Pharmacokinetic studies for the development of transdermal drug delivery systems.

by

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Chapter 5 Melanotan

5.1 Introduction

Over 1000 analogues of α-MSH have been synthesized and biologically evaluated by a group of researchers in Arizona (Hadley et al., 1998b). Several of these α-MSH analogues have exhibited superpotency and prolonged activity with respect to their melanogenic (skin tanning) properties. Among them only two α-MSH analogues, Melanotan I (MT-I) (Hadley et al., 1993;Levine et al., 1991) and Melanotan II (MT-II) (Dorr et al., 1996) have been extensively studied. Both Melanotan I and II have tanning capabilities, but because MT-II caused spontaneous penile erection as a side effect, it is now also being developed as an erectile dysfunction drug.. The tanning and penile erection capabilities of melanotan II have been shown in the study conducted by Dorr and co-workers (Dorr et al., 1996), and other studies on melanotan II have shown that the drug can initiate penile erection and increased sexual desire in some of the subjects (Hadley et al., 1998b;Wessells et al., 1998;Wessells et al., 2000). On the other hand MT-I has been further developed as a tanning agent (Dorr et al., 2000;Hadley and Dorr, 2006). The delivery of MT-I is problematic as it is a peptide drug with a short half-life, metabolic instability, relatively high polarity, and larger molecular size which limits its transport via gastrointestinal absorption (Hadley et al., 1998b). Therefore to date, the delivery of melanotan has been studied using intravenous injection, subcutaneous implant, and transdermal delivery.

Transdermal delivery of MT-I has been conducted and tested on mice (Levine et al., 1987). MT-I was topically applied to an area of the back of the mice and within 24-48 hours, eumelanin production was visible microscopically within hair bulb melanocytes in both treated and untreated areas. Thus, these results demonstrated that MT-I was successfully delivered through the skin and had the action of melanin production. However similar studies using rat skin showed poor penetration of MT-I (Dawson et al., 1988). In another in vitro study on human skin (Dawson et al., 1990), MT-I has successfully delivered across human skin and was measurable using radioimmunoassay (RIA) to test for quantity and biologic activity, respectively.

Preliminary clinial trials on MT-I to demonstrate tanning of skin were carried out in Arizona, USA by Norman Levine (Levine et al., 1991). Twenty eight healthy white men with history of either poor or good tanning skin were administered with 10

subcutaneous injection of MT-I. The results showed that a tan induced by Melanotan in the head and neck region, were produced in the same way as a natural tan and persisted for a similar time. This result has been considered the first demonstration of a stable drug candidate that could induce a natural tan in human beings. A study by Ugwu and co - workers (Ugwu et al., 1997) also showed a significant tanning of the forehead, arms, and neck following IV or SC dosing of MT 1 in three healthy voluteers.

In a pharmacokinetic study (Evans AM, 2002) conducted in 12 volunteers in Australia of a daily subcutaneous injection of 0.16mg/kg of Melanotan for ten days demonstrated that Melanotan has a half-life after subcutaneous administration of about 30 minutes with little or no activity in the plasma after 4 hours. There appeared to be negligible accumulation of Melanotan in subjects (Evans AM, 2002). A Phase II study conducted at two sites in Australia (Royal Prince Alfred Hospital, Sydney and Royal Adelaide Hospital, Adelaide) during 2003 recruited 81 subjects (61 Melanotan and 20 placebo). The subjects enrolled into the active arm received 30 subcutaneous injections of 0.16mg/kg/day of MT 1 for thirty days, given for every 10 days at the beginning of 3 consecutive months. Significant increases in skin melanin were seen in the active group compared to placebo ($p < 0.0001$) at the end of the 90 day study period (Datapharm Australia, 2004).

The safety and tolerability of a slow-release (depot) formulation of Melanotan is being tested in a human trial in Australia. This trial involves the subcutaneous insertion into the abdominal wall of biodegradable polylactide rods containing increasing doses of Melanotan. Subjects are being monitored for safety, pharmacokinetic levels of the drug in the blood and skin tanning effects over a 60 day period. Preliminary results with a 20mg and 40mg implant have shown the average skin melanin density increases from 2.81 + 0.33% to 4.03 + 0.29% within 10 days of starting treatment and persisted over the 60 day observation period. Adverse events were principally limited to the cosmetic effects (rapid tanning, freckling) induced by the rapid onset of melanogenesis. Other mild adverse events which have been reported less commonly include headache, satiety, rhinitis, chemical taste in the mouth, yawning, muscle twitching, diarrhoea, light-headedness and nervousness. Adverse events associated with sustained-release implants have been mainly restricted to irregular skin tanning, darkening of nevi (moles) and freckling. The darkening of moles and freckling are not strictly adverse events as they represent the natural effects of the drug, however the rapidity of the change was such as may be recorded as an unexpected event.

Recently, Clinuvel Pharmacetical Limited (previously known as Epitan Limited) has announced that it has successfully completed a phase II trial on injectable implants of Melanotan-I for the treatment of Polymorphous Light Eruptions (PMLE). The results show that Melanotan I can be an efficacious drug for the treatment of PMLE. They now plan to start the Phase III trial in nine centres across the European continent in early 2007 (Clinuvel Pharmaceutical Limited, 2006).

