Effects of a Superpotent Melanotropic Peptide in Combination With Solar UV Radiation on Tanning of the Skin in Human Volunteers

Robert T. Dorr, PhD; Gregory Ertl, MD; Norman Levine, MD; Chris Brooks, CCRC; Jerry L. Bangert, MD; Marianne Broome Powell, PhD; Stuart Humphrey, PhD; David S. Alberts, MD

Objective: Three phase 1 clinical trials of a superpotent melanotropic peptide, melanotan-1 (MT-1, or [Nle4-D-Phe7]-α-melanocyte-stimulating hormone) were performed to demonstrate safety for MT-1 therapy combined with UV-B light or sunlight.

Design: Open-label studies at 2 dose levels of MT-1 combined with small doses of UV-B to the neck or buttock or full sunlight to half of the back.

Setting: Dermatology clinics at the Arizona Health Sciences Center, Tucson.

Interventions: The first study randomized 4 subjects to MT-1 (0.08 mg/kg per day subcutaneously) and 4 subjects to injections of isotonic sodium chloride (9%) solution for 10 days, followed by neck irradiation with 3 times the minimal erythema dose (MED) of UV-B light. In the next study (n = 12), the MT-1 dosage was increased to 0.16 mg/kg per day for 10 days, with UV-B radiation (0.25-0.75 MED) given to a buttock site for 5 days during (n=7) or after (n=5) MT-1 administration. A final study randomized 8 subjects to 3 to 5 days of sunlight to half of the back or to sunlight plus 0.16 mg/kg of MT-1 for 5 days per week for 4 weeks.

Results: Tanning in the first study was achieved in 3 of 4 subjects receiving MT-1, and these subjects also had 47% fewer sunburn cells at the irradiated neck site. More skin sites darkened with the higher dose of MT-1 in the second study. In the third study, there was significantly enhanced tanning of the back in the MT-1 group, and this was maintained at least 3 weeks longer than the tanning in the sunlight-only controls, who required 50% more sun-exposure time for equivalent tanning.

Main Outcome Measure: There were no pathologic findings at any UV-B or sun-exposed sites in any subject. Toxic effects due to MT-1 were minor, consisting of nausea and transient facial flushing.

Conclusion: Melanotan-1 can be safely combined with UV-B light or sunlight and appears to act synergistically in the tanning response to light.

Arch Dermatol. 2004;140:827-835

The melanocortins include a family of peptide hormones that induce pigmentation by interaction with melanocortin-1 receptors (MC1R) in the epidermis.1 α-Melanocyte-stimulating hormone (α-MSH)1 is the primary pigmented hormone that is released from the pars intermedia of the pituitary gland in some animals and from UV-B–exposed keratinocytes and melanocytes in human skin. This 13–amino acid peptide binds to MC1R to induce cyclic AMP-mediated signal transduction, leading to the synthesis of melanin polymers from dopa precursors.2 Two types of melanin can be expressed in humans. The brownish-black pigment, eumelanin, is believed to convey protection from sun damage, whereas the reddish, sulfur-containing pigment, pheomelanin, is often expressed in light-skinned individuals who report poor tanning and burning in response to sunlight.2 Such poorly tanning, easily sun-burning populations (termed type I-II by the Fitzpatrick scale)3 typically possess mutations in the MC1R gene4 and are generally thought to be at greater risk of developing skin cancer.5,6

We have previously demonstrated that a superpotent derivative of α-MSH,7 melanotan-1 (MT-1, or [Nle4-D-Phe7]α-MSH), can induce tanning in human volunteers who are known to tan easily in response to sunlight (Fitzpatrick scale III-IV).8 In humans, MT-1 primarily induces eumelanin synthesis in the skin coincident with its tanning effect.9 Although melanotropins have been postulated to affect immunologic function,10-12 all of our prior trials reported only facial flushing and transient gastrointestinal up-
set, unless doses greater than those needed for tanning were administered.13

All of the previously reported clinical trials with MT-1 were performed in human volunteers who were instructed to avoid sunlight and use sunscreens with an sun-protective factor rating of 30 on all sun-exposed skin sites.8,9,13 Thus, the effect of MT-1 when combined with either sunlight or simulated UV-B radiation has not been tested. In the present report, we describe 3 pilot phase 1 clinical trials with MT-1 combined first with small doses of UV-B radiation delivered from a solar simulator, and then with direct sunlight. The intent of these studies was to examine whether MT-1 could be safely combined with small amounts of UV-B. A secondary end point that was investigated was whether there was additive stimulation of pigmentation or an alteration in the biological response of skin to UV-B, measured by the presence of sun-burn cells. In addition, a subset of patients receiving MT-1 and localized UV-B radiation underwent detailed analysis of 17 different B- and T-lymphocyte populations to evaluate any effect of MT-1 on immune function.

