Scandinavian Journal of Pain 4 (2013) 40–45

Contents lists available at SciVerse ScienceDirect

Scandinavian Journal of Pain

journal homepage: www.ScandinavianJournalPain.com

Original experimental

Central origin of pinprick hyperalgesia adjacent to an UV-B induced inflammatory skin pain model in healthy volunteers

Bernhard Rössler[a,b,c], Anna Paul[b,d], Maria Schuch[b], Martin Schulz[b], Thomas Sycha[b,d], Burkhard Gustorff[b,e,*]

[a] Department of Anaesthesia, General Intensive Care and Pain Management, Medical University of Vienna, Austria
[b] Vienna Human Pain Research Group, Department of Anaesthesia, General Intensive Care Medicine and Pain Control, Medical University of Vienna, Austria
[c] Medical Simulation and Emergency Management Research Group, Department of Anaesthesia, General Intensive Care and Pain Management, Medical University of Vienna, Austria
[d] Department of Neurology, Medical University of Vienna, Austria
[e] Wilhelminenspital, Department of Anaesthesia, Intensive Care and Pain Medicine, Vienna, Austria

ARTICLE INFO

Article history:
Received 15 April 2012
Received in revised form 17 August 2012
Accepted 3 September 2012

Keywords:
Sensitisation
Inflammation
Threshold
Ultraviolet
Neuropathic pain
Surrogate models
Quantitative sensory testing

ABSTRACT

Background and purpose: The UV-B model is an established pain model of different types of hyperalgesia in animal and human pain research. Beside the skin region of the sunburn in human volunteers pinprick hyperalgesia has been described in a large zone of non-inflamed skin adjacent to the sunburn. However, there are opposing results on the existence of pinprick hyperalgesia and most notably a controversial discussion is still on-going whether this mechanical hyperalgesia in the undamaged tissue adjacent to and at some distance from the site of inflammation is of peripheral or central origin. We therefore addressed this in our study by hypothesising that pinprick hyperalgesia around a circular spot of UV-B inflamed skin is not reduced by a superficial local anaesthetic block and therefore underlies centrally mediated mechanisms.

Methods: This exploratory study was conducted in a prospective, controlled, randomised, single-blinded fashion in relation to the study hypothesis in 12 healthy volunteers. Before circular irradiation with UV-B light (3-times the individual minimal erythema dose at both thighs), a strip of continuous intradermal local anaesthetic block with lidocaine 2% was established via two single plasmaphoresis hollow fibres. These were positioned perpendicular to one thigh overlapping on the midline of the leg at the distal part of the planned irradiation site, and compared with the contralateral control side without anaesthetic block. The local anaesthetic block was established and then maintained via a syringe pump. The area of pinprick hyperalgesia was measured by pricking on a large skin surface including 360° around the circular irradiation site. This was done with a slightly painful pin (256 mN) until 8 h after irradiation. Primary outcome was the area of pinprick hyperalgesia in the skin adjacent to the sunburn at 8 h.

Results: Large areas of mechanical hyperalgesia to pinprick surrounding the adjacent skin of the sunburn developed on both sides after 8 h without any significant difference between the side of the anaesthetic strip showing an area of $72.6 \pm 39.7\,cm^2$ (mean ± SD) and the control side ($59.1 \pm 20.1\,cm^2$); $p = 0.24$. Moreover, mechanical hyperalgesia to various pin stimuli of different strength was unchanged by the anaesthetic block.

Conclusions: This trial provides evidence that the development of mechanical hyperalgesia surrounding an experimental sunburn was not influenced by continuous peripheral afferent blockade with local anaesthetic at 8 h after UV-B irradiation. Our data support the hypothesis that in the UV-B model peripheral nociceptive afferent input of inflamed skin may enhance central hypersensitivity of mechanosensitive nociceptors in a larger receptive field far beyond the inflamed skin. Furthermore, these findings are in line with other pain models demonstrating comparable central hypersensitivity around the site of injury.

Implications: As for other pain models this finding provides further evidence that the UV-B model offers secondary mechanical hyperalgesia in addition to its known primary hyperalgesia. Consequently, this is a further validation for the utilisation of the UV-B model in human pain research.

DOI of refers to article: http://dx.doi.org/10.1016/j.sjpain.2012.11.006.
* Corresponding author at: Department of Anaesthesia, Intensive Care and Pain Medicine, Wilhelminenspital der Stadt Wien, Montlearstr. 37, A-1160 Vienna, Austria. Tel.: +43 1 49150 4001; fax: +43 1 49150 4009.
E-mail address: burkhard.gustorff@meduniwien.ac.at (B. Gustorff).

