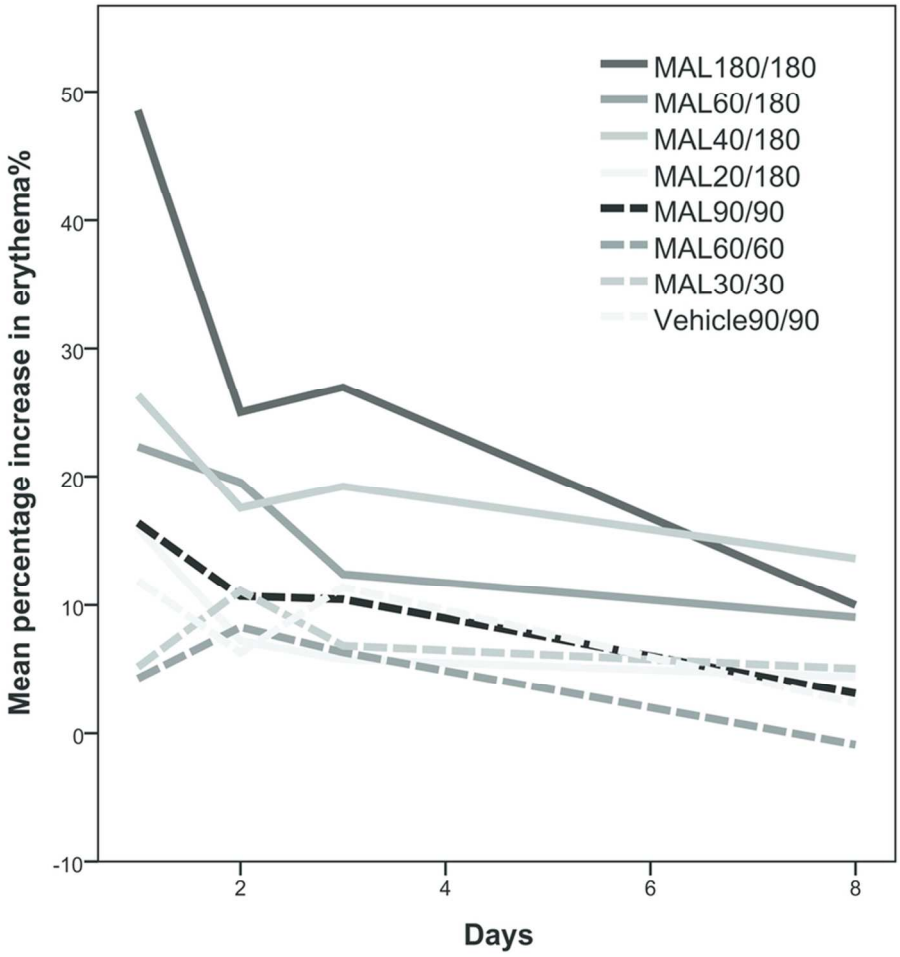
Figure 2. Mean decrease in erythema percentages for standard PDT treatment and 7 different altered regimens.

Figure 3A and 3B. Correlation between increase in erythema percentage just after illumination and photobleaching. A) Illumination after 180 min.; MAL20/180, MAL40/180, MAL60/180, MAL180/180. B) Illumination immediately after cream removal; MAL30/30, MAL60/60, MAL90/90, and MAL180/180.

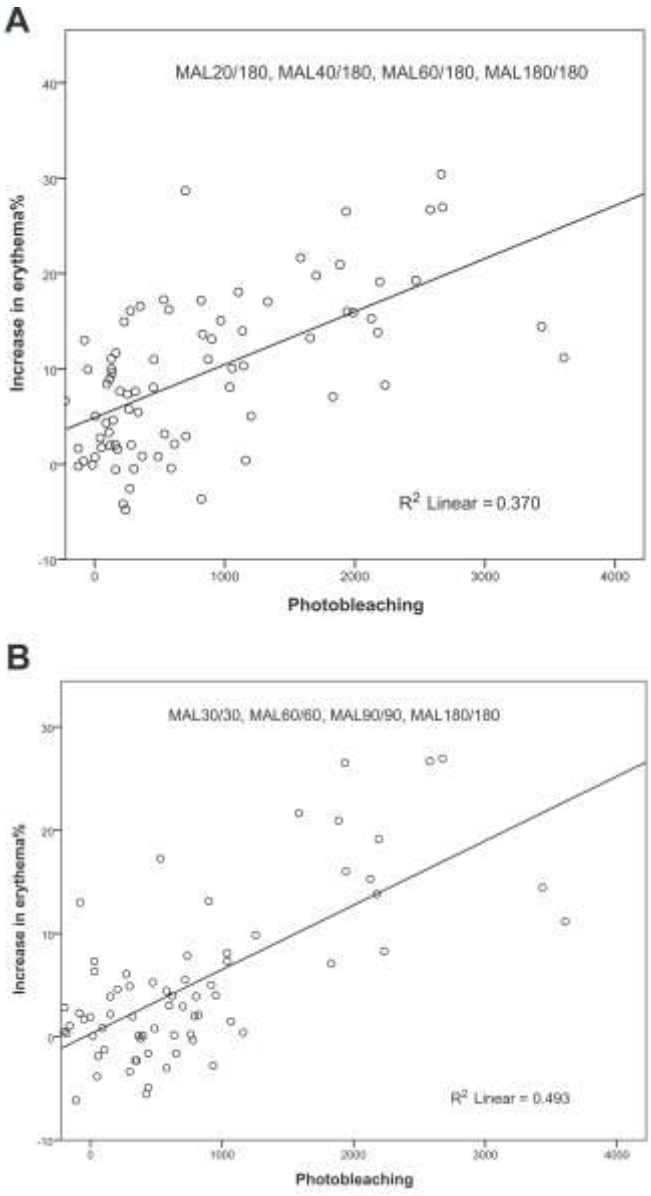
Figure 4. Mean pain score during 9 min. illumination.



