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ORIGINAL ARTICLE

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Factors determining the optimal body site and method for obtaining punch biopsies of human skin as a tissue in which to assess pharmacodynamic and pharmacokinetic endpoints in drug development studies

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Abstract There are potential advantages to detecting pharmacodynamic (PD) and pharmacokinetic (PK) endpoints in a tissue-based compartment such as the skin during the development of molecularly targeted drugs. We explored regional differences between inner arm, inner thigh, lower back and buttocks in 12 healthy male Caucasian volunteers in the tolerability of skin biopsy procedures; the Ki67 proliferation index; the frequency of detecting hair follicles and sweat glands; and the percentage of melanocytes. We also explored the amounts of tissue and protein obtained, and two separate methods of splitting biopsies for processing in mutually exclusive media. Biopsies from all body sites were well tolerated. The subjective ranking order was inner arm > buttocks = back > thigh. There were no statistically significant differences in the Ki67 labelling index ($P > 0.05$). The frequency of detecting sweat glands was the same in all body sites, but the frequency of detecting hair follicles was higher in back and buttock, compared to arm and thigh. The percentage of melanocytes was significantly lower in the buttocks compared to the back and thigh ($P < 0.05$), but not compared to the arm ($P = 0.07$). A 4-mm punch biopsy yielded a mean of 16.8 mg of tissue (range: 9–28 mg) and 160 μg of protein (range: 80–270 μg). In vivo sample splitting, by following a 2-mm punch with a 4-mm overpunch, had a shorter time from devascularisation to immersion into processing medium than ex vivo dissection of a 4-mm sample, which may be of importance to the assessment of labile endpoints. We conclude that multiple punch biopsies of the skin are feasible, with the buttocks representing the studied body site with the optimal balance between tolerability, hair follicle density and melanocyte density for obtaining tissue in which to assess PD and PK endpoints during drug development studies.

Keywords Skin biopsy · Drug development · Ki67 · Pharmacodynamic · Pharmacokinetic

Abbreviations IHC: Immunohistochemistry · CPU: Clinical Pharmacology Unit · EGFR: Epidermal growth factor receptor · PK: Pharmacokinetic · PD: Pharmacodynamic · GCP: Good clinical practice · ICH: International conference on harmonisation · mg: Milligram · μg: Microgram · rpm: Revolutions per minute · H & E: Haematoxylin and eosin · HPLC: High pressure liquid chromatography · LCMS: Liquid chromatography mass spectrometry · ELISA: Enzyme-linked immunosorbent assay · ANOVA: Analysis of variance · mm: Millimetre · p: Probability · mL: Millilitre · g: Gram · μL: Microlitre · NaCl: Sodium chloride · mM: Millimolar · CL: Confidence limit · Glsmean: Geometric least squares mean · n/a: Non-applicable

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Introduction

With increasing numbers of pharmaceutical agents being developed, for which the specific molecular targets relating to efficacy are known, there are clear advantages to being able to assess the extent of a drug's action on these targets early in the drug development process [5]. In addition to such pharmacodynamic (PD) endpoints, there is also growing interest in assessing drug and

metabolite concentrations directly in the target-expressing tissues for deriving pharmacokinetic (PK) endpoints, as there is some evidence that these may be more informative in explaining drug effects than plasma drug levels [9].

These approaches have been particularly vigorously pursued within the anti-cancer drug arena over the last few years. While anti-cancer drug-induced molecular effects observed in malignant tissue are the most likely to relate directly to clinical benefit [3], tissue heterogeneity and difficulties in tissue access may limit the practicality of this approach. If the same molecular markers present within the malignant tissue are also present within a more homogenous and more easily accessible normal tissue, then it may be possible to use the relevant normal tissue as a convenient surrogate for the malignant tissue. This normal-tissue based approach to aspects of oncology drug development has already been applied successfully to tissue derived from punch biopsies of the skin for a number of different drugs acting on the epidermal growth factor receptor (EGFR), both in determining proof of drug mechanism [1, 7, 14] and in estimating the optimal biologically effective dose of the drug [12]. Although, in the absence of extensive data, there are legitimate concerns about the directness of the surrogacy relationship between normal and malignant tissues, this approach is likely to be repeated across a broad range of anti-cancer drugs in the future, particularly those resulting in anti-proliferative effects. While proliferation endpoints may be particularly relevant to oncology drug development, they could also have much wider applicability, e.g. during the development of drugs used to treat connective tissue diseases or in transplantation medicine. If new drugs are well tolerated and non-genotoxic, then normal-tissue based PD and PK approaches may also be applied to early phase studies conducted in healthy human volunteers, in addition to those conducted in patients.

