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Pain sensitivity and experimentally induced sensitisation in red haired females

Trine Andresen[a,b], Dagmar Lunden[b], Asbjørn M. Drewes[a,b], Lars Arendt-Nielsen[a,*]

[a] Center for Sensory-Motor Interaction (SMI), Department of Health Science and Technology, Frederik Bajers Vej 7, Aalborg University, 9220 Aalborg, Denmark
[b] Mech-Sense, Department of Gastroenterology, Aalborg Hospital, Denmark

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ABSTRACT

Introduction and aim: Pain sensitivity has been linked to the melanocortin-1 receptor (MC1R) gene. A mutation in MC1R can result in pale skin and red hair in humans and may modulate pain responses in general. Human studies have shown that women with non-functional MC1R's were sensitive to experimental induced cold and heat pain. A study demonstrated that females with red hair required higher dose of anesthesia than females with dark hair to experience analgesia to electrical stimulation. Moreover, women expressing non-functional MC1Rs display greater analgesia from opioid analgesia. If redheads in general respond differently to pain and analgesics, this is of clinical importance. The aim of this study was therefore to investigate pain sensitivity and experimentally induced sensitisation in red haired females.
Method: Twenty healthy females with pale skin and red hair (mean age 32 years, range 20–55) and 20 healthy females with blond/dark hair (mean age 31 years, range 20–51) participated in this study. The pain tolerance thresholds to heat and pressure stimulation were determined. Hyperalgesia was induced experimentally by applying 0.075% topical capsaicin cream for 30 min. The secondary pin-prick hyperalgesic area was estimated with a calibrated filament (von Frey hair, 15 g) and the area of allodynia by a soft brush. This was done 0, 30, 60, and 90 min after cream removal.
Results: Neither heat nor pressure pain tolerance thresholds were changed in the two groups. The secondary pin-prick hyperalgesic areas were significantly smaller for red haired females than blond/dark haired females ($P = 0.014$). There were no significant differences in the allodynic areas.
Discussion: As the secondary hyperalgesic response evoked by topical capsaicin is a central phenomenon, the observed smaller pin-prick hyperalgesic area in the red haired females could indicate a central role of MCRs in development or maintenance of hyperalgesia. Central involvement of MC1Rs or dysfunction of peripheral MC1Rs activating central MC4Rs has been suggested to influence pain sensitivity. The difference observed between red haired and non-red haired females may have implications for pain management regimens as compounds interacting with sensitisation such as NMDA-antagonists or alpha-2-delta-ligands may exert different types of action in people with MC1R mutation.
Conclusion: The present study showed that red haired females were less sensitive to topical capsaicin induced pin-prick hyperalgesia compared with blond/dark haired females.
Implications: The smaller hyperalgesic area in redheads could be a manifestation of central anti-hyperalgesic involvement of MCRs and could have an influence on the treatment of pain as well as in studies investigating anti-hyperalgesic drugs.

1. Introduction

Part of the variability in pain sensitivity has been associated with genetic polymorphisms. Pain sensitivity has been linked to e.g. the melanocortin-1 receptor gene (MC1R) [1]. Red hair and pale skin are nearly always a result of a MC1R mutation. The red hair phenotype results from an excess pheomelanin (yellow-red) production due to dysfunctional MC1Rs. In contrast, when a normal MC1R is expressed, the predominant pigment produced is eumelanin (dark brown) resulting in a high eumelanin to pheomelanin ratio [2].

Studies in animals indicate that dysfunction in MC1Rs affects the pain perception and response to particular analgesics e.g. greater analgesia from the κ-opioid agonist pentazocine [3,4]. Studies with mice with non-functional MC1Rs demonstrated reduced sensitivity to different noxious stimuli and better analgesia to morphine-6-glucuronide [4]. In addition, human studies have shown that subjects with non-functional MC1Rs could tolerate higher electrical stimulation [4] and were more sensitive to cold and heat pain than subjects with functional MC1Rs [2]. Females with non-functional MC1R (red haired) have also been proven to display

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* Corresponding author. Tel.: +45 9940 8830; fax: +45 9815 4008.
E-mail addresses: LAN@HST.AAU.DK, susanne@hst.aau.dk (L. Arendt-Nielsen).