METHODS

DESIGN

A series of 3 phase 1 clinical trials were performed to evaluate the response of human skin to MT-1 when combined with sunlight or simulated UV-B radiation. Healthy subjects with an easily tanning skin type by history (Fitzpatrick type III-IV) were treated with subcutaneous MT-1 at 1 of 2 dose levels for 10 days. The effects on skin pigmentation were evaluated by serial reflectance measurements at 8 anatomic sites taken before and at the end of MT-1 treatment and up to 8 weeks after treatment. Placebo controls were used for protocol 1 (MT-1, 0.08 mg/kg per day), plus 3 times the minimal erythema dose [MED] of UV-B to a neck area) and protocol 3 (MT-1 plus midday sunlight to half of the back area). In protocol 2, subjects were randomized to receive UV-B exposure on the buttock at either the start of MT-1 treatment (group A) or immediately after finishing the 10-day course of MT-1 (group B).

SUBJECTS

Healthy subjects were recruited from newspaper advertisements and were screened to have skin types III or IV by the Fitzpatrick scale.7 Subjects could not have any history of skin diseases and were screened to have skin types III or IV by the Healthy subjects were recruited from newspaper advertising the 10-day course of MT-1 (group B). Randomized to receive UV-B exposure on the buttock at either the light to half of the back area). In protocol 2, subjects were randomized to receive 3 times the minimal erythema dose [MED] of MT-1 (protocol 1), or 0.16 mg/kg per day of MT-1 (protocols 2 and 3). Melanotan-1 was administered daily on Monday through Friday for 2 consecutive weeks in protocols 1 and 2 (10 total injections) and for 4 weeks in protocol 3 (20 total injections). The 0.16-mg/kg dose was used for protocols 2 and 3 following the observation of safety for MT-1 and UV-B in protocol 1. Melanotan-1 doses were not escalated beyond 0.16 mg/kg, since this is the maximally effective daily dose for tanning by MT-1 without sunlight.9

UV RADIATION

UV radiation was delivered by a solar simulator (Model 600 Multisport Solar Ultraviolet Simulator; Solar Light Co, Philadelphia, Pa) for protocols 1 and 2 or by a series of timed midday sun exposures in Tucson over March through June for protocol 3. The solar simulator instrument was initially calibrated against a standard white zinc oxide background. All subjects had their MED of UV-B radiation defined at the outset of each study. The MED was defined using a series of graded doses of UV delivered from the solar irradiation simulator. An individual subject's MED was determined visually following graded UV-B exposure to the opposite buttock prior to MT-1 dosing. The MED was defined as the amount of radiation (15.75-42 mJ/cm2) that produced faint erythema with 4 distinct borders 24 hours after UV-B dosing.

REFLECTANCE MEASUREMENTS

For all 3 protocols, pigmentation was included as a secondary end point. Pigmentation was measured as skin reflectance at 8 different anatomic body sites: the forehead, cheek, dorsal neck, inner forearm, scalp, abdomen, buttock, and anterior leg. Skin chromaticity was measured by light reflectance recorded on a Minolta CR200 Chromameter (Minolta Camera, Osaka, Japan). The reflected light is received and split into 3 fractions. Luminance (or L-scale) indicates relative brightness from black to white and decreases with tanning. A 1-unit decrease in luminance represents the threshold for detecting a tanning change by the naked eye.15-17 Luminance decreases with tanning (negative L-scale change from baseline) and increases with loss of pigmentation (positive L-scale change). There are 2 color scales: the a-scale for yellow to red, which does not change with tanning, and the b-scale, which indicates blue-yellow hue and increases with tanning.15-17 For these studies, only L-scale and b-scale responses were recorded and stored on a computer. Eight measurements per anatomic site were averaged at each time point. Each subject served as his own control and reflectance was serially measured at baseline (predosing), at the end of dosing, and for several weeks thereafter, usually until the reflectance values returned to the baseline level for each subject.