Traditionally, biopsies of the skin have been used to investigate areas that are diseased or dysfunctional, hence the required site of the biopsy has tended to be self-evident. When aiming to assess PK and/or PD endpoints in normal skin as part of a drug development study, faced with the entire normal skin area from which to choose, there is currently little in the literature to guide the physician in selecting the optimal body site from which to take punch biopsies. To address this issue we conducted the following study to determine whether certain factors differed between biopsies of the skin taken from different body sites in healthy male volunteers, including: the tolerability of the biopsies; variation in the architecture or density of pertinent histological structures likely to have high proliferative rates (such as hair follicles); and the proliferation index and the percentage of melanocytes present within the epidermis. Additionally, in order to maximise the potential for PK and/or PD analysis of skin samples we explored the feasibility of splitting the sample in various ways to allow immersion of the specimen in different processing media.

Materials and methods

Trial design

A prospective multiple skin biopsy study was conducted on healthy volunteers in the UK in full accordance with the Declaration of Helsinki and the International Conference on Harmonisation's (ICH's) guidelines on Good Clinical Practice (GCP).

Twelve healthy Caucasian males, within the age range 18–45, were recruited from the Clinical Pharmacology Unit (CPU) volunteer panel at AstraZeneca, Alderley Park, Cheshire, UK. Study specific exclusion criteria included the use of any regular medication or drugs of abuse, acute illness within 2 weeks of the start of the study, significant history of skin inflammation/infection/disease, history of reaction to local anaesthetic, or a personal or family history of abnormal wound healing or of scar formation. Direct sun or sunbed exposure to the skin areas intended for biopsy was avoided for the week prior to the study.

All skin biopsies were performed under aseptic conditions. Following a brief inspection of the skin to exclude overt pathology, approximately 1 ml of 1% lidocaine with 1:200,000 epinephrine (*AstraZeneca, Macclesfield, UK*) was injected into each proposed biopsy site. After local anaesthesia was achieved, a 2- or 4-mm biopsy punch (*Stiefel Laboratories Limited, Buckinghamshire, UK*) was used to obtain a core of epithelium and underlying connective tissue. Four separate skin sites were biopsied from each volunteer in the following order: inner upper arm, inner thigh, lower back and upper outer buttock. Haemostasis was achieved, if necessary, by local pressure. Wounds were covered with a sterile adhesive dressing and left to heal by secondary intention.

Within each subject the four biopsy sites were randomly allocated to one of four different tissue preparation and/or division patterns, a–d (Fig. 1). Following excision (for patterns a, b and c), or excision and ex-vivo division (for pattern d), the biopsy tissue was then either immediately immersed in 10% neutral-buffered formalin or placed in a cryotube and flash-frozen in liquid nitrogen and stored at −80°C. All aspects of the biopsy procedure were recorded in a biological sample log, referenced according to subject, tissue division pattern, date and time of the 24-h clock.

The volunteers returned to the CPU at 10 days (±3 days) post-procedure, for a tolerability assessment and an inspection of the biopsy sites.