1877-8860/$ – see front matter
http://dx.doi.org/10.1016/j.sjpain.2012.09.001

1. Introduction

Ultraviolet-B (UV-B) light induces an inflammatory response in the skin ("sunburn") leading to primary hyperalgesia [1]. This response can be used as a suitable experimental model for pain research in animals and humans [2,3]. In a previous study, we have shown that an area of pinprick hyperalgesia develops surrounding the zone of primary hyperalgesia [2]. This occurs in the undamaged tissue adjacent to and at some distance from the site of inflammation.

In other pain models, this type of mechanical hyperalgesia in the area surrounding the site of injury is thought to be due to an excitatory state of the central nervous system and is therefore classified as secondary hyperalgesia [4]. In the UV-B model however there are opposing results on the existence of pinprick hyperalgesia and a controversial discussion is still on-going whether the mechanical hyperalgesia in skin regions adjacent to the inflammation is of peripheral or central origin [3,5]. According to the concept of central hypersensitivity primary afferent nociceptor activity elicits secondary mechanical hyperalgesia in the skin adjacent to the sunburn area. However, this hypothesis remains controversial and proof in human UV-B-studies is lacking. Efforts to characterise the sensory and nociceptive system have led to a wide range of testing methods including mechanical and thermal stimuli [6,7]. Based on previous published and unpublished findings of the authors, a selection of pinprick hyperalgesia, allodynia, vasomotor reaction, pressure pain and thermal sensitivity have been chosen for this study [2].

In a human pain model with intradermal electrical stimulation it had been demonstrated previously that the spreading of mechanical hyperalgesia or allodynia from the region of nociceptive input was not interrupted by an intradermal superficial anaesthetic strip of lidocaine continuously applied by an intradermal microdialysis fibre [4]. This technique allows blocking locally the extension of peripheral superficial nociceptor activity as typical pattern of primary hyperalgesia. In case of peripherally triggered mechanical hyperalgesia the block results in an absence of mechanical hyperalgesia distal from the block. If however mechanical hyperalgesia remains despite the block, a central origin of the hyperalgesia can be assumed [4].

We hypothesised that pinprick hyperalgesia around a circular spot of UV-B inflamed skin is not reduced by such a superficial anaesthetic block and underlies therefore central nervous system mechanisms.

2. Materials and methods

2.1. Study design

This exploratory study was conducted in a prospective, controlled, randomised, single-blinded fashion in relation to the study hypothesis. The study consisted of one period lasting about 10 h for each subject. The subjects provided their own control, as the local anaesthetic block was applied only to one leg, the other leg being the control. Allocation of the anaesthetic block to the left or right thigh via microdialysis fibres was determined by randomisation.

2.2. Study population

Following approval of the protocol by the local Ethics Committee of the Medical University of Vienna, 12 healthy volunteers aged 19–40 yrs (6 males, 6 females) participated in this study after having given their written informed consent. The participants were blinded to the hypothesis and thus not informed about the intention of the measurements. Individuals had a body mass index between the 15th and 85th percentile and were right-handed and drug-free. Volunteers were treated "per protocol" and dropouts were replaced.

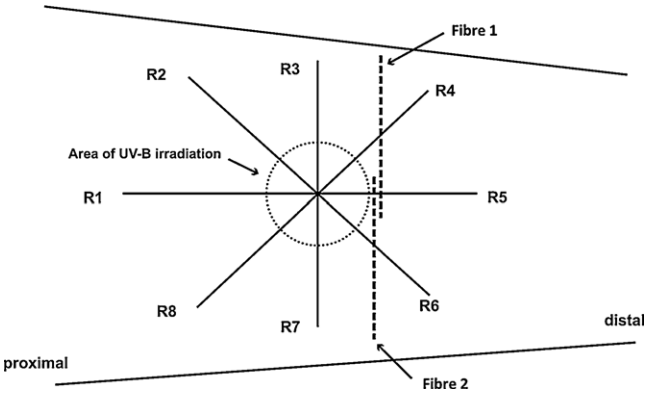

Fig. 1. Demonstrate the area of planned UV-B irradiation, eight radial spokes (R1–R8), and two microdialysis fibres on a thigh.

2.3. Randomisation

Opaque, sealed envelops containing the randomised, computer-generated patient allocations were prepared by a person not otherwise involved in the study. Subjects were allocated to receive the local anaesthetic block on either the right or the left thigh. Randomisation was gender balanced. Each subject was studied for one session.

2.4. UV irradiation and local anaesthetic strip

Individual minimal erythema dose (MED) of UV-B light (Sellasol, Sellas Medizinische Geraete GmbH, Gevelsberg-Vogelsang, Germany; wavelength 290–320 nm) was set at the middle of the non-dominant forearm at least 24 h before study irradiation [2,4]. On the study day, two single plasmaphoresis hollow fibres (0.4 mm diameter, Asahi Medical Co., Japan) were inserted intradermally on the anterior side of one thigh. Both microdialysis fibres were oriented transversally to the long axis of the thigh along the distal border of the intended UV-B-spot, overlapping on the midline of the leg by 3–4 mm to ensure a continuous 10 cm-long strip of local anaesthetic (see Fig. 1). Insertion of the fibres was performed under local cryoanaesthesia using ice packs, ensuring a painless procedure.