END POINTS

The primary end point in the trials was safety, assessed both clinically and by histopathologic examination of irradiated skin sites. The pathologist (J.L.B.) was kept blinded to the treatment group. Two secondary biological end points were evaluated in protocols 1 and 2. In protocol 1, the number of UV-B-induced sunburn cells was evaluated in histologic sections from a 3-mm punch biopsy specimen of neck skin that was irradiated prior to MT-1 dosing immediately after injection. The powdered drug was diluted to a concentration of 20 mg/mL, and therefore all subcutaneous injection volumes were less than 1.0 mL. All doses were subcutaneously administered into the upper arm or thigh using a 25-gauge needle. The doses were calculated using actual body weight to deliver either 0.08 mg/kg per day of MT-1 (protocol 1), or 0.16 mg/kg per day of MT-1 (protocols 2 and 3). Melanotan-1 was administered daily on Monday through Friday for 2 consecutive weeks in protocols 1 and 2 (10 total injections) and for 4 weeks in protocol 3 (20 total injections). The 0.16-mg/kg dose was used for protocols 2 and 3 following the observation of safety for MT-1 and UV-B in protocol 1. Melanotan-1 doses were not escalated beyond 0.16 mg/kg, since this is the maximally effective daily dose for tanning by MT-1 without sunlight.9

©2004 American Medical Association. All rights reserved.

Downloaded From: by a Non-Human Traffic (NHT) User on 11/29/2018
ated 24 hours earlier with 3 MED of UV-B radiation individualized for each subject. Sunburn cells were quantified by the method of Gilchrest and modified as described by Cesarini et al. Four punch biopsy specimens were obtained at baseline (no UV-B); on the last day of dosing (at the UV-B site and an adjacent nonirradiated site); and 2 months after dosing at the UV-B site only. In protocol 2, a panel of immunologic parameters was evaluated by flow cytometry quantitation of immune cell markers from peripheral blood samples. Blood samples drawn from 7 patients treated with MT-1 (0.16 mg/kg per day × 10 days) were collected in acid-citrate-dextrose Vacutainer tubes. The analysis of blood leukocytes included the number of lymphocytes, monocytes, and granulocytes as well as subset populations of B cells (CD19+), T cells (CD3+), and natural killer cells (CD16+/CD56+). Ratios of the B- and T-cell populations and T-helper and T-suppressor/cytotoxic cells were determined. The analysis was performed using the BD Simulset system (Becton Dickinson Bioscience, San Jose, Calif). The HECA 452 antibody for skin-associated T cells kindly provided by Louis Picker, PhD, University of Texas, Austin. Identified T-cell subsets included T cells that were positive for the activation markers, HLA-DR, interleukin 2 receptors (CD25+), transfer in receptor (CD16+/CD56+), and Lymphocytes that “home” to the skin (expressing the cutaneous lymphocyte-associated antigen (CLA/HECA452+). For these studies, blood was withdrawn by peripheral venipuncture at 2 predosing times, at the completion of the 10 doses of MT-1, and 10 days after the completion of dosing (ie, the time at which the tanning response is generally maximal). The white blood cell fraction was separated by Ficoll centrifugation, and different subtypes of lymphocytes and monocytes were detected and quantified by automated fluorescence-activated cell sorting (FacScan; Becton Dickinson Biosciences).

**STATISTICAL ANALYSES**

Analysis of variance (ANOVA) was performed on the reflectance (tanning) data in all 3 protocols. A paired t test was performed for each subject value at baseline and at the 4 different postdosing time points. Differences in the number of sunburn cells between saline (isotonic sodium chloride [9%] solution) placebo-treated subjects, and those receiving MT-1 in protocol 1 were also tested using ANOVA followed by a χ² test. The time of sunlight exposure to a given level of skin tanning in protocol 3 was also compared using ANOVA. The immunologic parameters were compared at 2 baseline time points and then on the day of dose 5, the day of dose 10, and 10 days after dosing ended. The 95% confidence intervals were analyzed for the 2 baseline values and between the mean of those baseline values and the other time points. The Bonferroni adjustment was used to correct the P value for the multivariate tests, such that a significant difference would be .05 for the 17 immunologic variables or <.003 for significance.