Tolerability assessment

At 10 days (±3 days) post-procedure, each subject was asked to rate the skin biopsy sites in terms of preference (1, 2, 3, 4), with 1 being the most preferred and 4 being the least preferred site. If certain sites were preferred

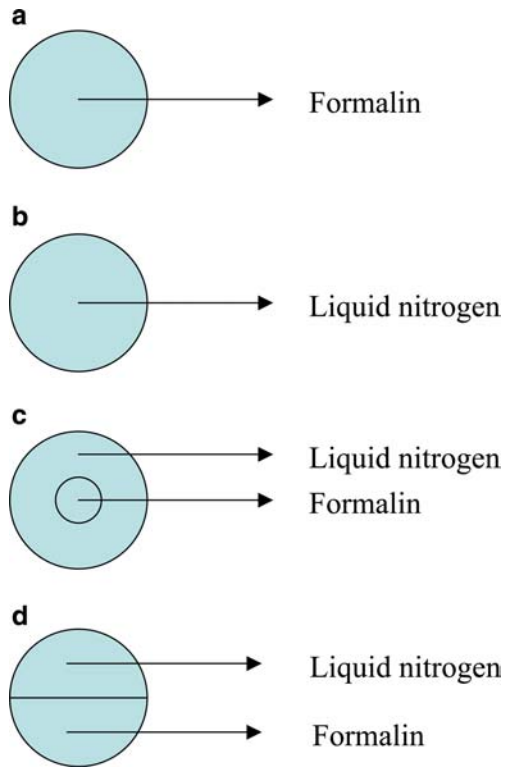

Fig. 1 Tissue preparation and/or division patterns **a**–**d** (plan view of skin surface). **a** 4 mm diameter disc for formalin fixation. **b** 4 mm diameter disc for liquid nitrogen preservation. **c** Concentric over punch (in vivo tissue division): 2 mm diameter disc for formalin fixation followed by 4 mm diameter rim-donut for liquid nitrgoen preservation. **d** 4 mm diameter disc halved (ex vivo tissue division) by dissection perpendicular to the plane of the skin, cutting down from the excision surface towards the skin surface. Half of the bisected disc sent for formalin fixation and half for liquid nitrogen preservation

equally, the sites were given the same ranking number. Subjects were also asked, on a body-site-by-body-site basis, whether they would agree, in theory, to having four biopsies, each involving a separate anaesthetic injection, within 24 h from around the same body site.

Estimation of amount of tissue/protein obtained and histological analysis

For the biopsy specimens flash-frozen in liquid nitrogen, samples were weighed (after subtracting the weight of the cryotube) and suspended in ice-cold RIPA buffer (1 g Trizma pH 7.4 (#T-7693 Sigma), 0.9 g NaCl (#S-5886 Sigma) and 1 ml of 100 mM EDTA per 100 ml), to which was added a cocktail of 1% phosphatase inhibitors 1 (#P-2850 Sigma), 1% phosphatase inhibitors 2 (#P-5726 Sigma) and 1% protease inhibitors (#P1860 Sigma) immediately before use. The optimum volume of RIPA buffer varied depending upon initial sample weight, for samples < 0.015 g 100 µl buffer was used, for samples > 0.015 g 150 µl buffer was used. The samples were then homogenised using an Ultra Turrax T25 basic (8-mm range) (#TWK-610-010M Fisher Scientific) and refrozen at −80°C. The homogenates were thawed on ice and vortexed to ensure good suspension. Ice-cold detergent mix 10% NP-40 (#I-3021 Igepal CA-630 Sigma), 10% sodium deoxycholate (#D6750 Sigma) was added to 10% final volume and stood on ice for 30 min, with vortexing of the mix every 5 min. The samples were then centrifuged at 13,000 rpm for 10 min at 4°C. The supernatants were removed and stored at −80°C. The protein concentration and total amount of protein in each sample were determined using a BCA Protein Assay Kit (#23225 Pierce).

For the biopsy specimens immersed in formalin, following a uniform fixation period of 25–30 h, the tissue was embedded into paraffin blocks. Serial paraffin wax sections (4–6 µm) were cut and picked up onto Superfrost Plus slides (*Surgipath, Peterborough, UK*). The slides were marked with study, subject, biopsy and section number, and dried at 37°C overnight. The sections were subsequently stained by standard methods with Haematoxylin and Eosin (H & E) or for Ki67. Ki67 was detected by Ventana SCC1 antigen retrieval, followed by incubation with DAKO M7240 mouse monoclonal MIB-1 (1:100 dilution for 1 h) using Ventana DABMAP secondary antibody for visualisation.