The microdialysis fibres were filled with lidocaine 2% (Astra Zeneca GmbH, Wedel, Germany) at a rate of 10 ml/h until a drop of local anaesthetic appeared at the tip of each fibre and then perfused at a constant flow rate of 5 μl/min (0.3 ml/h) via a syringe pump infusion line (Fresenius Kabi AG, Bad Homburg, Germany), driven by a motor pump (Braun, Austria).

Establishing the local anaesthetic block took approximately 45 min and anaesthesia was confirmed using painful mechanical stimuli (512 mN). Baseline measurements were performed and immediately afterwards a circular spot of 4.2 cm diameter – the outer border just proximal to the microdialysis fibres – was irradiated with UV-B light at 3-times MED. The leg serving as the control was also irradiated. The rest of the skin was covered with a UV-protecting tissue. The areas of pinprick hyperalgesia and allodynia were assessed at 4 and 8 h after irradiation.

2.5. Endpoints

2.5.1. Main outcome variables

The area of pinprick hyperalgesia on test side vs. control 8 h after irradiation was chosen as primary outcome.

2.5.2. Additional outcome variables

Additionally, the mean of the three diameters crossing the anaesthetic strip (spokes 4, 5, 6) were compared to the corresponding 3 diameters on the non-blocked side 4 and 8 h after irradiation. Furthermore, the mean of the three diameters crossing the anaesthetic strip (spokes 4, 5, 6) were compared to the mirror 3 diameters on the same side (spokes 8, 1, 2) 4 and 8 h after irradiation, and the size of spokes 1–8 were chosen test side vs. control 4 and 8 h after irradiation.

The S/R function was tested at two sites on the midline of the leg, in the area of pinprick hyperalgesia halfway between the microdialysis fibres and the distal outer margin of the hyperalgetic area and for control on the mirror side proximal of the sunburn. Additionally, the S/R function was assessed within the sunburn, at the site of primary hyperalgesia.

Furthermore, heat pain perception threshold (HPPT), cold pain threshold (CPT), warmth perception threshold (WPT), and pressure pain threshold (PPT) were chosen at 4 and 8 h after the start of the irradiation in the field of primary hyperalgesia. Neither thermal perception and pain thresholds nor pressure pain thresholds were assessed within the area of mechanical hyperalgesia, since own unpublished data (under review) showed absence of the respective hyperalgesia at all time points of assessment of the present study.

Furthermore, we assessed superficial blood flow as a marker for inflammatory strength at the irradiated site and the surrounding area.

Additionally, we assessed the impact of the local anaesthetic block on the sunburn, hence proximal effects by assessing thermal thresholds and PPT in the field of primary hyperalgesia on the lidocaine perfusion side as well as on the control side.

2.6. Methods of evaluation, sequence of measurements

Measurements were performed in the following standardised sequence: Laser Doppler perfusion imaging (LDI), pinprick testing and allodynia, WPT, CPT, HPPT and PPT.

The area of pinprick hyperalgesia was measured by pricking the skin with a slightly painful pin (256 mN, *The PinPrick*, MRC Systems, Heidelberg, Germany), starting approximately 10 cm away from the edge of the sunburn and moving inwards towards the sunburn at 5 mm intervals along 8 radial spokes (see Fig. 1). Spoke number 1 runs in a cranio-caudal axis from the outside to the centre of the sunburn, representing the 12 o'clock position in the irradiated area. Spoke number 2 runs in an angle of 45° clockwise to the first spoke, again from the outside to the centre of the sunburn. Consequently, as depicted in Fig. 1, spokes 4, 5, and 6 cross the anaesthetic strip.

The pinprick activates cutaneous nociceptors, and hyperalgesia was defined as a change in the perception of the stimulus intensity, usually described as "more intense", "sharper" or "more painful" pricking. The first spot of such perception was marked with a pen and the distance to the centre of the sunburn was measured. The area of pinprick hyperalgesia was determined from these 8 distances by calculating the area of the octagon. To avoid bias between measurements, the marks were removed immediately after recording.

The area of dynamic allodynia was assessed in a similar manner using a brush (SENSELab-Brush 05, Somedic, Hörby, Sweden, 200–400 mN) to strike the skin rectangular to the radial spokes.

Within the area of pinprick hyperalgesia, S/R function was evaluated by applying a set of seven custom-made weighted pinprick stimuli (8, 16, 32, 64, 128, 256, 512 mN, *The PinPrick* MRC Systems, Heidelberg, Germany). Volunteers were asked to rate pain intensity of the stimuli on a numerical rating scale from 0 (no pain) to 100 (worst pain) [6]. Five series of all ten stimuli were applied in a randomised, balanced order. Mechanical pain sensation (MPS) was calculated as the geometric mean of all pain ratings for both static and dynamic stimuli using log-transformed data for statistical analysis.