**RESULTS**

**PROTOCOL 1**

This pilot study evaluated the effect of a low dose of MT-1, 0.08 mg/kg per day, plus 3 MED of UV-B light to a small site on the neck. The number of sunburn cells following 3 MED of UV-B radiation was evaluated at the site on the neck 24 hours after irradiation. There were 4 male subjects in each arm of the study who were randomized to receive UV-B plus MT-1 or saline placebo. Significant tanning was observed in 3 of 4 subjects treated with MT-1, with responding sites limited to the forehead (2 of 4), cheek (3 of 4), and neck (2 of 4). These are as detailed in Table 1. None of the saline-treated controls displayed any changes in pigmentation, and 1 (patient 4) showed significantly lighter pigmentation. This latter observation might suggest that the subjects were avoiding sunlight exposure and using sun-protective factor 30 sunscreen as instructed. Importantly, microscopic examination of the irradiated skin biopsy specimens showed no pathologic interactions (skin irritation, ulceration, or histopathologic change) between MT-1 and UV-B light. In contrast, there was a significant reduction in the number of sunburn cells in those subjects receiving MT-1 plus 3 MED of UV light. These effects are summarized in Table 2. Despite large variability between groups, the analysis shows that 24 hours after the UV-B dose was delivered, subjects receiving MT-1 had a 47% reduction in the number of sunburn cells at the irradiated site compared with the saline-treated controls (P = .001; Table 2). This protective effect was achieved despite the fact that only 1 subject in the MT-1 group (patient 7) achieved a statistically significant change in skin reflectance on the
The common adverse effect was flushing of the upper body that occurred variably within minutes after MT-1 injection. About half of the subjects experienced a median of 830 skin pigmentation varied for different subjects. The most responsive skin sites were the forehead, cheek, and scapula, with 6 of 11 subjects responding at all 3 sites. The dorsal neck area was much less responsive, with only 3 of 11 subjects exhibiting significant changes in both luminance and b-scale reflectance values. The buttock, abdomen, and anterior leg exhibited significant skin darkening in 4 or 5 of the 11 subjects in this trial, and this differs from our prior study. Another major difference was the prolonged duration of tanning in this trial. The results in Table 4 show that many subjects had still not returned to their baseline reflectance values at the final evaluation at week 6 (4 weeks after MT-1 dosing ended). Finally, there was significant further enhancement of skin tanning at the 3 dose sites for UV-B delivered to the buttock on the early group A schedule or the later group B schedule. Both schedules of UV-B exposure produced significant darkening at the UV-B treated sites compared with adjacent nonirradiated skin (Table 5). Table 5 also shows no difference in buttock tanning at the UV-B site between the early or late addition of UV-B to the 10-day MT-1 regimen. Importantly, there were no toxic effects noted at any of these sites. Adverse effects in protocol 2 were quantitatively and qualitatively similar to our previous studies. The most common adverse effect was flushing of the upper body that occurred variably within minutes after MT-1 injection. About half of the subjects experienced a median of

### Table 2. Effect of Melanotan-1 on Sunburn Cell Response to 3 MED of UV-B Radiation to the Neck (Protocol 1)*

<table>
<thead>
<tr>
<th>Treatment (n = 4)</th>
<th>Baseline (No UV-B)</th>
<th>Day 10 of Dosing</th>
<th>2 Months’ Dosing of 3 MED of UV-B, Neck Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonirradiated Neck Site</td>
<td>3 MED of UV-B, Neck Site</td>
<td>3 MED of UV-B, Neck Site</td>
</tr>
<tr>
<td>Saline (0.001 mL/kg per day × 10 d)</td>
<td>0.25 (0.5)</td>
<td>55.5 (16.5)</td>
<td>33 (16)</td>
</tr>
<tr>
<td>Melanotan-1 (0.08 mg/kg per day × 10 d)</td>
<td>0 (0)</td>
<td>0.75 (1.5)</td>
<td>23.5 (26.4)*</td>
</tr>
</tbody>
</table>

Abbreviation: 3 MED, 3 times the minimal erythema dose.

*Data are mean (SD) number of sunburn cells. P<.01 by χ² analysis compared with isotonic sodium chloride solution (saline) control.