All of the above forms of administration are expensive to administer, time-consuming and inconvenient to the subject. If this is to become an over the counter (OTC) product, there is a need to establish the pharmacokinetics of a more user-friendly dosage regimen. In this study, the TDS® system has been combined with MT-I to assess the capabilities of the system to deliver MT-I for the assessment of tanning and it's pharmacokinetic properties. This is a more convenient mode of application compared to a subcutaneous implant and IV injection. The expected difference in bioavailability in a subcutaneous versus a transdermal dose has resulted in the selected dose escalation from 1 mg up to 40mg administered for 10 consecutive days in this study. The total dose of up to 400mg was received by subjects based on the tanning and toxicity criteria. This criteria and full dosage regime will be explained in the methods section.

5.2 Study Objectives

The main objective of this study was to assess the ability of the TDS® system to deliver MT-I systemically to induce the production of skin's melanin and give the tanning effect in healthy Caucasian subjects.

The second objective of this study was to assess the pharmacokinetic properties and therapeutic dosage of MT-I in conjunction with the safety and tolerability of MT-I in healthy subjects.

5.3 Study Approval

The study was approved by the St. Thomas' Hospital Research Ethics Committee. Reference no. 04/Q0702/120, dated 11th. November 2004.

5.4 Subjects

This study involves 30 healthy Caucasian subjects enrolled over a period of 9 months.

5.5 Study Methodology

5.5.1 Study treatment

The TDS® system as prepared under GMP manufacturing and FDA guidelines by Natural Vitamin Co., Las Vegas, Nevada, USA and dispensed by Transdermal Technologies Inc. (TTI) Lake Park, Florida, USA. TTI was sent TDS® and Melanotan to be combined and formulated by Controlled Therapeutics, Glasgow, United Kingdom. TDS® - Melanotan 1, 5, 10, 20, and 40mg/mL solutions were supplied in metered pump dispenser with one entire spray containing 0.2mL solution.

5.5.2 Design

This study was a dose escalation study starting with a 1mg dose for Cohort 1 and ending with a 40mg dose for Cohort 5. Each cohort contained 6 subjects who received a dose for 10 continous days. The detail of the cohorts and doses are shown in the Table 5.1. The lowest dose (1mg/day) is approximately $1/10^{th}$ of the effective dose established in previous studies of ten daily IV doses of 0.16mg/kg/day [100mg delivered over 10 days to a 62kg person] (Ugwu et al., 1997).

Table 5.1 Doses and corresponding cohorts for TDS-Melanotan administration

Cohort (Treatment) Number	No. of subjects	Dose (mg/day)	Total Dose (mg)
1	6	1	10
2	6	5	50
3	6	10	100
4	6	20	200
5	6	40	400

5.5.3 Screening Evaluation

Prospective subjects attended the study site for a screening visit within 4 weeks of study commencement. The nature of the study, the procedures and the risks were fully explained. Before any screening procedures occured they had signed an Informed

Consent Form for Screening and an Informed Consent Form for tissue storage. At the screening sessions, blood samples were taken for routine biochemical, haematological, endocrinological and serological screenings. A urine specimen was also obtained for urinalysis and drug screening and pregnancy testing for females. A further 10mL of blood was taken for the assessment of MC1R genotype. Each subject was then provided with Uritainer and requested to collect all of their urine over the following 24-hour period for the measurement of 24-hour urinary cortisol levels. Routine physical examinations, inclusion/ exclusion criteria and assessment of skin type were also conducted during the screening. Skin type has been recorded based on Fitzpatrick scale (Fitzpatrick, 1988) as list down below.

i) Type 1: Never tans, always burns

ii) Type 2: Sometimes tans, mostly burns

iii) Type 3: Mostly tans, sometimes burns

iv) Type 4: Always tans, never burns

In addition to the subject's skin type assessed according to Fitzpatrick classification, a more definitive assessment of skin type will be assessed according to the "melanocortin-1 receptor" (MC1R) genotype (Palmer et al., 2000). A 10mL blood sample was collected into a standard EDTA blood tube for this purpose. After mixing well, the tube was labelled and stored refrigerated (not frozen) until further analysis.

5.5.4 Study Procedures

5.5.4.1 Dose escalation strategy

Upon acceptance into the study, volunteers were allocated subject numbers and hence to a Cohort number for administration of Melanotan at one of the pre-assigned doses. Cohorts 1, 2, and 3 were run in parallel. Cohort 4 for the 20mg dose was started approximately 1 month after completion of day 30 of the first three cohorts, after review of all of the haematology and clinical biochemistry results at Day 30. Upon completion of a review of all of the results, (and found) no related grade 3 or 4 toxicity as defined by the National Cancer Institute Common Toxicity Criteria (National Cancer Institute, 1999), no abnormal pathology results and no serious adverse events, another six

subjects were enrolled in Cohort 4 to receive the 20mg/day dose. The above procedures were also carried out for the enrolment of Cohort 5 (40mg/day dose).

5.5.4.2 Treatment procedures (per cohort)

5.5.4.2.1 Day 0

Subjects reported to the Study Centre at approximately 0830 hours on Study Day 0. The volunteers underwent a check-in procedure during which Informed Consent for Study Participation was obtained. A urinary pregnancy test was conducted on females to make sure that they were not pregnant. Subjects then had skin reflectance measurements performed according to the procedures outlined in Section 5.5.8. Prior to discharge from the Study Centre, subjects were given Diary Cards which they were asked to fill in on a daily basis to record daily exposure to sun, applications of sunscreen to the skin, concurrent medications and any adverse events. Subjects were requested to bring these diary cards to the Study Centre at each study visit for review.