For the evaluation of thermal thresholds (HPPT, CPT, WPT) a Thermal Sensory Analyser (small thermode, 18 mm × 18 mm; TSA-2001, Medoc Ltd., Ramat Yishai, 30095, Israel) was used. The volunteers were asked to respond to the thermal stimuli by pushing a response button. Measurements were performed through the method of limits starting at 32 °C and increased at a rate of 1 °C/s to a maximum of 53 °C. Before testing began, subjects were trained in a standardised manner to allow familiarisation with the testing systems and to determine individual baseline thresholds. The thermal sensory tests were repeated four times with a 15 s interstimulus rest period and results were averaged. The participants were instructed in a standardised manner and trained twice at screening and at the study day.

PPT was determined using a manual pressure algometer (Wagner Instruments, Greenwich, CT, USA) at the same testing locations as before. The contact area was 1 cm^2. After putting the algometer on the skin surface, the pressure was increased at a rate of 50 kPa/s until the subject's pain threshold was reached. PPT assessment was repeated three times and the results averaged.

Superficial blood flow (SBF) and flux of the irradiated site and of the surrounding area was measured with laser Doppler perfusion imaging (LDI, DRT4, Moor Instruments Ltd., Millwey, England) at three time points: prior to UV-B exposure – to obtain a measure of baseline perfusion, and at 4 and 8 h after irradiation. This laser wavelength was 670 nm, the beam was 1 mm in diameter, with a maximum power output of 1 mW. The laser scanned a region of about 8 cm × 8 cm from 20 cm distance, measuring superficial perfusion. The images were analysed using dedicated image-processing software (LDI, DRT4, Moor Instruments Ltd., Millwey, England). SBF served as a surrogate for inflammatory intensity. Mean red blood cell flux (FLUX) is an arbitrary unit calculated from the velocity and number of red blood cells.

Superficial blood flux was measured in the erythematous sunburn area and the surrounding tissue on the leg with the anaesthetic block and compared to its corresponding site on the control leg.

2.7. Statistical methods

Data analysis was performed by repeated measure ANOVA, Bonferroni post hoc test and paired *t*-test. Data from Stimulus/Response (S/R) function, WPT and PPT were log transformed before analysis [7]. All data with a normal distribution are presented as mean ± standard deviation. S/R function parameters were normally distributed in log-space and were transformed logarithmically before statistical analysis. *p*-values of less than 0.05 were considered to be statistically significant. Statistical analysis was carried out using GNU R-Statistics, Version 2.11.

3. Results

3.1. Baseline data

Six men and 6 women were included in the trial. The average age was 25.2 ± 3.5 years, average height was 176 ± 9 cm

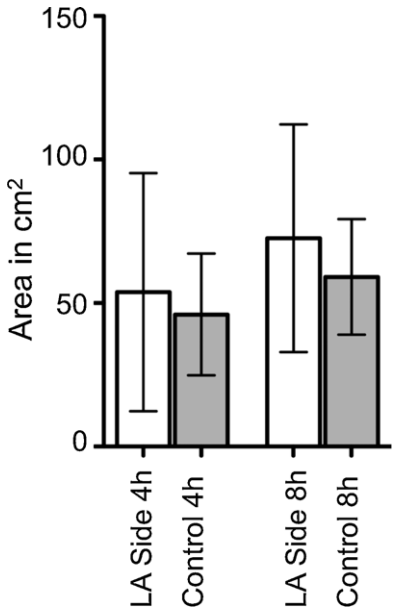

Fig. 2. Compares areas of pinprick hyperalgesia on the side of intradermal local anaesthetic (LA) block and the contralateral irradiated thigh (control) at 4 and 8 h ($p = 0.24$). Standard deviations are provided for each column. The size of the area is illustrated on the *y*-axis.

and average weight was 71 ± 13 kg. Mean body mass index was 22.8 ± 1.9 kg/m^2. All 12 subjects completed the trial. Prior to commencing irradiation, the effectiveness of the anaesthetic block was verified in each subject.

3.2. Primary endpoint

All volunteers developed an area of pinprick hyperalgesia in the area surrounding the sunburn on both legs after 8 h. No significant difference was detected between the side with the anaesthetic block and the control side ($p = 0.24$, 72.6 ± 39.7 cm^2 (±SD) and 59.1 ± 20.1 cm^2, respectively). For details see Fig. 2.

3.3. Secondary endpoints

3.3.1. Size of spokes

The mean of the three diameters crossing the anaesthetic strip (spokes 4, 5, 6) was 53 ± 23 mm (mean ± SD) on the blocked side and 49 ± 17 mm on the non-blocked side at 8 h. At 4 h, the mean of the three diameters crossing the anaesthetic strip (spokes 4, 5, 6) was 45 ± 26 mm (mean ± SD) on the blocked side and 44 ± 16 mm on the non-blocked side. *p*-value 0.24.