Sections were assessed, in a blinded fashion, by a consultant on the UK specialist list for pathology. The percentage of Ki67 positive cells and melanocytes were scored within the entire basal epithelial monolayer. Sweat gland acini and hair follicles, in whole or in part, were scored on the basis of whether they were present or absent in the sections.

Statistical analysis

The percentages of Ki67 positive cells and melanocytes within the basal epidermis were formally statistically analysed using an analysis of variance (ANOVA) model including terms for body site, biopsy preparation technique and subject. The model was used to investigate the presence of systematic differences between body sites and biopsy preparation techniques. The data on percentages of melanocytes and Ki67 positive cells appeared log-normally distributed and so was log-transformed prior to analysis. A statistical significance level of $P < 0.05$ was used. No formal statistical analysis was undertaken on data on the presence/absence of hair follicles and sweat glands due to the small size of the dataset and the potential confounder of biopsy section size. No formal statistical analysis was conducted on the tolerability ranking dataset.

Results

All biopsies, including timings and biopsy site locations, were obtained as planned. All biopsy sites showed evidence of normal healing when assessed 10 days

(±3 days) post-procedure. The timings, from devascularisation of the biopsy specimen to immersion into processing medium (either liquid nitrogen or formalin) according to biopsy preparation technique and body site, are shown in Table 1. Of note, for biopsy methods a–c, most samples were placed into the processing medium within the same minute, with the rest being completed within two adjacent minutes of the 24-h clock. For biopsy method d the majority of samples were completed within two adjacent minutes of the 24-h clock, with some samples having timings falling within three adjacent minutes and only one for each particular processing medium (formalin or liquid nitrogen) completing within the same minute.

There was some macroscopic evidence of warping of the 2-mm punch biopsies post-fixation and pre-embedding that was not apparent in the 4-mm discs or 4-mm rim-donuts, but this did not affect the ease of sectioning. No subjective differences in the quality of tissue architecture were apparent on H & E between the different methods of sampling.

The arithmetic mean weights, with minima and maxima shown in parentheses, of the samples immersed in liquid nitrogen according to the different biopsy preparation techniques were as follows: 4-mm discs (method b) = 16.8 mg (9–28 mg); 4-mm diameter rim-donuts (method c) = 12.9 mg (7–18 mg); 4-mm bisected discs 9 mg (2–18 mg). The arithmetic mean amounts of protein, minima and maxima, obtained from the samples immersed in liquid nitrogen according to the different biopsy preparation techniques were as follows: 4-mm discs (method b) = 160 μg (80–270 μg); 4-mm diameter rim-donuts (method c) = 100 μg (5–218 μg); 4-mm bisected discs 85 μg (25–155 μg).

The results of the analysis of the quantitative histological scoring of the sections for the percentages of Ki67 positive cells and melanocytes in the basal epidermis, and the number and presence/absence of hair follicles or sweat glands by biopsy site, are shown in Table. 2 and 3, respectively. The sample obtained by method d from the inner thigh of one individual was shown, on H & E staining, to contain only subcutaneous fat and no epithelium. Consequently only 35 of the 36 specimens were used for the quantitative histological analysis. As the other half of the same sample placed in liquid nitrogen liberated only 25 μg of protein, it is likely that an inappropriate biopsy procedure rather than inappropriate division of the sample accounted for these observations. Three of the 35 skin samples (1 × back, 1 × buttock, 1 × thigh) revealed evidence of focal regenerative changes with intradermal melanophages and lymphocytes, in keeping with reparative damage most likely due to trauma or eczema. Because chronic inflammation could alter both the percentage of Ki67

Table 1 Maximum possible time-intervals between biopsy devascularisation and immersion of specimen into processing medium according to body site and biopsy preparation technique