5.5.4.2.2 Days 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10

On Day 1, subjects attended the Study Centre at about 0730 hours. Subjects were checked for any concomitant medications and had a blood pressure (BP) check before insertion of a cannulae into an antecubital vein of the left arm. A 10 mL blood sample was obtained before the treatment application. The treatment dose was sprayed topically to the inner aspect of the right inner upper arm and gently rubbed into the skin. A surgical glove was worn when applying the treatment to prevent self-dosing. The applied drug was allowed to dry for approximately 5 minutes prior to the subject covering the arm with clothing. Following dosing, subjects remained under observation at the Study Centre. Serial 10mL blood samples were collected at 20min, 40min, 1, 1½, 2, 2½, 3, 4, 5, 6, 8, 10 and 12 hours post dose. On the remaining treatment days (2 – 10), a 10mL blood sample was drawn just prior to the treatment being applied. The treatment was applied at the same application sites for 10 continuous days. On day 5 and 10, skin reflectance measurement was carried out by using a spectrophotometer. On day 10, a blood sample was taken for pathology assessment and subjects were provided with a Uritainer and requested to collect their urine over the following 24 hour period. Subjects were asked to return the Uritainer on the following day for 24-hour urinary cortisol measurement.

5.5.4.2.3 Day 20 (+/- 1 day)

On this day, subjects attended Study Centre at about 0830 hours for Skin reflectance measurements. Subjects also were also assessed for concomitant medication, vital signs and adverse events.

5.5.4.2.4 Day 30 (+/- 1 day)

On this day subjects attended the Study Centre at about 0830 hours for skin reflectance measurements (Section 5.5.8) prior to blood collection for safety measurements (haematology, biochemistry and endocrinology), and a urine sample was obtained from female subjects for pregnancy testing. Subjects were then provided with a Uritainer and repeat the urine collection procedure as mentioned above.

On each of the above visits, subjects were assessed for concomitant medication, vital signs and adverse events. Subjects were also reminded to record their daily activity related to sun exposure, any adverse events and medication taken, in the diary book supplied.

5.5.4.3 Restrictions

5.5.4.3.1 UV Exposure

Subjects were required to refrain from excessive exposure to UV light from 1 week prior to the study start until after the Day 30 visit. Subjects were not permitted to use UV beds and had to apply sunscreen to all exposed body regions during prolonged ($\geq$ 30 minutes full sun) sun exposure.

5.5.4.3.2 Concurrent Medications

Subjects were instructed to abstain from medications (prescribed or over-the-counter, including herbal remedies, but excluding oral contraceptives) deemed to be significant by the Principal Investigator for the 7 days preceding the dosage phase (Days 1-10), and throughout the study (until Day 30). Subjects were asked to inform Study Personnel if any additional medication was required. All the details of concomitant medications were recorded in the subject's Diary and Case Report Form (CRF).

5.5.5 Parameters for Evaluation

5.5.5.1 Skin Reflectance Measurements

Skin reflectance measurements were performed on Days 0, 5, 10, 20 and 30. Reflectance by the skin, of wavebands of light that are 15 nm wide were measured at 20 nm intervals in the wavelength range of 400 to 700nm with a Minolta 2500d spectrophotometer. Refer to Appendix 22 for the whole procedures of the skin reflectance measurements. Nine different anatomical sites were assessed as follows:

Site 1 : Forehead

Site 2 : Left cheek

Site 3 : Right side of neck

Site 4 : Left shoulder (scapula)

Site 5 : Left inside upper arm

Site 6 : Right inside upper arm

Site 7 : Left medial forearm

Site 8 : Right side of lower back

Site 9 : Left calf

Four skin reflectance parameters were measured, in triplicate, on an area of 8mm x 8mm at each anatomical site, these are: L-value, b-value, R_{420} and R_{400} (the latter two readings were used to calculate Melanin Density (MD)). Reflectance measurements were recorded as L-values, corresponding to luminescence or brightness from black to white, and b-values, representing colour hues from blue to yellow (Porges et al., 1988;Seitz and Whitmore, 1988). Visually apparent tans are typically associated with $\geq$ 1 unit decrease in the L-value and $\geq$ 1 unit increase in b-value, compared to baseline (Ugwu et al., 1997). In addition, the density of cutaneous melanin was estimated from reflectance at that site of wavebands of light centred at 400 nm and 420 nm according to the results of Dwyer et al. (Dwyer et al., 1998;Dwyer et al., 2000). The equation used is:

$$MD = 100 \times (0.035307 + 0.009974(R_{420} - R_{400}))$$

Where;

MD = Melanin density

R_{400} and R_{420} = Reflectance at 400 nm and 420 nm, respectively.