### Table 3. Skin Type Characteristics for Subjects in Protocol 2‡

<table>
<thead>
<tr>
<th>Patient No.†/ Sex/Age, y</th>
<th>Skin Type‡</th>
<th>MED, mJ</th>
<th>Day When Given UV-B§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>63/F/54</td>
<td>III</td>
<td>31.5</td>
<td>1-5</td>
</tr>
<tr>
<td>67/M/24</td>
<td>III</td>
<td>21.0</td>
<td>1-5</td>
</tr>
<tr>
<td>69/F/52</td>
<td>III</td>
<td>26.25</td>
<td>1-5</td>
</tr>
<tr>
<td>70/F/45</td>
<td>III</td>
<td>26.25</td>
<td>1-5</td>
</tr>
<tr>
<td>72/F/36</td>
<td>III</td>
<td>36.75</td>
<td>1-5</td>
</tr>
<tr>
<td>73/F/61</td>
<td>IV</td>
<td>31.5</td>
<td>1-5</td>
</tr>
<tr>
<td>78/M/29</td>
<td>III</td>
<td>26.25</td>
<td>1-5</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65/M/41</td>
<td>IV</td>
<td>31.5</td>
<td>15-19</td>
</tr>
<tr>
<td>66/M/27</td>
<td>III</td>
<td>26.25</td>
<td>15-19</td>
</tr>
<tr>
<td>71/F/49</td>
<td>III</td>
<td>31.5</td>
<td>15-19</td>
</tr>
<tr>
<td>74/M/24</td>
<td>IV</td>
<td>26.25</td>
<td>15-19</td>
</tr>
<tr>
<td>77/F/40</td>
<td>IV</td>
<td>26.25</td>
<td>15-19</td>
</tr>
</tbody>
</table>

Abbreviation: MED, minimal erythema dose.

*Protocol 2: melanotan-1 (0.16 mg/kg per day for 10 days) + UV-B radiation to the buttock for 5 days.
†Group A received UV-B radiation to the buttock in the first 5 days of melanotan-1 dosing; group B received UV-B radiation to the buttock for 5 consecutive days, starting 3 days after the last melanotan-1 dose.
‡Fitzpatrick scale for tanning vs burning by personal history.
§Five daily doses of UV-B to the buttock at 0.25, 0.5, and 0.75 MED of radiot.
3 instances of this self-limited reaction at some time during the 2-week dosing period. The flushing reactions lasted from a few minutes up to an hour and were not associated with other sequelae. A mild sensation of nausea was reported in 4 of the 11 subjects. This effect was typically noted after the second or third injection of MT-1 and lasted for 30 minutes up to several hours. Because of the mild severity, antiemetic therapy was not required in any subject, but a few subjects described mild anorexia late in the evening on injection days. Fatigue was also reported in about one third of the subjects, usually in the afternoon of the first few days of injections. This was variable in intensity, but was never severe enough to require bed rest or drug discontinuance. Similar to the flushing reactions, the episodes of afternoon fatigue did not recur or increase in intensity with successive MT-1 doses.

Seven subjects in protocol 2 had 5 serial blood samples collected before, during, and after receiving MT-1 to determine whether the acute drug regimen induced changes in 17 different types of white blood cells. The first 2 samples were baseline samples, drawn prior to dosing, about 8 weeks apart and were compared statistically. This analysis showed that for all 17 parameters in the 2 baseline samples, zero was contained in the 95% confidence interval. The average of these 2 baseline samples was then calculated and used for comparison with the other 3 time points: on the day of doses 5 and 10 and 10 days after dosing ended. The results show that 2 parameters, T-memory cell (P = .05) and T-cell LCA2 cell (P = .01) levels, were approximately doubled at the time of dose 5. At the time of dose 10, the T-helper LCA cell levels were decreased by about 50% (P = .01). There were no significant changes noted at the last time point (10 days after the last dose was delivered). However, these individual statistical differences did not remain significant after applying the Bonferroni correction for multiple analyses.

**PROTOCOL 3**

This open-label trial in 8 subjects with type III-IV skin evaluated the effects of a prolonged schedule of MT-1: 0.16 mg/kg per day for 20 injections over 4 weeks. This was combined with full sunlight exposure to one half of the back for 3 to 5 days until a visual tan was apparent. The sunlight exposures were given either at the start of MT-1 dosing (n = 3) or after 10 of the total 20 doses had been administered (n = 3). One subject in the latter group dropped out midway through dosing because of time commitments, and therefore only 2 subjects were available for analysis. A control group of 3 subjects received the same sun-exposure regimen to the back without any MT-1 (Figure 1) to allow for a comparison of the time to achieve comparable tanning of the exposed hemi-back site.