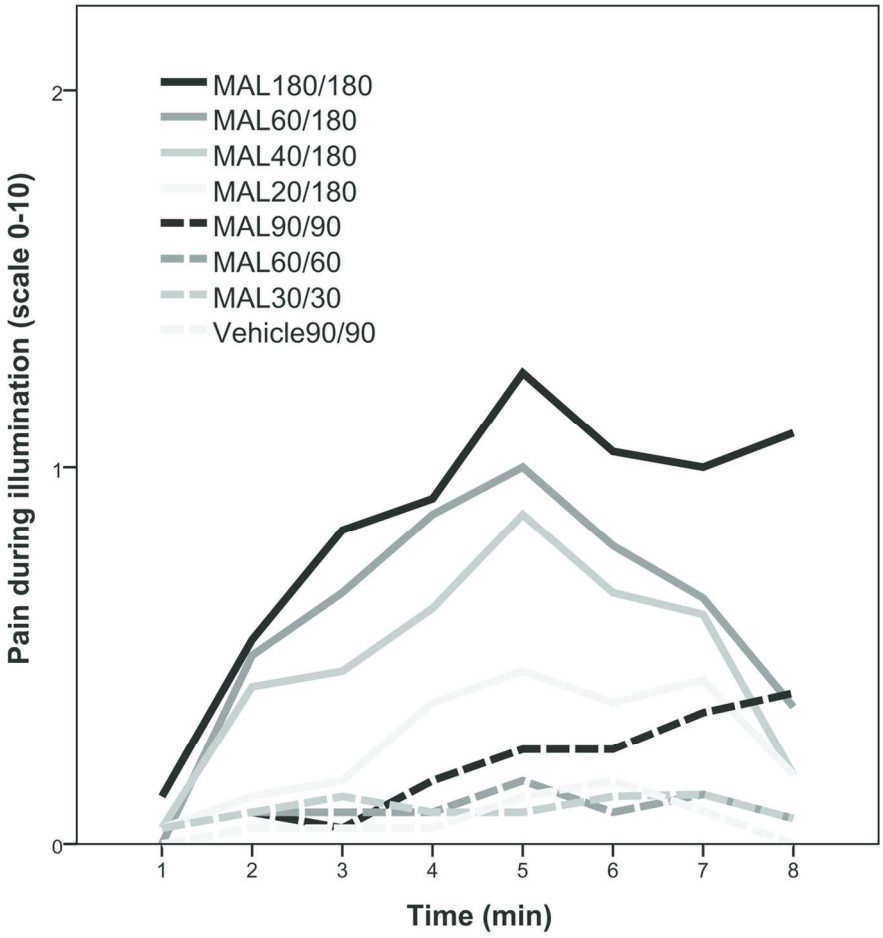
Mean fluorescence over time after incubation with MAL. Error bars indicate standard deviation.
80x78mm (300 x 300 DPI)



Mean decrease in erythema percentages for standard PDT treatment and 7 different altered regimens.
81x79mm (300 x 300 DPI)



Correlation between increase in erythema percentage just after illumination and photobleaching. A) Illumination after 180 min.; MAL20/180, MAL40/180, MAL60/180, MAL180/180. B) Illumination immediately after cream removal; MAL30/30, MAL60/60, MAL90/90, and MAL180/180.
145x254mm (300 x 300 DPI)



Mean pain score during 9 min. illumination.
127x122mm (300 x 300 DPI)