Biopsy preparation technique/processing medium	Site	No. of samples devascularised and placed into processing medium within:		
		0–1 min[a]	1–2 min[b]	2–3 min[c]
a	Arm	1	2	0
	Thigh	3	0	0
	Back	2	1	0
	Buttock	3	0	0
	Total	9	1	0
b	Arm	2	1	0
	Thigh	2	1	0
	Back	3	0	0
	Buttock	1	2	0
	Total	8	4	0
c/F	Arm	2	1	0
	Thigh	3	0	0
	Back	3	0	0
	Buttock	3	0	0
	Total	11	1	0
c/N	Arm	2	1	0
	Thigh	3	0	0
	Back	1	2	0
	Buttock	2	1	0
	Total	8	4	0
d/F	Arm	1	2	0
	Thigh	0	1	2
	Back	0	2	1
	Buttock	0	3	0
	Total	1	8	3
d/N	Arm	1	2	0
	Thigh	0	2	1
	Back	0	2	1
	Buttock	0	3	0
	Total	1	9	2

Processing techniques a–d as described in Fig. 1; N liquid nitrogen; F formalin

[a]Starting and finishing within the same minute of the 24-h clock

[b]Starting during the first minute and finishing during the second minute of the 24-h clock

[c]Starting during the second minute and finishing during the third minute of the 24-h clock

positive cells and the number of melanocytes, data from these three specimens were excluded from the analysis of the endpoints presented in Table 2.

The analysis of Ki67 showed that there were similar percentages of positive basal cells in samples from all body sites. The geometric mean percentage values, maxima and minima, by body site were as follows: for the arm 24.4% (12–36%), thigh 22.6% (12–42%), back 24.3% (16–42%) and buttocks 15.4% (4–43%). Although there were slightly lower percentages in the buttock samples compared to the other sites the difference among body sites did not reach statistical significance ($P = 0.2$) when the ANOVA model was applied (Table 2).

The percentage of melanocytes present in the basal cells appeared to be lower in samples taken from the buttock compared to other sites. The geometric mean, maxima and minima, by body site were as follows: for the arm 8.1% (1.6–71%), thigh 12.4% (2.5–81%), back 13.8% (2–65%), and buttock 2.5% (0–13%). This was statistically significant ($P = 0.04$) for the differences between body sites. Specifically, the percentages found in the buttocks were significantly lower compared to both the thigh and the back (Table 2).

Table 3 summarises the number of subjects with any sweat glands or hair follicles present in the samples taken from different body sites. There appeared to be little difference between body sites in the number of subjects with sweat glands present. In contrast, a greater number of samples from back and buttocks appeared to have hair follicles present compared to those samples taken from the arm or thigh. The average number of hair follicles was also greater in the back and buttock samples (data not shown).

With regard to the tolerability of the procedure, for the inner arm all 12 subjects agreed, in theory, to having four biopsies done from the same body site within a 24-h period. The numbers agreeing to the other body sites were 11 for inner thigh, and 10 each for buttock and lower back. In terms of rankings of the preferred body sites for a single biopsy, the overall order across the 12 volunteers was inner arm > buttock = lower back > inner thigh.

Discussion

The skin is an easily accessible tissue that offers, when the primary target of a drug is present, the potential for assessing both PD change and the tissue concentrations of the drug or metabolite responsible for these changes. The advantages of such an approach in drug development would include the early demonstration of a drug's effects on its proposed molecular target (proof of mechanism) to manage better the financial and safety risks associated with continued development, and the potential for determining an initial readout of a drug's exposure:response relationship [1, 7, 12, 14].

In order to assess a variety of both PD and/or PK endpoints in skin, samples may have to be processed by mutually exclusive protocols. For example, while immunohistochemistry (IHC) endpoints are traditionally assessed from formalin fixed specimens, formalin is currently an inappropriate processing medium if the tissue is to be analysed by high pressure liquid chromatography (HPLC) or liquid chromatography mass spectrometry (LCMS) for drug or metabolite concentrations; by enzyme-linked immunosorbent assay (ELISA) or western blotting for specific protein levels; or by northern blotting or gene array analysis for transcript levels. For these latter examples, liquid nitrogen is often the processing medium of choice. In theory, samples taken from within the same small area of skin may be more likely to have comparable PK exposures and lower background PD variability than comparisons made between samples taken from more disparate sites. Although multiple biopsies close to the same area of skin would permit placement of skin in different media, the

Table 2 Analysis of the percentage of Ki67 positive cells, and of melanocytes, in the basal epidermis of skin samples from the arm, thigh, back and buttock

