Topical Liposomal Rose Bengal for Photodynamic White Hair Removal: Randomized, Controlled, Double-Blind Study

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ABSTRACT

Background: Blond and white hair removal by laser is a complicated task with weak satisfactory results due to the deficiency in laser-absorbing chromophore.

Objective: To investigate if repetitive sessions of photodynamic therapy (PDT) using external application of liposomal Rose bengal (RB) photosensitizer followed by intense pulsed light (IPL) exposure enables removal of gray and white hair.

Materials and Methods: Rose bengal loaded in liposomes (LRB) was constructed, prepared in hydrogel, and was studied for some pharmaceutical properties. Penetration and selective hair follicle damage in mice skin were studied. Topical gel containing LRB was used for treating fifteen adult females who were complaining of facial white terminal hair. Unwanted facial hair was treated for three sessions at intervals of 4–6 weeks using intense pulsed light (IPL). At each session, the treatment area was pre-treated with topical LRB gel, while a control group of another 15 patients applied placebo gel before IPL treatment. Evaluations included hair regrowth, which was measured 4 weeks after each treatment session and at 6 months follow-up by counting the number of terminal hair compared with baseline pretreatment values. Treatment outcomes and complications if any were also reported.

Results: Average hair regrowth in the LRB group was 56% after 3 treatment cycles. After six-months follow up, average terminal hair count compared with baseline pretreatment showed 40% reduction and no recorded side effects. A significant difference (P<0.05) was seen compared with the control group; the clinical results were promising.

Conclusions: Photodynamic hair removal using rose bengal-encapsulated liposomal gel in combination with IPL treatment showed significant efficacy in the treatment of white hair compared with a control group.


INTRODUCTION

Laser removal of dark hair can be accomplished with a variety of melanin absorbing chromophore wavelengths. Such light systems include 694-1064 nm laser systems.1 Laser hair removal of blonde and white hair was nearly impossible or had disappointing results due to the deficiency in laser energy absorbing chromophore in hair.

Photodynamic therapy as an alternative technique used to target white hair included the use of photosensitizer, such as 5amino-levulinic acid, which also led to targeting of pilosebaceous structures, was carried out.2 Exogenous melanin encapsulated liposomes (Meladyne) also have been studied as introduced target for white, grey, and light blond hair with unsatisfactory results by investigators.3

In the present study, we aimed to evaluate the efficacy of photodynamic therapy using topical application of liposomal RB gel followed by IPL exposure, and its success for removal of white hair.

MATERIALS AND METHODS

Phosphatidylcholine from soy bean (PC), Cholesterol (Chol), Chloroform analar grade, Rose Bengal (RB), and phosphate buffer (PBS) type were purchased from Sigma Chemical Company (St. Louis, MO). Triton X100 was also purchased from Sigma Chemical Company (St. Louis, USA). Gel formulations were prepared from carboxy methyl cellulose CMC (Al Nasr Company ARE). Propyl paraben, methyl paraben of pharmaceutical grade, and USP 25 were purchased from Normest Company for Scientific Development (53-fourth stage, 10th of Ramadan City, Egypt).

Methodology

A mixture of PC and Chol in molar ratios of 1: 0.2 was dissolved in 5 ml chloroform. A thin film formed from the lipids when deposed from the organic solvent using rotary evaporator (type VV2000 Heidolph-Elektso, Germany) under vacum. Five ml of RB (1 mg/ml) in phosphate buffered saline (PBS) (pH = 7) was added to the dried film in the rotary evaporator above transition temperature (Tc) at 50°C for two hours for complete hydration. To reduce liposomes size liposomal suspension was subjected...
to sonication in ultrasonic water bath (type Retsch, Germany) for 30 minutes then extruded using a stainless steel extrusion device (Lipex Biomembranes, Vancouver, BC, Canada) at 50°C through polycarbonate filters (Nuclepore Corporation, Pleasanton, CA) to an initial pore size of 0.1 μm. Free un-entrapped MB was separated by high speed centrifuge (Kontron, Italy) on 10,000 rpm for 30 minutes. The supernatant was measured for free un-entrapped RB and the pellets were re-suspended in fresh PBS for further studies.