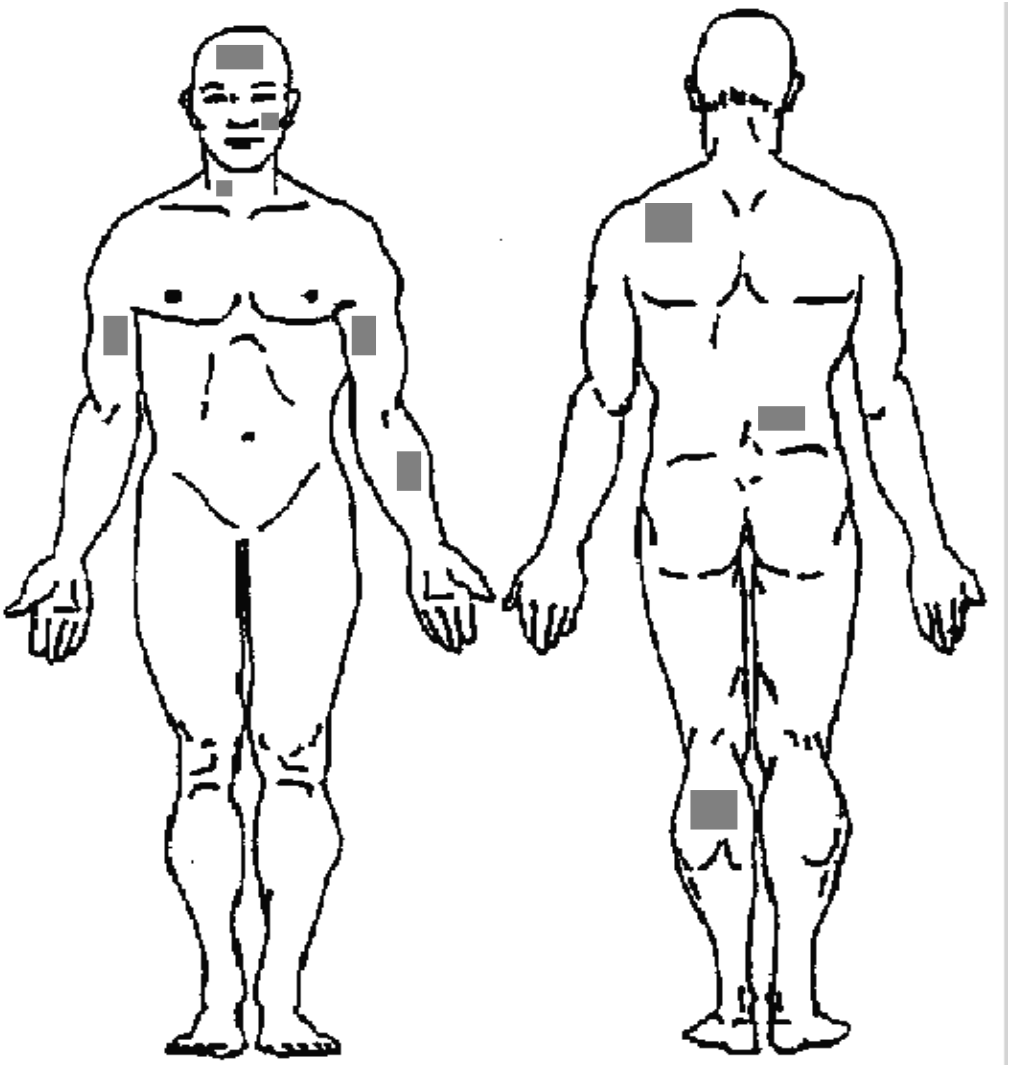

Figure 5.1 A body diagram shows the 9 anatomical sites (grey shaded area) for the skin reflectance measurement.

5.5.5.2 Melanotan Assay

Melanotan concentrations will be measured in plasma using a validated LC-MS-MS method. At the time this thesis was submitted, the method is still under development. Therefore, the melanotan plasma concentration and pharmacokinetic analysis cannot be reported.

5.5.5.3 MC1R Genotype

A 10 mL blood sample was collected from each subject at the time of screening for the MC1R genotype. The blood samples for the MC1R genotype will be analysed by any available technique used in the genetic laboratory such as oligonucleotide DNA microarray (Hacia et al., 1999). However, the method is still under development, therefore the data cannot be presented in this thesis.

5.5.6 Statistical Analysis of Skin Reflectance Measurements

For each subject the change, from baseline, on Days 5, 10, 20, and 30 were calculated for Melanin Density (MD), L, and b for each anatomical site. The analysis of variance (ANOVA) was performed for the MD, b, and L values on all of the sites, subjects, and observation days together with sites and Days as a fixed factors and subjects as a random factor. The Dunnett Simultaneous Test was used to test if the differences between day 5, 10, 20, and 30 and the baseline was significant. The level of statistical significance was set at α-level of 0.05.

5.6 Results

Thirty healthy Caucasian subjects, divided into 5 cohorts, successfully participated in this study. One subject from Cohort 3 refused to finish the study for personal reasons, but was replaced by another subject. All of the subjects followed the protocol well and restricted their sun esposure. The average length of sun exposure in all of the subjects studied for the whole 30 days study period was, mean [range] 25 [0-82] hours. After completing the 30 day study on each Cohort, no significant skin tanning was observed in any of the subjects. The results of MD, b and L at day 5, 10, 20, and 30 also showed no difference compared to baseline for all anatomical sites and all of the cohorts accepted the final cohort, the 40 mg dose was where site 7 (left medial forearm) showed a significant increase and a decrease in the MD and ‘b’ values, respectively. Therefore, only the results from cohort 5 will be presented and discussed.

The analysis of skin reflectance parameters by each subject had found the MD, b and L values were inconsistent throughout the 30 day observation period for all the subjects and the sites studied. Only site 7 (left medial forearm) appeared to have a significant increase in MD and b values, and a significant decreased in L values for most of the subjects. For simplicity, the analysis for MD, b, and L were performed at each site. The plot of mean MD and b change from baseline showed no increase for all of the anatomical sites except site 7 (left medial forearm) (Figure 5.2 and Figure 5.3). Similarly, the plot of mean L change from baseline also showed no decreased for all the anatomical sites except for site 7 (Figure 5.4). Further detail on site 7 also showed most of the subjects had an increase in MD and b, and this correlated with the decrease in L values from day 0 to day 30. Figure 5.5, Figure 5.6, and Figure 5.7 show the plots of MD, b, and L change from baseline for site 7 for all of the subjects in cohort 5. Whilst Figure 5.8 shows clearly the plot of mean MD, b, and L values change from baseline for site 7.