P-value for site difference (all sites)	Site	Glsmean (%)	Ratio vs buttock	Lower 95% CL	Upper 95% CL	*P*-value for site contrast
Ki67						
0.20	Buttock	0.16				
	Thigh	0.24	1.49	0.901	2.46	0.11
	Arm	0.24	1.52	0.954	2.41	0.07
	Back	0.24	1.53	0.948	2.46	0.08
Melanocytes						
0.04	Buttock	0.03				
	Thigh	0.14	5.16	1.56	16.67	0.01
	Arm	0.08	2.79	0.93	8.41	0.07
	Back	0.11	4.14	1.32	12.95	0.02

Table 3 Skin samples scored for the presence/absence of hair follicles or sweat glands, according to body site

N number of subjects

	Arm ($N = 9$)	Back ($N = 9$)	Buttock ($N = 9$)	Thigh ($N = 8$)
Sweat glands	6	5	5	5
Hair follicles	1	4	5	2

current study has demonstrated the practicality of splitting skin within the same 4-mm area utilising both ex vivo and in vivo techniques. Noting the limitation that the data on timings were only taken to within a single minute of the 24-h clock, ex vivo splitting, by bisecting a 4-mm punch biopsy disc, does appear to take longer on average, from devascularisation of the sample to immersion into processing medium, than in vivo splitting using an overpunch technique (Fig. 1c, d, Table 1). Although the time differences were small, these may be significant for very labile endpoints, such as the phosphorylated epitopes present on many signal transduction pathway molecules [8].

We measured the amounts of tissue and protein obtained from liquid nitrogen preserved skin biopsies procured through the techniques shown in Fig. 1b–d. Even though the amounts of subcutaneous tissue were not controlled for in this current study (i.e. it was not explicitly directed in advance whether subcutaneous tissues should or should not be limited or trimmed during devascularisation, or during ex vivo dissection) it was apparent, both from simple geometry and in practice, that the method for obtaining and splitting skin biopsies influenced the size, and by direct extension the amount, of tissue and protein available for subsequent analysis. For an entire 4-mm punch biopsy disc the arithmetic mean weight of tissue obtained was 16.8 mg (range: 9–28 mg), yielding a mean amount of protein of 160 μg (range: 80–270 μg). Undoubtedly, the difficulties inherent in processing very small tissue samples including, for example, exaggerated percentage losses from material retained on the homogenisation equipment, will have contributed to the wide variability noted in protein recovery even among samples of the same initial size. Although the weight measurements on our skin specimens contained unspecified amounts of potentially irrelevant subcutaneous fat, and the cells of most interest, unlike those in a tumour, may not be uniformly distributed throughout the stratified skin epithelium, broadly speaking these amounts of tissue and protein are still within the practicable range of many standard laboratory protein and RNA analysis techniques [13]. The size of biopsy that was placed into formalin did not seem to influence the quality of the tissue used within the subsequent histometric or IHC analyses.

With regard to the optimal body site from which to obtain skin biopsies, where this has been stated in previous anti-proliferative drug development studies, often the upper chest or back has been used [1, 7]. We have avoided these areas primarily because of their well-documented risk of keloid scar formation [2]. Given that keloid scarring may take from months to years to manifest after the initial injury—although this may make the upper body acceptable as a biopsy site for those with short prognosis diseases—it remains a significant disincentive to using this area in both patients with longer prognoses and in healthy volunteers. Within the current study we assessed regional differences in key features potentially relevant to PD endpoints, among biopsies obtained from four, low keloid-risk, easily accessible body sites: the inner upper arm, inner thigh, lower back and upper outer buttock.

We did not demonstrate any statistically significant differences in the percentages of Ki67 positive basal cells between the four body sites (Table 2), implying that, in the absence of other factors, for proliferation endpoints there may be little to chose between them. For future studies looking at other primary endpoints, we would suggest that body site differences, or lack of differences, should, however, be confirmed on a marker-by-marker basis.