Gel Formulations
In an aluminum foil covered beaker, propyl (0.2%) and methyl paraben (0.1%) were dissolved in 60 ml distilled water followed by the addition of 40 ml freshly prepared LRB (0.1%) with gentle agitation. Carboxy methyl cellulose was then sparkled to the solution with continuous agitation at 50 rpm until the gelling process was complete. Hydrogel was left overnight at 4°C for removal of air from the gel. Another gel formula containing the same concentration of FRB was prepared following previous procedure.

Percentage of Drug Encapsulation (Encapsulation Capacity)
One ml liposomal suspension was dissolved in 1 ml 0.1% triton X100 and the concentration of RB was measured through following its absorption spectrum and estimated from previously prepared standard calibration curve in triton x 100. The encapsulation capacity (EC) estimated through the following relationship: EC = (concentration of loaded drug / Initial concentration) x 100.

Release of RB From Hydrogel
Equal amounts (1 ± 0.15 g) of gel containing FRB and gel containing LRB were placed in 6 dialysis bags (Nuclepore Corporation, Pleasanton, CA) of MW 12,000 kD, of 0.5 mm pore size. Dialysis bags were placed in 100 ml phosphate buffer of pH = 5.5 (skin pH) at 32 ± 0.5°C (skin temperature) as acceptor solution in a dissolution equipment (Electro lab model TDT-08L, India). Paddles were fitted to 100 ml glass dissolution vessels at a constant rate of 100 rpm. Each type of gel had four samples (n=3).

Kinetics of RB released From Gel
To study the kinetics of RB release, the release data for the two gel formulae LRB and FRB were fitted to zero, first, second, and Higuchi’s diffusion control models using coefficient of variation for data analysis (StatistiXL for MS Excel software). The release kinetics were estimated by applying the highest linear correlation coefficient and the lowest coefficient of variation (CV%). The release rate constants and half-lives of the drug from each formula were calculated.

Skin Permeation Study
Drug permeation through the skin in vivo was evaluated on healthy albino mice. Animals were treated through the standard regulations of institutional guidelines. Thirty male albino mice of age 60 ± 15 days and weight of 170 ± 20 grams were used and divided into 3 groups, 10 in each named control, FRB and LRB group. Gel formulations (0.5 ± 0.05 mg) containing FRB and LRB were applied on shaved dorsal skin on FRB and LRB groups, respectively, for 30 minutes. Then, each group was subdivided into 2 groups. The first subgroup was irradiated 10 minutes with light of wavelength 550 nm emitted from an 80-mW LED (Photon Scientific, Egypt) at an energy density of 4.8 J/cm². Autopsies were taken from the animals from the second subgroup after 30 minutes of gel application and fixed without staining, while animals from the irradiated group were left for 24 hours. Then, autopsies were fixed and stained with H & E and studied histopathologically. A Carl Zeiss MicroImaging microscope (Carl Zeiss Micro-Imaging, Thornwood, NY) was used to examine and photograph the processed tissue sections (skin and underlying tissue of 5 μm thickness) using transmitted light bright field observation (halogen illumination), and an Olympus CKX41 inverted microscope with a mercury lamp fluorescence unit (U-RFLTSO) and a green filter cube (510–550 nm) for excitation. Beyond 590 nm emission was used to examine the depth of penetration of RB into the tissue by following the emitted orange fluorescence of RB. The fluorescence images were captured using a Canon PowerShot A650 IS digital camera. This approach allowed RB present in the tissue to be readily imaged (as orange RB fluorescence on a dark background). Sections from the control animals, which did not receive RB or H&E counterstaining, showed no detectable autofluorescence.