**Table 4. Reflectance Changes in 11 Subjects Given Melanotan-1, 0.16 mg/kg per Day for 10 Injections (Protocol 2)**

<table>
<thead>
<tr>
<th>Anatomic Site</th>
<th>Baseline L-Value</th>
<th>Change From Baseline L-Value by Week:†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Forehead</td>
<td>61.59</td>
<td>-2.47 (5)</td>
</tr>
<tr>
<td>Cheek</td>
<td>61.06</td>
<td>-2.58 (5)</td>
</tr>
<tr>
<td>Neck</td>
<td>60.35</td>
<td>-2.16 (3)</td>
</tr>
<tr>
<td>Abdomen</td>
<td>67.29</td>
<td>-1.26 (2)</td>
</tr>
<tr>
<td>Scapula</td>
<td>64.30</td>
<td>-0.9 (6)</td>
</tr>
<tr>
<td>Buttock</td>
<td>68.25</td>
<td>-0.24 (0)</td>
</tr>
<tr>
<td>Forearm</td>
<td>65.59</td>
<td>-1.68 (4)</td>
</tr>
<tr>
<td>Leg anterior</td>
<td>64.67</td>
<td>+0.55 (0)</td>
</tr>
</tbody>
</table>

*Mean baseline luminance (L-value) for all 11 subjects completing the study (6 in group A [UV-B radiation to the buttock on the first 5 days of melanotan-1 dosing]; 5 in group B [5 consecutive days of UV-B radiation to the buttock, starting 3 days after the last melanotan-1 dose]).†Data are mean change in absolute luminance (number of subjects [of 11] showing a significant decrease in luminance and increase in b-scale value [data not shown for b-scale differences]).

**Table 5. Increased Tanning at UV-B–Irradiated Buttock Sites in Melanotan-1(MT-1)–Treated Subjects (Protocol 2)**

<table>
<thead>
<tr>
<th>UV-B Sequence</th>
<th>No. of Patients</th>
<th>Reflectance Differences at UV-B Site*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-Scale</td>
</tr>
<tr>
<td>First 5 d of MT-1 dosing</td>
<td>6</td>
<td>-1.95 (0.59)†</td>
</tr>
<tr>
<td>After MT-1 dosing</td>
<td>5</td>
<td>-1.87 (0.44)†</td>
</tr>
</tbody>
</table>

*Absolute mean (SD) reflectance differences for 0.75 times the minimal erythema dose of UV-B at the buttock site and the adjacent, nonirradiated buttock site.†P < .05 by paired t-test analysis: UV-B treated vs untreated buttock sites.

©2004 American Medical Association. All rights reserved.
non–sun-exposed back sites. This differs dramatically from the onset of maximal tanning at both sun-exposed and non–sun-exposed back sites returned to baseline after 7 weeks (Figure 1). The difference is even more striking when one considers that the subjects receiving only sunlight (no MT-1) received almost twice as much total sun exposure to the back as compared with the combination group (Table 6).

Since the group receiving MT-1 in protocol 3 experienced the greatest cumulative drug exposure to date, the question of adverse effects has special importance. The most common adverse effect was again facial and upper truncal flushing, which occurred variably. There were 9 instances of flushing in 3 of the 5 subjects. As in our prior studies, the onset was within minutes of MT-1 injection, and it typically resolved within 30 to 60 minutes. The most serious adverse effect was nausea, which was experienced by 2 of the 5 subjects. This began within 40 minutes of the first injection in patient 817. To prevent nausea, the next 3 MT-1 injections in this subject were given after 10 mg of prochlorperazine (Compazine; GlaxoSmithKline, Research Triangle Park, NC) had been administered orally. Another subject (patient 816) also experienced nausea after the second dose and received 10 mg of oral prochlorperazine before the next 3 doses. Latter MT-1 doses were given with no antiemetics, and there was no significant nausea. The only other reported adverse effect was afternoon fatigue or somnolence, which was reported in 3 subjects. For example, during week 3, one subject described a 2-hour period of fatigue after each injection. In another subject, general fatigue was described throughout the second week of the injections, with some persistence over the weekend (when no MT-1 was administered). All of these adverse effects were of mild intensity, and there was no evidence of cumulative toxic effects. Indeed, most adverse effects were reported during the first 2 weeks of the 4-week regimen, and only 1 instance of flushing and 1 instance of fatigue were reported in the last (fourth) week of MT-1 dosing.