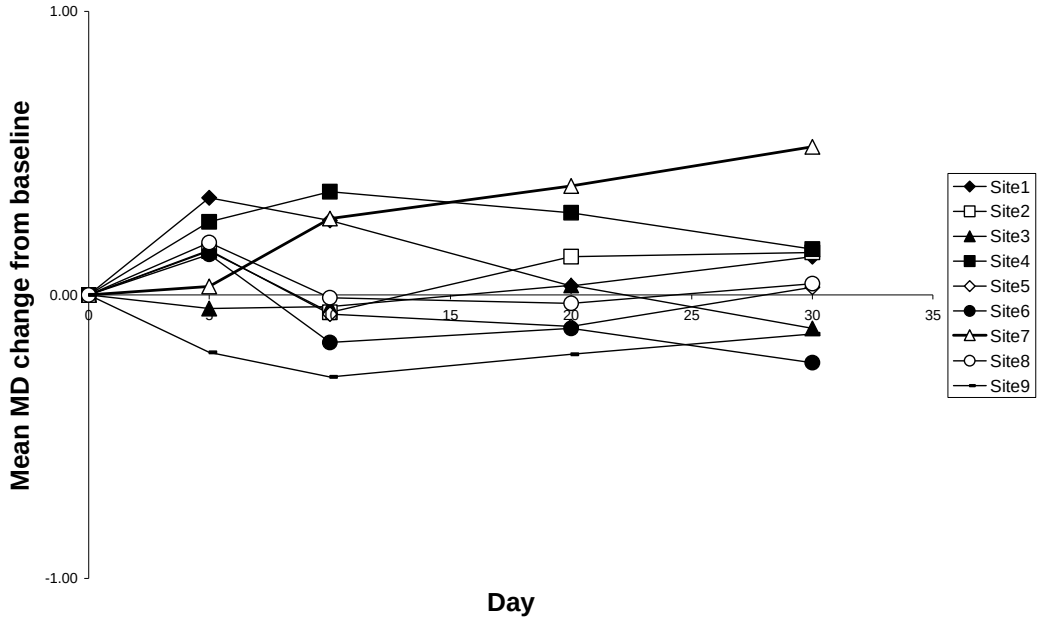

Figure 5.2 Plot of mean MD change from baseline for all the anatomical sites in Cohort 5

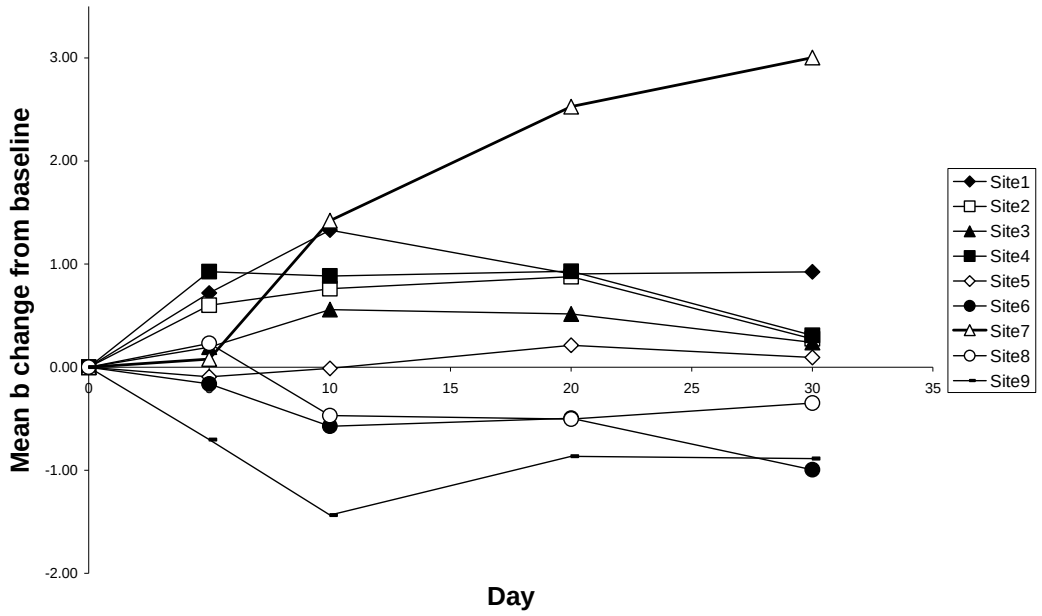

Figure 5.3 Plot of mean b change from baseline for all the anatomical sites in Cohort 5