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Statistical Analysis
The unpaired student’s t-test was used to determine the statistical significance of the differences between drug release from the two gel formulations containing LRB and FRB. The differences were considered significant when the \( P \)-value was less than or equal to 0.05. The final data are expressed as the mean ± standard error (SE) and mean ± standard deviation (SD).

Clinical Study
Two groups (Treatment group A and Control group B), each of fifteen female subjects with white terminal facial hair, were entered into the study. Subjects were between 45 and 65 years of age and Fitzpatrick skin types III–IV. All subjects had white facial hair that could be counted before and after treatments. Study exclusions included oral retinoid use within 1 year of study treatments, recent ultraviolet exposure, intake of photosensitizing drugs, and a history of laser, light, or electrolysis as a depilatory method to the treatment area for 1 year prior
to treatment. Signed informed consent was obtained from all
subjects prior to treatment, and they were asked not to epilate
their hair during the whole study, just to shave.

They all were photographed with digital photography with the
same patient’s camera equipment (Fuji S2pro), lighting, and po-
position before first, after each, and last treatment. Patients were
then randomized into one of the two groups.

Before each IPL treatment, the number of white, terminal hair
was manually counted by an observer with a 6_ magnifying
apochromatic optical loupe within 3cm² of a well-defined re-

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region of a treatment area. To be reproducible, the areas were
marked, photographed before each treatment, and then the
hair was epilated. For the treatment group A, topical liposomal
RB gel was applied for 30 minutes under dark occlusion. While
for the control group B, a placebo gel of the same consistency
and color was applied following the same mode of application.

For both groups, treatment was performed with an IPL system
using the iPulse i200+ (manufactured by Cyden Ltd,UK) with a
fluence of 6 J/cm². Additionally patient and investigator were
blinded to the study arms.

Percentage of hair reduction was defined as the average num-

ber of terminal hair present at each treatment compared with
the average number of similar hair noted at baseline. Similar
counts were taken 6 months after the final treatment. Subjec-
tive patient evaluations and adverse effects were also recorded
at each follow-up visit. A patient satisfaction scale was insti-
tuted at the last follow-up visit. The following scale was utilized:
(1- completely satisfied, 2- satisfied, 3- moderate satisfaction,
4- not satisfied, 5- not satisfied at all).

Statistics
Comparisons between the terminal hair counts before the first
and after the last treatment were performed using the 2-tailed
\( t \) test for paired samples. Differences were considered signifi-
cant when \( P \leq 0.05 \).

Liposomal characterization showed encapsulation efficiency
of 95.33% ± 0.13 %. Figure 1 shows a photomicrograph of the
prepared liposomes loaded RB. Physical characterization of
the prepared hydrogels were transparent and non-gritty. Fig-
ure 2 represents drug released from free RB and RB loaded
in liposomes at different time intervals. There was an insig-
nificant difference \( (P > 0.05) \) in drug release between the 2 gel
formulae for the first 15 minutes. Drug released from FRB gel
was a significantly higher \( (P < 0.05) \) than from LMB gel at time
intervals starting from 30 minutes up to 60 minutes. There was
a linear relationship between RB released concentration and
time fitting zero order kinetics for LRB gel and FRB gel with lin-

er regression of 0.99 ± 0.01 and 0.89 ± 0.02 respectively. The
release rate constants of RB from LRB gel and FRB gel was \( K_{LRB} = 0.242 ± 0.09 \text{ min}^{-1} \) and \( K_{FRB} = 0.469 ± 0.03 \text{ min}^{-1} \), respectively.
Rose Bengal half lives in FRB gel and LRB were \( T_{1/2} = 0.6 ± 0.1 \text{ h} \) and \( 0.52 ± 0.3 \text{ h} \), respectively.

Animal Skin Permeation Study
Histological fluorescent photographs of sections of the ani-
mal skin post 30 minutes gel application are shown in Figures
3a,b. Figure 3a showed a marked fluorescence of the stratum
cornium, which is the superficial layer of the epidermis while
in Figure 3b the fluorescence was markedly shown in deeper
epidermal layers and a fluorescent hair shaft was observed
reflecting the penetration of RB loaded in liposomes all epi-
dermal layer.