The mean of the three diameters crossing the anaesthetic strip (spokes 4, 5, 6) was 53 ± 23 mm (mean ± SD) and 50 ± 17 mm for the mirror 3 diameters on the same leg (spokes 8, 1, 2) at 8 h after irradiation, while the mean of the three diameters crossing the anaesthetic strip was 45 ± 26 mm (mean ± SD) and 40 ± 16 mm for the mirror 3 diameters on the same leg at 4 h after irradiation. *p*-value 0.09. Dimensions of each of the eight spokes at the time point 8 and 4 h after irradiations are stated in Table 1. No statistically significant difference was found for any spoke comparing intervention with control side.

3.3.2. Area of hyperalgesia to pinprick after 4 h

No significant difference was found after 4 h between the area of pinprick hyperalgesia treated with the lidocaine block and its control ($p = 0.24$, 53.8 ± 41.5 cm^2 and 46.0 ± 21.2 cm^2, respectively).

3.3.3. Mechanical stimuli

Comparison of the data collected during the mechanical S/R function tests showed no statistically significant difference between the measurements taken distally from the lidocaine block compared to the corresponding proximal site. The mean values of the pain ratings on the NRS Scale at 8 h were: 2.4 ± 2.5 proximal site vs. 2.2 ± 2.5 at the distal site ($p = 0.85$).

The mean values of the pain ratings on the NRS Scale at baseline proximal: 1.6 ± 2.0 vs. 2.4 ± 2.5 at 8 h proximal site ($p = 0.07$). The mean values at baseline distal were 1.5 ± 2.3 vs. 2.2 ± 2.5 at 8 h distal site ($p = 0.18$). The mean values of the pain ratings at 8 h primary hyperalgesia site were 4.0 ± 3.1 vs. proximal site 2.4 ± 2.5 ($p = 0.0001$), and vs. distal site 2.2 ± 2.5 ($p = 0.0002$).

3.3.4. Area of allodynia

As for the hyperalgesic areas elicited by pinpricking, the extent of the allodynic areas did not differ significantly between the treatment and control sides ($p = 0.73$). At 4 h, areas were 14.0 ± 2.5 cm^2 for the lidocaine perfusion side and 16.5 ± 8.1 cm^2 for the control, and at 8 h areas were 16.0 ± 6.5 cm^2 for the lidocaine perfusion side and 15.5 ± 4.0 cm^2 for the control.

3.3.5. Measurements within the sunburn

Within the sunburn thermal perception, thermal pain and pressure pain thresholds as well as blood flow were not affected by the lidocaine perfusion. Data for WPT, HPPT, CPT, PPT, and FLUX are provided in Table 2.

4. Discussion

4.1. Mechanical hyperalgesia in the UV-B model

The major finding of this study is that a superficial intradermal local anaesthetic block in the immediate proximity distal to the area of primary hyperalgesia of a circular sunburn did not reduce the development of a large area of pinprick hyperalgesia in non-inflamed skin surrounding the sunburn 8 h after irradiation compared to a control sunburn. Additionally, pinprick hyperalgesia was unchanged by the anaesthetic block. These data support the

Table 1
Demonstrate the lengths of eight radial spokes from the edge of the hyperalgetic area to the centre of the sunburn 8 and 4 h after irradiation, on the intervention side (LA: local anaesthesia, i.e. strip side) vs. control side. Spoke number 1 runs in a cranio-caudal axis from the outside to the centre of the sunburn, representing the 12 o'clock position. Spokes 2–8 are positioned in a clockwise direction at a 45° angle to the previous spoke. Spokes crossing the anaesthetic strip are marked in italics.

	Spoke 1	Spoke 2	Spoke 3	*Spoke 4*	*Spoke 5*	*Spoke 6*	Spoke 7	Spoke 8
8 h LA side mean ± SD (mm)	57 ± 15	45 ± 19	42 ± 12	*50 ± 27*	*60 ± 23*	*49 ± 19*	43 ± 17	47 ± 21
8 h control side mean ± SD (mm)	50 ± 16	43 ± 13	39 ± 14	*45 ± 12*	*58 ± 15*	*44 ± 21*	44 ± 14	38 ± 16
p-value	0.1	0.72	0.51	*0.59*	*0.75*	*0.52*	0.83	0.1
	Spoke 1	Spoke 2	Spoke 3	*Spoke 4*	*Spoke 5*	*Spoke 6*	Spoke 7	Spoke 8
4 h LA side mean (mm)	42 ± 19	34 ± 19	37 ± 15	*36 ± 24*	*55 ± 26*	*44 ± 25*	36 ± 16	46 ± 22
4 h control side mean (mm)	38 ± 15	37 ± 17	33 ± 12	*38 ± 13*	*54 ± 17*	*40 ± 16*	36 ± 18	37 ± 14
p-value	0.52	0.69	0.36	*0.82*	*0.93*	*0.54*	0.99	0.16

Table 2
Demonstrates the warmth perception threshold (WPT), heat pain perception threshold (HPPT), cold pain threshold (CPT), and pressure pain threshold (PPT), as well as the Flux 4 and 8 h after irradiation, comparing the measurements in the field of primary hyperalgesia from intervention side (LA) to control. Bonferroni post hoc analysis calculated for FLUX LA 4 h vs. 8 h: $p = 0.007$; control 4 h vs. 8 h: $p = 0.009$. No statistically significant difference was found between LA and control side at 4 h ($p = 0.75$) or 8 h ($p = 1.00$).