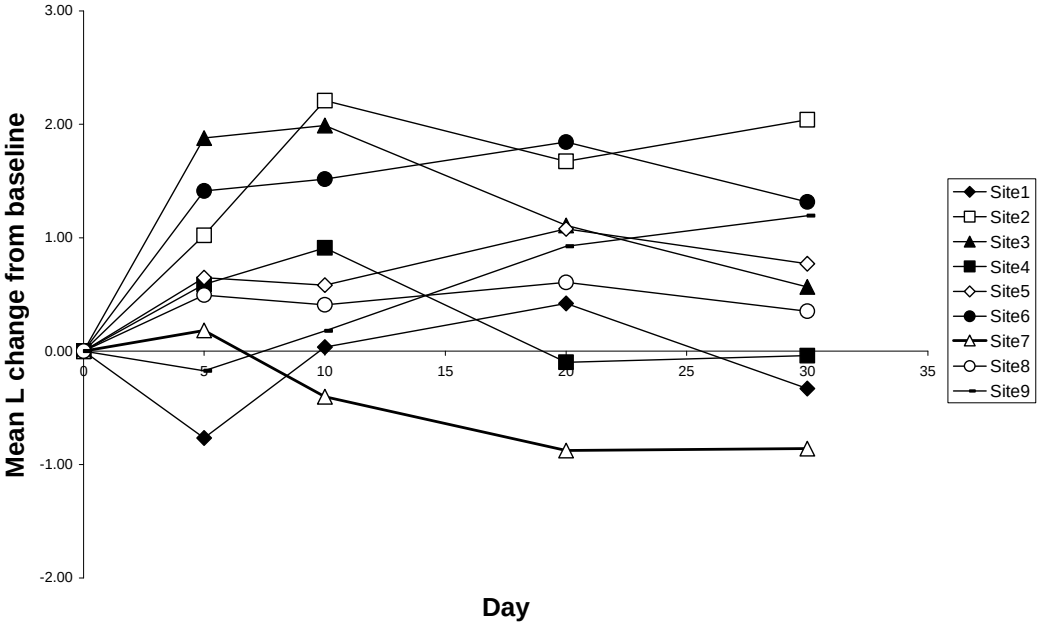

Figure 5.4 Plot of mean L change from baseline for all the anatomical sites in Cohort 5

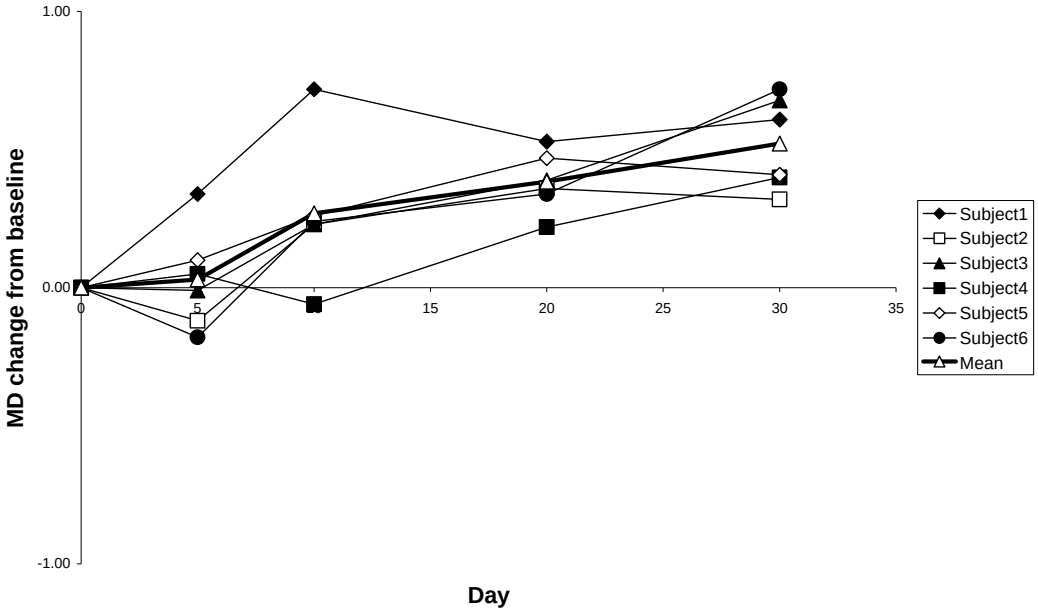

Figure 5.5 Plot of MD values change from baseline for site 7 for all the subjects in Cohort 5.

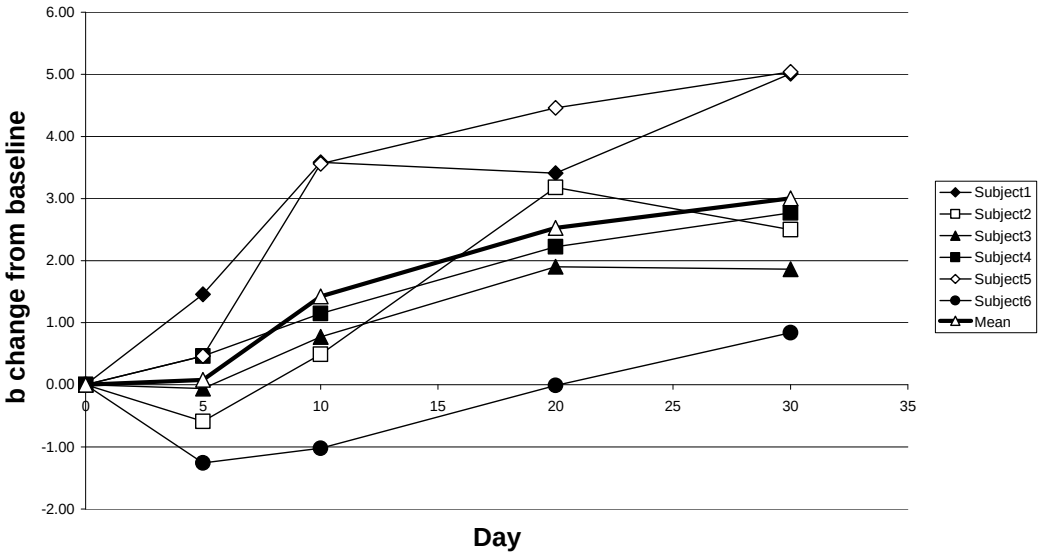

Figure 5.6 Plot of b values change from baseline for site 7 for all the subjects in Cohort 5.