Light microscope examination after IPL exposure revealed:
The stained histological sections of irradiated skin treated
with LRB displayed damaged and degenerated (necrotic) hair follicles located in the deep dermal layer and the stratum corneum remained intact, indicating the migration of LRB into deeper skin layers (Figures 4a, 4b).

Results of Clinical Study
Patients using the liposomal RB showed an average decrease of hair density of 44% 12 weeks after 3 treatment sessions (54 hairs ± 9.1 before therapy vs 30 hairs ± 6.7). \( P \) value < 0.05. (Figures 5a, 5b)

Patients using the placebo gel had a decrease of hair density of 16% (60 hairs ± 8.3 before therapy vs 50 hairs ± 9.0) after therapy. \( P \) value > 0.05.

Six months after therapy, terminal hair count of the liposomal group showed 40% reduction (54 hairs ± 9.1 before therapy versus 32 hairs ± 5.2 after therapy; \( P \) value < 0.05 compared with baseline pretreatment. (Figures 6a, 6b)

The control group using placebo gel showed 11% reduction of hair growth after 6 months (60 hairs ± 8.3 before therapy vs 53 hairs ± 2.3 after therapy. \( P \) value > 0.05.

The differences of the results between the treatment group and the control group were significant (\( P < 0.05 \)) after 3 treatment sessions as well as after 6 months (Table 1).

However, in the control group, patients and investigators noticed that hair reduction was very weak and clinical correlation
was disappointing. No changes regarding hair thickness or color could be observed.

Side Effects
There were no permanent side effects like hypo- or hyperpigmentation seen in our study, only mild erythema in most of the patients mainly due to the effect of IPL exposure.

Patient Satisfaction
Mean satisfaction rate was 3.0 in the liposomal RB group and 4.7 in the control group ($P<0.05$).

DISCUSSION
Destroying hair follicles with lasers and other light sources has revolutionized the ability to eliminate unwanted hair. The primary principle of laser-assisted hair removal is damaging stem cells in the bulge area or replacing the hair follicle at the level of the dermis with connective tissue through thermal injury. To selectively damage a hair follicle, laser energy has to be absorbed by a chromophore within a period equal to or less than the thermal relaxation time of the hair follicle.

Melanin is the endogenous chromophore, which is present in the hair shaft, the outer root sheath of the infundibulum, and the matrix area. It's concentration is dependent on the amount of pigment, which is responsible for hair color. The technique of laser-assisted hair removal has been described with the ruby laser (694 nm), alexandrite laser (755 nm), diode laser (800 nm), and neodymium:yttrium aluminum garnet (Nd:YAG) laser (1064 nm) for long-term hair reduction. The mentioned lasers target melanin to achieve selective photothermolysis of the hair follicles. The same mechanism of action is used by intense pulsed light sources (IPL) that consist of a noncoherent filtered flash lamp that emits wavelengths ranging from 500 to 1200 nm. It has also been successfully used for permanent hair reduction.

Both techniques, using either coherent (laser) or noncoherent (IPL) light, require a chromophore as a target. The endogenous
chromophore melanin, which is the target for laser hair removal, is not present in all types of hair. As a result, there is a lack of pigment in blond, gray, and white hair that led to the idea of external chromophore application. Nanni et al were the first to report on topically applied carbon particles suspended in mineral oil, which were massaged into the hair follicles in combination with a Qswitched Nd:YAG laser treatment.9

Although permanent hair removal was not achieved, the concept of exogenous chromophores was followed further. Based on this idea, photodynamic therapy may be a useful approach for hair removal. It involves the combination of non-ionizing radiation with topical photosensitizer that tends to localize in the follicular epithelium and photochemical destruction of hair follicles, no matter what hair color or growth cycle could potentially be obtained.10 A pilot study where topical aminolevulinic acid had been applied and shown to promote photoepilation, but with adverse effects.11 These previous data have led the authors to delineate another photosensitizer for treating white hair with less adverse effects.