	LA	Control	*p*-value
WPT 4 h	37.3 ± 1.7 °C	36.5 ± 1.5 °C	0.5
WPT 8 h	37.1 ± 1.5 °C	36.7 ± 1.2 °C	
HPPT 4 h	41.3 ± 2.5 °C	40.6 ± 2.7 °C	0.61
HPPT 8 h	40.9 ± 2.3 °C	40.0 ± 2.1 °C	
CPT 4 h	12.9 ± 10.7 °C	14.4 ± 9.4 °C	0.97
CPT 8 h	13.9 ± 9.4 °C	14.7 ± 8.7 °C	
PPT 4 h	378.2 ± 78.0 kPa	425.6 ± 132.1 kPa	0.64
PPT 8 h	365.4 ± 104.2 kPa	381.0 ± 119.8 kPa	
FLUX 4 h	65.4 ± 17.6	60.4 ± 22.0	0.002
FLUX 8 h	82.6 ± 10.2	80.2 ± 9.9	

hypothesis that mechanical hyperalgesia in the area around the sunburn is not mediated by only a peripheral mechanism. These results are in line with results collected from other pain models demonstrating central hypersensitivity [4,8].

Development of thermal and mechanical hyperalgesia within the inflamed area is in line with other human and animal data [2–4,9]. Our data on mechanical hyperalgesia in the non-inflamed skin surrounding the UV-B induced inflammation concur with results from previous sunburn studies [2,10,11]. The size of the area of pinprick hyperalgesia found was relatively large compared to the sunburn spot. The area of allodynia was smaller compared to the area of pinprick hyperalgesia. This is a pattern also found in other pain models of secondary hyperalgesia and a further hint for two different mechanisms of hyperalgesia [12–15].

Behavioural tests in rats showed no change in mechanical thresholds, i.e. an absence of mechanical hyperesthesia in the area adjacent to the location of UV-B irradiation [5]. In our study we did not test mechanical detection threshold but hyperalgesia. Another explanation of this difference between the rat and the human model may be spatial summation of afferent input since the rat paw covers several dermatomes compared to one large dermatome at the thigh in human subjects.

4.2. Mechanical hyperalgesia in other models of pain

The central origin of mechanical hyperalgesia has been demonstrated in several pain models as intradermal application of capsaicin, electrical stimulation and thermal injuries [16–19]. Anaesthetic strips as used in our study demonstrated the central origin of mechanical hyperalgesia in the intradermal electrical pain model [4].

4.3. Trigger of secondary hyperalgesia

Since the role of peripheral sensitisation has recently been emphasised and changes in nociceptor coding properties have been suggested as an explanation for mechanical hyperalgesia, we aimed to block the peripheral nociceptors spreading distally from the inflamed skin, which may mediate peripheral sensitisation into the non-inflamed skin [3]. Our data demonstrate clearly that this block does not affect the development of neither mechanical hyperalgesia nor allodynia. This counts for both the size of the area of mechanical hyperalgesia and allodynia and the mechanical S/R function on either side of the local anaesthetic strip.

Another hypothesis is that hyperalgesia is modulated by pro-inflammatory cytokines and chemokines such as interleukins and TNF-α [20–22]. For the sunburn model, this release of pro-inflammatory substances has also been demonstrated in humans [23–25]. However, for the non-irradiated area adjacent to the sunburn this neuropeptide modulated hypersensitivity has up until now not been demonstrated.

As for other pain models our data support the assumption that in the UV-B model an increase of spontaneous nociceptor activity in inflamed skin may enhance responsiveness of these mechanosensitive nociceptors in a larger receptive field beyond the inflamed skin via the central nervous system [4,26]. Nociceptive afferent input and increase of spontaneous activity are regarded the trigger for the development of secondary hyperalgesia. The size of the sunburn may therefore play a crucial role. This also may explain inconsistency in the proof or respective lack of proof of mechanical hyperalgesia adjacent to irradiated tissue in the sunburn model [5,9,27]. While our study group has applied relatively large sunburn areas (between 19.6 cm^2 and 13.9 cm^2), other groups have worked with significantly smaller and partially differently shaped areas [2,5,27].