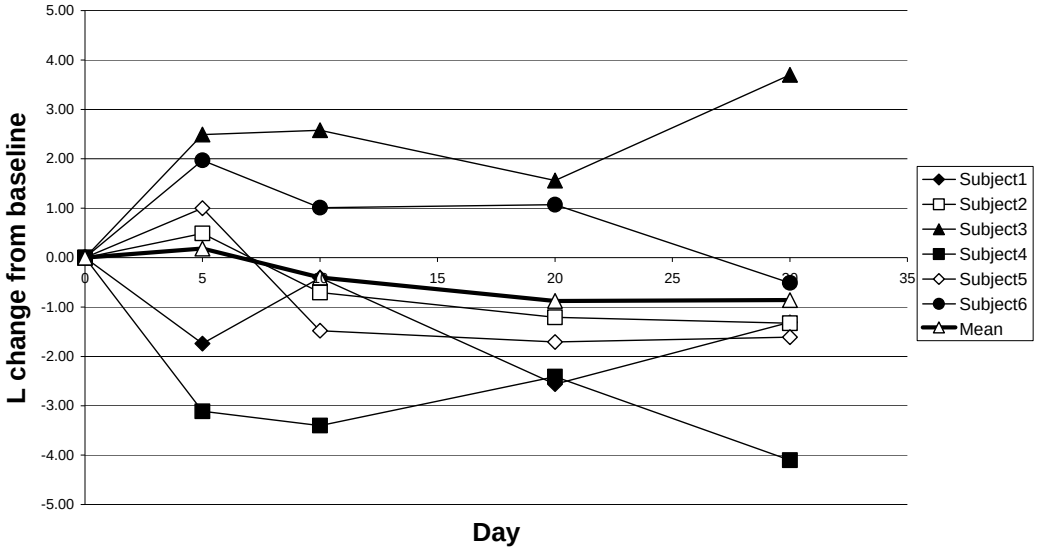

Figure 5.7 Plot of L values changed from baseline for site 7 for all the subjects in Cohort 5.

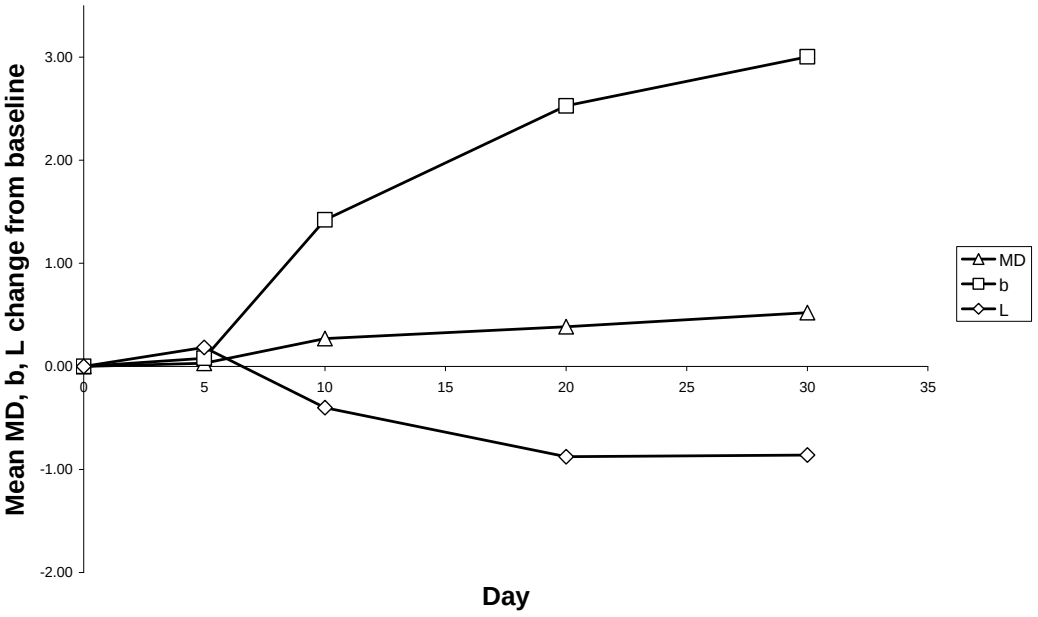

Figure 5.8 Plot of mean MD, b, and L change from baseline for site 7

The analysis of variance (ANOVA) was performed for the MD, b, and L values on all sites and subjects together for the difference between Day 5, 10, 20, and 30 from the baseline (n = 270 observations). No significant differences were observed between day 5, 10, 20, and 30. The 95 % confidence interval (CI) and the p values are summarised in Table 5.2. The ANOVA performed for MD, b, and L on each site also showed no difference between day 5, 10, 20, and 30 for all sites except site 7. The MD and b values for site 7 were statistically different at day 10, 20 and 30. Although the mean L values were decreased at day 10, 20, and 30, the differences were not statistically significant. The confidence interval and the p values of MD, b, and L for site 7 are summarised in the Table 5.3.

The analysis of all the blood samples for dose 40 mg also showed that MT I were not detected in plasma in any of the sampling point in all the subjects. This result showed that the TDS system may not be able to deliver MT I systemically and that is the likely answer for the lack of tanning in all the subjects studied.