The primary goal of the present study was to characterize the safety of MT-1 combined with small amounts of UV light. The results show that the synthetic superpotent melanotropin, MT-1, can be safely combined with small amounts of UV-B from a solar simulator or with brief exposures to full sunlight. The latter combination produced a marked enhancement of skin tanning, with the most rapid onset seen for sunlight added at the start of MT-1 dosing. We have further shown that MT-1 can be administered for 20 days over 4 weeks at a daily dose of 0.16 mg/kg, without producing cumulative, more intense, or new adverse effects. The trials also confirm that the 0.16-mg/kg dose of MT-1 is superior to the 0.08-mg/kg dose used in the original clinical study in terms of the degree of tanning, as well as the number of anatomic sites that responded by darkening. For example, in our original study of a 0.08-mg/kg dose in 28 male subjects, significant skin darkening was only observed on the forehead, cheek, and neck. In contrast, the results from protocol 2 show that significant darkening can be achieved at most body sites. In some cases this included the buttocks, wherein MC1R densities are reported to be

using the validated standards of reflectance for the laboratory scales. As expected, these tans were limited to the sun-exposed site, and the nonexposed back sites actually lightened slightly (increased L-values) over the course of the study (Figure 1, open square). In addition, the sun-exposed back sites returned to baseline reflectance values 7 weeks after the sun exposures (L-value).

Figure 2 compares the effect of the combination of sunlight exposure begun on the first day of MT-1 dosing with the degree of tanning achieved on the same subjects’ non–sun-exposed back site. This comparison shows that the combination of MT-1 plus sunlight produced rapid and profound skin darkening at the sun-exposed back site (Figure 2, solid symbols). This was significantly greater than the darkening produced by MT-1 alone at the contralateral (nonexposed) back site (Figure 2, open symbols).

The absolute change in reflectance units for the combination was profound, involving the largest reflectance changes recorded: a mean 15-unit decrease in luminance and a mean 10-unit increase in b-scale values.

When sun exposure was added to MT-1 at the end of the first 2 weeks of dosing, there was a similar increase in darkening at the sun-exposed back site compared with the non–sun-exposed back site (Figure 3). However, in this case, the onset of darkening was delayed by approximately 2 weeks. This represents a significant difference compared with sunlight delivered on the first day of MT-1 dosing (Figure 2). Figures 2 and 3 show that the duration of darkening was remarkably prolonged for all subjects receiving sunlight plus MT-1. In this case, it did not matter whether sunlight was added at the start or middle of the MT-1 dosing period. Indeed, at the conclusion of reflectance monitoring at 11 weeks, all MT-1–treated subjects were as dark as they were at the onset of maximal tanning at both sun-exposed and non–sun-exposed back sites. This differs dramatically from the sun-only controls, wherein reflectance values had all
very low. We were also able to demonstrate enhancement of pigmentation at sites receiving concomitant UV-B radiation from the solar simulator. However, there was 1 female subject in protocol 2 with type IV skin by history who did not respond at any skin site to the 0.16-mg/kg dose of MT-1. This is the first observation of a completely nonresponsive individual, and there is no clear explanation at this time for the total lack of response.

The 50% reduction in the number of sunburn cells seen in protocol 1 suggests that MT-1 may be able to reduce the biological consequences of solar radiation. However, there are several caveats. First, this was a small pilot study involving only 8 subjects, and only 4 subjects received MT-1. Second, there was minimal tanning of the neck induced by the 0.08-mg/kg dose of MT-1 used. Finally, there was substantial variability between subjects in the number of sunburn cells produced by 3 MED of UV-B radiation. This variability suggests that large sample sizes will be required to determine whether a reduction of UV-B–induced sunburn cells comprises a reliable surrogate of a solar-protective effect of MT-1. However, a preliminary description of a phase 2 trial with positive results showing a 50% reduction in sunburn cell development with the 0.16-mg/kg dose of MT-1 in subjects with skin types I and II was recently reported by EpiTan Ltd, Melbourne, Australia (http://www.epitan.com.au).

If the goal of sun exposure is simply to obtain a tan, then MT-1 in combination with a minimal amount of sunlight should provide a tan, which reduces the need for substantial solar exposure. This might considerably reduce the damage to skin from solar exposure. The results, therefore, have important implications relative to the use of a tanning booth to acquire a tan, which has recently been associated with nonmelanoma skin cancer risk. It should also be noted that the degree of darkening induced by MT-1 in the absence of solar or UV light is rather minimal even after 20 days of administration. It is therefore the synergistic action of sunlight and MT-1 on the duration and intensity of the pigmentary response that is remarkable.