Rose bengal (RB) is a known type II xanthene photosensitizer with a high absorption coefficient in the visible region of the spectrum.12 RB has been used as a promising sensitizer in wastewater treatment due to its water solubility, absorption in the visible region, good quantum yield of singlet oxygen, and inexpensiveness.13

The limited clinical application of RB arises from the low lipid solubility, which limited its capacity to cross biological barriers such as the cell membranes. Encapsulation in liposomes was seen as a promising approach to overcome these disadvantages.

Liposomes14 have been developed as drug carriers to modify the solubility of drugs to help target the drug to its sites of action, or to modify the release profile of the therapeutic agent. Liposomes have been shown to reduce photosensitizers’ toxicity and protecting them from metabolism and immune responses to enhance the clinical effects.15

Our clinical study relates to a photodynamic method for removal of facial white hair using a a photochemical mean. It involves the administration of topical (Rose bengal as a photosensitizer loaded in small size liposome ≤ 0.1 µm), to the treated area and exposure of that skin to IPL energy capable of activating RB. It has been found from the experimental and skin permeation studies that RB photosensitizer composition can penetrate into the hair follicle and are only found at low levels in other surrounding tissues. Delivery of RB into the target tissue was enhanced and facilitated by the use of liposome having a diameter that increases the probability of preferential delivery, and/or that selectively attaches to the hair follicle. Alternatively, the attachment may occur after the active RB is released from liposome, either passively or by attractive or chemical forces produced by IPL beam.16 Based upon previous considerations, topical liposomal RB gel was applied for only 30 minutes under occlusion on patients complaining of facial white terminal hair before irradiation with IPL, whose wavelength ranges between 530 and 560 nm, that corresponds to the absorption spectrum of RB.

Photosensitization reactions type II induced by RB excitation, the generation of singlet oxygen (1O2) and other ROS,17 are known to induce organelle photodamage leading to Programmed Cell Death (PCD) in the form of apoptosis, and necrosis.18,19 We relayed our clinical results on the previous mechanisms.

Previous studies for white hair removal are difficult to compare with our study, as some of the treatment regimens are very different from our standard regimen, and to the best of our knowledge, there is no recent reference using liposomal RB as a PD sensitizer in the treatment of white hair. In the present study, counting of hair and evaluation of percent of hair reduction were performed by the same evaluating dermatologist in all patients. The dermatologist was blinded to treatment status.

These factors are very important in an effort to obtain a high reliability of the evaluation. We consider the relatively stable hair count in the control group to be a confirmation that IPL has mainly no role in removal of white hair. With the 44% reduction in the mean count of white hair of treated group versus 16% reduction in the mean white hair count of control group, only after 3 sessions, liposomal RB-PDT showed to be more effective than ALA-PDT and without the acute adverse effects that occur just
after treatment with ALA. Both the evaluating dermatologist and the patients found a good improvement in hair count after liposomal RB-PDT treatment. Patients were moderately satisfied with the treatment at the 12-week and at the follow-up visit.

Generally, mild to moderate pain is an adverse effect of PDT.20 The collateral heating may cause pain and other undesirable consequences but in our study, most of our patients had no pain, also no serious adverse side effects were recorded because the light used was within the so-called optical window.21 Lack of endogenous chromophore absorbing in this wavelength range implies that the target has to be selectively loaded prior to IPL treatment with some appropriate exogenous chromophore (RB).

CONCLUSION
These studies demonstrate that RB can be delivered into the hair follicle loaded in liposomes and formulated in hydrogel, leading to selective damage. The selectivity was confirmed by animal histological findings in this study. In addition, clinical results have shown that this treatment is safe and appears to provide long-term effect with considerable patient satisfaction. Further larger studies are required to confirm our findings.

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DISCLOSURES
None of the authors have disclosed any relevant conflicts of interest.

REFERENCES