The proliferative drive, as assessed by the levels of activated EGFR, within certain skin adnexae, notably sweat glands and hair follicles, does appear to be higher than in the basal epidermis index [1]. In our own hands, given the caveat of a relatively small data set, using Ki67 to represent the general proliferative index, this picture was certainly supported on the basis of the arithmetic means for hair follicles (arithmetic mean 27.9%), compared to the basal epithelium (arithmetic mean 23.9%), although not for sweat glands (arithmetic mean 3.1%). In contrast, when using the geometric means the values for sweat glands and hair follicles (0.8% and 13.3%, respectively) were both less than the geometric mean Ki67 index for the basal epithelium (21.3%). If a higher proliferative drive or a higher key biomarker signal were to be present, it could make such adnexae more attractive than the basal epidermis as structures in which to assess certain PD endpoints, hence regional differences in the frequency of these skin adnexae could be important in the choice of the biopsy site. In the current study, as both the size of biopsy taken and the amount of tissue on the section of each biopsy block that was examined were variable, comparisons based on absolute numbers of adnexae are unlikely to be informative. Instead, although subject to the same limitations, we made crude estimates based purely on the presence or absence of these structures (Table 3). These estimates suggest that, while there appeared to be little difference between body sites in terms of sweat glands, biopsies taken from the lower back or buttocks were more likely to have hair follicles present than those taken from the inner arm or inner thigh. These findings are entirely consistent with observations in the literature of regional differences in the rate of healing of superficial partial thickness burns that re-epithelialise from such skin adnexae [10]. Regional differences in capillary density were not assessed in the current study, but are well documented in the plastic surgery literature because of their impact on skin flap and graft surgery [11]. Whether regional differences in skin density is an additional anatomical variable relevant to drug development studies, that could, for example, affect the PK profile of drug exposure between body sites, is currently unclear.

The percentage of melanocytes among the basal cells was lower, in a statistically significant manner, for biopsies taken from the buttocks compared with both

the thigh and the back ($P < 0.05$), but not the arm ($P = 0.07$) (Table 2). The relevance of this finding relates to: the potential interference of melanin in the quantitative assessment of certain PD biomarkers (e.g. if optical densitometry is used) [7]; the potential for melanin to bind certain drugs non-specifically [6]; and to melanin as a surrogate measure of ultraviolet light exposure, which is known to affect a number of different functions within the skin, including proliferation [4]. Consequently, we would suggest that skin biopsies from regions with low levels of melanocytes are preferable for assessing both PD and PK endpoints. In addition, as in the current study, we would suggest that sun or sunbed exposure should be standardised for participating subjects both before and during any future drug trial involving skin biopsies. While the impact of ultraviolet light exposure is likely to be most marked in Caucasians, these principles will be equally applicable to non-Caucasians. We have not addressed racial differences within the current study, but given the potential for pigmentation to differ markedly between individuals both within and between races, in addition to all the other factors contributing to inter-subject variability, we would suggest that, whenever possible, individuals in PD and PK studies involving skin biopsies should act as their own controls.

All the four body sites were well tolerated with volunteers agreeing, in theory, to having four biopsies taken from the arm, thigh, buttocks and back in 100, 92, 83 and 83% of cases, respectively. Across the 12 volunteers, the body sites for a single biopsy were ranked at 10 days (± 3 days) post-procedure in the order: inner arm > buttocks = lower back > inner thigh.

In conclusion, we have shown that skin biopsies from four different body sites, all with a low documented risk of keloid scar formation, are well tolerated in healthy male Caucasian volunteers, with the inner arm being, subjectively, the most preferred site. The amounts of tissue and protein obtainable from manipulations of 4-mm punch biopsies of the skin from these sites are within the practicable range of a number of standard protein and nucleic acid analytical techniques. If the same skin sample has to be split for immersion into different processing media, e.g. for mutually exclusive PD and PK assessments, both in vivo and ex vivo splitting techniques are possible, but the in vivo over-punch splitting technique is faster. Even though the inner arm ranked highest for tolerability, on the basis of: the same Ki67 proliferation index in the basal epithelium; a higher potential for detecting potentially relevant skin adnexae (hair follicles) compared to inner arm and inner thigh; and a lower percentage of melanocytes compared to inner thigh and lower back, we would suggest that the buttocks represent the optimal body site of those studied for human skin biopsies in which to assess PD and PK endpoints during drug development studies.

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