The native hormone, α-MSH, has been reported to have broad anti-inflammatory activities in experimental
models of inflammation. These effects include inhibition of arthritis in a rat model, reduction of endotoxin-induced liver inflammation in a septic shock model, and improved survival in a model of endotoxia and peritonitis. These effects may be mediated by α-MSH-induced inhibition of the synthesis and activity of cytokines such as interleukin 1 in neutrophils. Direct effects on neutrophil migration and superoxide dismutase production have also been reported. Protocol 2 indirectly addressed the issue of immunologic activity for MT-1 in humans receiving 10 injections of 0.16 mg/kg. At the end of the 2-week dosing period, we could not demonstrate any significant changes in the absolute numbers of 17 different white blood cell subtypes in the peripheral blood of 7 of these subjects. However, the effectiveness of these peripheral blood cells to mount an immunologic reaction was not evaluated, and therefore we cannot rule out an alteration in immune response induced by MT-1. On the other hand, no infections have been observed in any of the approximately 100 healthy subjects treated with MT-1 to date. Thus, while the question of whether MT-1 induces immunologic alterations in humans is still largely unanswered, we do know that it does not acutely alter the number of several classes of white blood cells in the peripheral blood. The lack of an immunologic effect for MT-1 is also consistent with a study in mice wherein the native hormone blocked contact hypersensitivity reactions, but MT-1 did not.

The other adverse effects of MT-1 seen in this study are similar to those previously reported. Nausea induced by MT-1 was seen in about 20% of the current subjects and required antiemetic treatment in only 2 subjects. This effect may be mediated by interaction of MT-1 with melanocortin-3 receptors (MC3Rs), which have been found in the gut tissues of animals. The fact that these mild gastrointestinal adverse effects were infrequent and were not cumulative suggests that any activation of MC3R by MT-1 is not dose limiting. The biochemical pathway for the other MT-1 adverse effects, notably facial flushing and fatigue, are not known. However, the acute flushing reactions in the upper trunk may be caused by the production of the vasodilatory molecule nitric oxide, which has been observed following MSH binding to MC1R on keratinocytes and melanocytes. The only other adverse effect, fatigue, was also seen in our prior phase 1 dose-escalation study, wherein it was dose-dependent in severity. In our present study, fatigue of a mild nature was noted at some time in about one third of the subjects. Similar to the other toxic effects, fatigue did not recur with each dose and was not cumulative in intensity when it did recur. Whether these effects are mediated by binding to MC3R and MC4R, which are found in the brain, is not known.

Perhaps the most important observation in the 3 clinical studies of MT-1 is the observation of marked tanning synergy with the combination of UV-B light (protocol 2) or sunlight (protocol 3). The degree of skin darkening measured at both light exposed sites was significantly greater than that achieved with UV light, sunlight, or drug alone. Indeed, the tanning observed in the sun-exposed back in protocol 3 is the most intense we have ever measured. Furthermore, the combination of MT-1 plus sunlight produced a long-lasting tan at the sun-exposed back sites, which had not begun to return to baseline reflectance values 11 weeks after MT-1 dosing started. This is significantly longer than what we have seen previously using a 2-week course of MT-1, 0.16 mg/kg. The 4-week course of MT-1 used in protocol 3 also represents the largest cumulative exposure to drug to date. Importantly, we saw no new adverse effects or more intense adverse effects with this doubled exposure to MT-1.

Current studies with MT-1 are being performed in Australia by Epitan Ltd. They are investigating the effect of a MT-1–induced tan on sun damage in a population of individuals at high risk for sun injury, namely, those with a history of actinic keratoses and poorly tanning skin types (type I–II by Fitzpatrick scale). In addition, genetic phenotyping is planned to evaluate whether the drug is active in individuals with polymorphisms at the MC1R gene locus. A 1-month depot formulation has also been developed following observations of enhanced efficacy of slowly released drug in animal models. These studies will help to define whether MT-1 has potential as a chemoprotectant for human skin cancer.

Accepted for publication January 23, 2004.

This work was funded entirely by Program Project Grant POI CA-27502 from the National Cancer Institute, National Institutes of Health, Bethesda, Md (Dr Alberts).

The studies were conducted under an Investigator-Sponsored Investigational New Drug Application (No. 35121) from the US Food and Drug Administration (submitted May 24, 1990) (Dr Levine). Dr Humphrey is the Manager—Clinical Development at EpiTan Ltd, Melbourne, Australia, and provided detailed critiques of the manuscript and analysis of the data. These studies were conducted entirely at the Arizona Cancer Center, Tucson, between 1994 and 1996. Statistical analyses were provided by Mikel Aiken, PhD, who headed the Biometry Core Service of the Arizona Cancer Center at the time the studies were performed.

Correspondence: Robert T. Dorr, PhD, Arizona Cancer Center, 1515 N Campbell Ave, Tucson, AZ 85725-5024 (bdorr@accc.arizona.edu).

References