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greater analgesia from the κ-opioid agonist, pentazocine than a blond control group [3]. In another study females with red hair required a higher dose of the anesthetic desflurane than females with dark hair to experience analgesia to strong electrical stimulation [5]. These studies suggest that MC1R gene variants could possibly modulate pain responses in general. If redheads in general respond differently to pain and analgesics, this is of clinical importance.

The aims of the study were to evaluate: (1) pain sensitivity to different phasic experimental stimulus modalities, and (2) capsaicin induced hyperalgesia in females with red hair and blond/dark hair.

2. Materials and methods

2.1. Study design and subjects

The study included 40 healthy females, who were divided into two matched groups with either red hair (mean age 32, range 20–55) or non-red hair (blond/dark) (mean age 31, range 20–51). All subjects gave their informed consent according to the Declaration of Helsinki. The study was approved by the local Ethical Committee (N-20080013). Red hair was defined as subjects having fair skin color, freckles, and hair color between burnt orange to bright copper. In addition, they should be sensitive to ultraviolet irradiation.

All females were tested from 6th to 14th day of their menstrual cycles if they were not in their menopause. None of the subjects had chronic pain conditions or skin lesions at test sites. None were pregnant, and none took pain medication. Furthermore, the volunteers were not allowed to take any analgesics 24 h prior to study day.

Power calculation suggested that 14 subjects would provide significant difference in pain tolerance threshold (data from heat stimulation from previous study performed in this group). α was 0.05, and the power of the statistical analysis was set to 0.95. Therefore, 20 volunteers with red hair and 20 with non-red hair were enrolled.

2.2. Pressure stimulation

Pressure stimulation was performed with a handheld algometer (Type 2, Somedic production AB, Sweden) with probe size 1 cm^2 in diameter, 30 kPa/s force increase and applied to the left arm 10 cm distal to the elbow on the lateral site of the deep flexor muscle. The subjects were instructed to press a button when they reached the pain tolerance threshold (PTT), and the stimulation stopped.

2.3. Heat stimulation

Ten cm proximal from the wrist on the right volar forearm, an area (9 cm^2) of the skin was heated with a computerized 'Thermo Tester' (TSA II NeuroSensory analyzer, Medoc Ltd., Ramat Yishai, Israel). The temperature increased from a baseline of 32 °C to a maximum of 52 °C with a rate of 1 °C/s. The subjects were told to press a button when the heat tolerance threshold was reached. Three successive stimulations were performed, and between each of the stimulations the temperature returned to baseline. The average of the three stimulations was computed and used for the data analysis.

2.4. Topical capsaicin induced hyperalgesia

Ten cm proximal to the left hand wrist over the mid volar forearm, an area (9 cm^2) of the skin was heated with the computerized 'Thermo Tester' for 5 min at 45 °C. The area was marked with a pen, and capsaicin cream (0.075% capsaicin cream, Aalborg Hospital Pharmacy, Denmark) was applied for 30 min within this area. The application site was defined as the primary hyperalgesic area, and the area surrounding the application area was defined as the secondary pin-prick hyperalgesic area. Immediately after removing the capsaicin cream, the secondary hyperalgesic area and the allodynic were assessed (denoted $t = 0$).

The secondary pin-prick hyperalgesic area was assessed with a calibrated von Frey filament (Touch Test Sensory Evaluator Kit, von Frey 15 g, Stoelting Europe, Dublin, Ireland), and the allodynic area was assessed with a soft brush. The stimulation with the von Frey filament and brush started in normal skin distant away from the primary area and continued towards the centre of the application site until the subjects reported a clear change in sensation. This was performed in eight radial directions. The borders from normal to sensitized skin were marked with a pen and drawn on a transparency film, and the areas were then calculated with a specialized computer program (Trust, 1200 wireless tablet, Trust International BV, Dordrecht, The Netherlands).

Thirty minutes after removing the cream, rekindling (5 min at 40 °C) of the primary area was performed with the computerized thermostat. Immediately after, the secondary area was again quantified the same way as before (denoted $t = 30$). This was repeated 60 min (denoted $t = 60$) and 90 min (denoted $t = 90$) after removal of the cream [6].

2.5. Statistics

The software package SigmaPlot 11.0 (Systat software Inc., San Jose, CA, USA) was used for the statistical analysis. Two-sided unpaired *t*-test was used to test for significant differences between females with red hair and females with non-red hair for pressure and heat stimulation. If the data were not normalised, a Mann–Whitney rank-sum test was used. Two-way analysis of variance was used to test for the overall effect over time for the capsaicin model with the factors: (1) time, and (2) hair color. A *P*-value ≤ 0.05 was considered as significant.