Various mechanisms may contribute to secondary hyperalgesia in the UV-B model. Previous trials demonstrated that NSAIDs, Cox-2-selective inhibitors and opioids can be targeted to reduce secondary hyperalgesia in the UV-B-model [10,11,28]. Gabapentin, a typical centrally acting anti-hyperalgesic drug, did have a hypoalgesic effect in the model of temporal pain summation [29]. Nevertheless, it did not change secondary hyperalgesia in the UV-B induced inflammatory skin pain model at a single dose of 600 mg [11]. N-methyl d-aspartate (NMDA)-receptor antagonists have not been studied in humans in this model, although the NMDA-receptor may be involved as it is in other models [19].

4.4. Limitations

It is the advantage of the use of intradermal microdialysis fibres to maintain anaesthesia reliably over a prolonged period, compared to multiple small injections of local anaesthetic [12,17,30]. We ascertained that anaesthesia was functional before applying irradiation, to exclude any interference with the developing inflammation. This excludes any breakthrough of afferent nociceptive input during our study. However, our data are not suitable to exclude, that the application of the microdialysis fibres, although pain-free during transient cryoanaesthesia, may have induced peripheral sensitisation.

Within the area of sunburn neither thermal perception, thermal pain, pressure pain thresholds nor blood flow were affected by the anaesthetic strip, indicating a well-defined small area of local anaesthesia with no relevant dispersion. Systemic effects cannot be excluded in our study. However, compared to intravenous doses of about 200 mg lidocaine delivered to a 70 kg subject, the systemic impact of intradermal application of 6 mg/h lidocaine in our study can be regarded as minimal [31].

We did not study the maximum hyperalgesia in the UV-B model for practical reasons, which peaks at 24 h. However, for the testing of our hypothesis, the extent of mechanical hyperalgesia was large and sufficient. This was a single-blinded study and we cannot exclude investigator bias. However, all parameters are consistent at all time points and measure sites and confirm the respective results.

4.5. Conclusion

In conclusion, this trial provides evidence for the central origin of secondary mechanical hyperalgesia in the UV-B sunburn pain model. Continuous peripheral afferent blockade with local anaesthetic did not alter the development of mechanical hyperalgesia at 8 h after UV-B irradiation. This confirms previous findings in other pain models and provides further evidence that the UV-B model

offers secondary hyperalgesia in addition to its known primary hyperalgesia.

Conflict of interest statement

There are no possible conflicts of interests by any of the authors.

Acknowledgements

The authors' work was supported by Glaxo-Smith-Kline (UK) and in part by a competitive research grant of the Wiener Wissenschafts-, Forschungs- und Technologiefonds (Vienna Science and Technology Fund, LS07-040). Furthermore, the authors thank Dr. Ulrich Thaler for his support in the development of this manuscript.