No serious adverse events were reported by all the subjects studied. From 30 subjects, only 6 subjects reported headache at some point during the 30 day study period, but were only mild and lasted for a short period of time. Two subjects caught a cold and one subject experienced stomach pain, but this was not related to the study drug. All of the subjects tolerated the treatment applications and there was no local reaction observed at the application site.

Table 5.2 The confidence interval (CI) and p values for MD, L, and b obtained from ANOVA for the differences between Day 5, 10, 20, 30 from baseline for all the observation sites and subjects

Day	MD		L		b	
	CI	p	CI	p	CI	p
Day 5	-0.1466 - 0.3736	0.6591	-0.7446 - 1.920	0.6556	-0.6167 - 1.014	0.9366
Day 10	-0.2312 - 0.2890	0.9962	-0.5072 - 2.158	0.3604	-0.5414 - 1.089	0.8283
Day 20	-0.2149 - 0.3053	0.9799	-0.5901 - 2.075	0.4560	-0.3591 - 1.271	0.4523
Day 30	-0.1999 - 0.3203	0.9450	-0.7757 - 1.889	0.6963	-0.5247 - 1.106	0.7977

Table 5.3 The confidence interval (CI) and p values for MD, L, and b obtained from ANOVA for the differences between Day 5, 10, 20, 30 from baseline for all the subjects in site 7.

Day	MD		L		b	
	CI	p	CI	p	CI	p
Day 5	-0.1802 - 0.2402	0.9863	-1.6360 - 2.0022	0.9963	-1.2250 - 1.3820	0.9995
Day 10	0.0598 - 0.4802	0.0098	-2.2210 - 1.4172	0.9374	0.1180 - 2.7250	0.0302
Day 20	0.1748 - 0.5952	0.0004	-2.6960 - 0.9422	0.5286	1.2230 - 3.8300	0.0002
Day 30	0.3132 - 0.7335	0.0000	-2.6790 - 0.9588	0.5445	1.7000 - 4.3070	0.0000

5.7 Discussion and Conclusion

Although the primary endpoint, the skin tanning, was not achieved in this study, the analysis of the skin reflectance data have showed a significant increase in the MD and b and the decrease in L values at one site of the body. However, the difference is only significant for MD and b, but not for L. Therefore we cannot conclude that the TDS®-Melanotan worked.

A few suggestions can be made from the above observations. At the doses used the TDS® system may not capable of delivering the necessary therapeutic dosage of MT 1 through the skin to induce enough production of melanin to give a tanning effect. This is may be because of MT-I is a peptide which is by its nature has a large molecular weight, is hydrophilic, and is a polar compound (Bodde et al., 1989) presenting difficulties for effective transdermal drug delivery. Oral administration for peptides is not feasible as peptides are rapidly metabolised in the intestinal tract by pancreatic and intestinal proteolytic enzymes to form a smaller structure (Steffansen et al., 2005) Also the large molecular size of peptides almost restricts the compounds from entering enterocytes in the intestine (Steffansen et al., 2005). Therefore the delivery of a peptide through the skin presents a challenge for the future development of the TDS® system. These can be confirmed with the result from plasma analysis of all the subjects in 40mg dose group which were found no MT I detected at any time points.

Another parameters that can be considered to influence the tanning capabilities is the MC1R (Melanocortin 1 receptor) polymorphism. MC1R is a G-protein–coupled receptor existing in skin melanocytes, that can activates adenylate cyclase to elevate cyclic adenosine monophosphate levels upon stimulation by the proopiomelanocortin-derived peptides α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropic hormone (Thody and Graham, 1998). Stimulation of MC1R by α-MSH and other pro-opiomelanocortin (POMC) peptides leads to eumelanogenesis and is central to the tanning response of human skin under UV exposure (Rouzaud et al., 2005;Suzuki et al., 1999). MC1R also regulates the balance of two melanin types, the red/yellow pheomelanin and black/brown eumelanin.

The MC1R gene has found to be highly polymorphic in Caucasian populations (Rouzaud et al., 2005). More than 30 allelic variants of the human MC1R gene have been identified, mostly in European and Australian populations (115,116,120,153).

Among the variants reported, R142H, R151C, R160W, and D294H are known to be associated with a red hair phenotype (116, 118, 120, 154). It can be seen in a study on a series of individuals from a general Irish population. 75 % contained a variant in the MC1R gene, with 30% containing two variants. The Arg151Cys, Arg160Trp, and Asp294His variants were significantly associated with red hair and fair skin (poor tanning capabilities) ($p = 0.0015$, $p < 0.001$, and $p < 0.005$, respectively) (Smith et al., 1998). UV induced tanning maybe ineffective in numerous 'fair-skinned' individuals, which contain functional disruption of MC1-R (D'Orazio et al., 2006).

Although the MC1R genotype may contribute to the poor tanning capabilities as discuss above, the significant of this factor to the result obtained in this study is highly unlikely, especially since the blood results of the highest dose, 40mg, were unable to confirm the absorption of MT I.

Editors' note: After completion and submission of this thesis, representatives of the Epitan Corporation notified the company that they were aware that (1) the drug would not likely show up in the blood stream at the levels dose and (2) no significant Melanin density shifts would occur unless and until the subjects were exposed to sunlight. They wanted the study blinded to these facts. The company representative, upon learning of these results, concluded the TDS system was clearly delivering the peptide.