References

[1] Modir JG, Wallace MS. Human experimental pain models 1: the ultraviolet light UV-B pain model. Methods Mol Biol 2010;617:159–64.
[2] Gustorff B, Anzenhofer S, Sycha T, Lehr S, Kress HG. The sunburn pain model: the stability of primary and secondary hyperalgesia over 10 hours in a crossover setting. Anesth Analg 2004;98:173–7.
[3] Bishop T, Ballard A, Holmes H, Young AR, McMahon SB. Ultraviolet-B induced inflammation of human skin: characterisation and comparison with traditional models of hyperalgesia. Eur J Pain 2009;13:524–32.
[4] Klede M, Handwerker HO, Schmelz M. Central origin of secondary mechanical hyperalgesia. J Neurophysiol 2003;90:353–9.
[5] Bishop T, Marchand F, Young AR, Lewin GR, McMahon SB. Ultraviolet-B-induced mechanical hyperalgesia: a role for peripheral sensitisation. Pain 2010;150:141–52.
[6] Rolke R, Magerl W, Campbell KA, Schalber C, Caspari S, Birklein F, Treede RD. Quantitative sensory testing: a comprehensive protocol for clinical trials. Eur J Pain 2006;10:77–88.
[7] Rolke R, Baron R, Maier C, Tölle TR, Treede RD, Beyer A, Binder A, Birbaumer N, Birklein F, Bötefür IC, Braune S, Flor H, Huge V, Klug R, Landwehrmeyer GB, Magerl W, Maihöfner C, Rolko C, Schaub C, Scherens A, Sprenger T, Valet M, Wasserka B. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. Pain 2006;123:231–43.
[8] Koltzenburg M. Neural mechanisms of cutaneous nociceptive pain. Clin J Pain 2000;16:131–8.
[9] Benrath J, Gillardon F, Zimmermann M. Differential time courses of skin blood flow and hyperalgesia in the human sunburn reaction following ultraviolet irradiation of the skin. Eur J Pain 2001;5:155–67.
[10] Sycha T, Gustorff B, Lehr S, Tanew A, Eichler HG, Schmetterer L. A simple pain model for the evaluation of analgesic effects of NSAIDs in healthy subjects. Br J Clin Pharmacol 2003;56:165–72.
[11] Gustorff B, Hoechtl K, Sycha T, Felouzis E, Lehr S, Kress HG. The effects of remifentanil and gabapentin on hyperalgesia in a new extended inflammatory skin pain model in healthy volunteers. Anesth Analg 2004;98:401–7.
[12] LaMotte RH, Lundberg LE, Torebjork HE. Pain, hyperalgesia and activity in nociceptive C units in humans after intradermal injection of capsaicin. J Physiol 1992;448:749–64.
[13] Kilo S, Schmelz M, Koltzenburg M, Handwerker HO. Different patterns of hyperalgesia induced by experimental inflammation in human skin. Brain 1994;117:385–96.
[14] Liu M, Max MB, Robinovitz E, Gracely RH, Bennett GJ. The human capsaicin model of allodynia and hyperalgesia: sources of variability and methods for reduction. J Pain Symptom Manage 1998;16:10–20.
[15] Lang S, Klein T, Magerl W, Treede RD. Modality-specific sensory changes in humans after the induction of long-term potentiation (LTP) in cutaneous nociceptive pathways. Pain 2007;128:254–63.
[16] Pedersen JL, Kehlet H. Hyperalgesia in a human model of acute inflammatory pain: a methodological study. Pain 1998;74:139–51.
[17] Serra J, Campero M, Ochoa J. Flare and hyperalgesia after intradermal capsaicin injection in human skin. J Neurophysiol 1998;80:2801–10.
[18] Koppert W, Ostermeier N, Sittl R, Weidner C, Schmelz M. Low-dose lidocaine reduces secondary hyperalgesia by a central mode of action. Pain 2000;85:217–24.
[19] Koppert W, Dern SK, Sittl R, Albrecht S, Schuttler J, Schmelz M. A new model of electrically evoked pain and hyperalgesia in human skin: the effects of intravenous alfentanil, S(+)-ketamine, and lidocaine. Anesthesiology 2001;95:395–402.
[20] Cunha FQ, Poole S, Lorenzetti BB, Ferreira SH. The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. Br J Pharmacol 1992;107:660–4.
[21] Saade NE, Nasr IW, Massaad CA, Safieh-Garabedian B, Jabbur SJ, Kanaan SA. Modulation of ultraviolet-induced hyperalgesia and cytokine upregulation by interleukins 10 and 13. Br J Pharmacol 2000;131:1317–24.
[22] Lindenlaub T, Sommer C. Cytokines in sural nerve biopsies from inflammatory and non-inflammatory neuropathies. Acta Neuropathol 2003;105:593–602.
[23] Strickland I, Rhodes LE, Flanagan BF, Friedmann PS. TNF-alpha and IL-8 are upregulated in the epidermis of normal human skin after UVB exposure: correlation with neutrophil accumulation and E-selectin expression. J Invest Dermatol 1997;108:763–8.
[24] Feldmeyer L, Keller M, Niklaus G, Hohl D, Werner S, Beer HD. The inflammasome mediates UVB-induced activation and secretion of interleukin-1beta by keratinocytes. Curr Biol 2007;17:1140–5.
[25] Angst MS, Clark JD, Carvalho B, Tingle M, Schmelz M, Yeomans DC. Cytokine profile in human skin in response to experimental inflammation, noxious stimulation, and administration of a COX-inhibitor: a microdialysis study. Pain 2008;139:15–27.
[26] Cervero F, Gilbert R, Hammond RGE, Tanner J. Development of secondary hyperalgesia following nonpainful thermal stimulation of the skin a psychophysical study in man. Pain 1993;54:181–9.
[27] Harrison GI, Young AR, McMahon SB. Ultraviolet radiation-induced inflammation as a model for cutaneous hyperalgesia. J Invest Dermatol 2004;122:183–9.
[28] Sycha T, Anzenhofer S, Lehr S, Schmetterer L, Chizh B, Eichler HG, Gustorff B. Rofecoxib attenuates both primary and secondary inflammatory hyperalgesia: a randomized, double blinded, placebo controlled crossover trial in the UV-B pain model. Pain 2005;113:316–22.
[29] Enggaard TP, Mikkelsen SS, Zwisler ST, Klitgaard NA, Sindrup SH. The effects of gabapentin in human experimental pain models. Scand J Pain 2010;1:143–8.
[30] Weidner C, Klede M, Rukwied R, Lischetzki G, Neisius U, Skov PS, Petersen LJ, Schmelz M. Acute effects of substance P and calcitonin gene-related peptide in human skin—a microdialysis study. J Invest Dermatol 2000;115:1015–20.
[31] Kawamata M, Takahashi T, Kozuka Y, Nawa Y, Nishikawa K, Narimatsu E, Watanabe H, Namiki A. Experimental incision-induced pain in human skin: effects of systemic lidocaine on flare formation and hyperalgesia. Pain 2002;100:77–89.