For measurements of the intra-individual variance, the intra-correlation coefficient (ICC) was calculated. ICC evaluates each subject's ability to reproduce the response between repeated measurements. An ICC > 0.6 was considered as an indicator of good reproducibility of the pain model used. The overall inter individual variance was calculated with the coefficient of variance (CV) expressed in %. The CV reflected the overall variability of the pain model.

3. Results

There were no significant differences in height, weight, and age between the red-haired and the non-red haired females (all $P > 0.5$).

3.1. Pressure and heat stimulation

Neither pressure pain nor heat pain tolerance thresholds were different in the two groups (pressure: $P = 0.8$; heat: $P = 1.0$) (Table 1).

Table 1
Representation of the results for pressure and heat stimulation, no significant difference was observed between red haired and non-red haired women.

	PTT (kPa/s)	HTT (°C)
Red hair	713.6 ± 403.8	47.4 ± 2.8
Non-red hair	673.5 ± 226.1	47.8 ± 1.7

Data are shown as mean ± S.D. *Abbreviations*: PTT = pressure tolerance threshold; HTT = heat tolerance threshold.

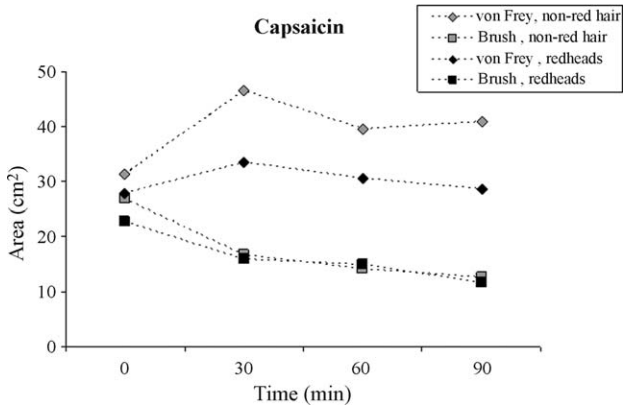

Fig. 1. The mean of the hyperalgesic/allodynic areas at each time point for the capsaicin test. Gray ◊ represents the secondary pin-prick hyperalgesic area assessed with von Frey, and gray □ represents the allodynic area assessed with brush for blond/dark haired females. Black symbols represent data from red haired females.

3.2. Topical capsaicin induced hyperalgesia

The pin-prick hyperalgesic area was significantly smaller in the red haired females ($P = 0.014$) compared with blond/dark haired females. The areas of allodynia did not change in red haired and non-red haired females at any time ($P > 0.5$) (Fig. 1). The subjects' abilities to estimate hyperalgesic and allodynic areas for the repeated measurements performed at 30, 60, and 90 min were reproducible (hyperalgesic area: ICC > 0.7; allodynic area: ICC > 0.7). The overall variability (CV) for the pin-prick hyperalgesic area was <10% and <16% for the allodynic area. These results reflect that the topical capsaicin model is valid and reproducible over the 90 min.

4. Discussion

The present study showed that females with red hair (usually associated with melanocortin-1 receptor polymorphisms) developed smaller pin-prick hyperalgesic areas after topical capsaicin compared with blond/dark haired females. The two groups showed similar pain sensitivity to high intensity heat and pressure pain stimulation.

4.1. Pain sensitivity

MC1R expression has been identified in human pituitary tissue, glia cells, and in cells of the human periaqueductal gray matter. Current available information does not seem to provide the basis for evaluating which specific mechanism may influence the pain sensitivity due to MC1R mutations. The central nervous system is not a major site of MC1R expression. Nevertheless, central involvement of MC1Rs or dysfunction of peripheral MC1Rs activates central MC4Rs which has been suggested to influence pain sensitivity [2,7].

It is well established that sensitivity to one type of pain does not correlate with sensitivity to another type of pain as the underlying mechanisms differ between pain modalities [8,9]. In the present study the pain responses to superficial (heat) and deep somatic (pressure) tissue stimulation were investigated and no differences were found between the two groups. In the study by Liem et al. redheads were found more sensitive to cutaneous heat pain stimulation than non-red haired females [2]. The different results in the two studies could be due to different temperature rates (0.5 °C/s versus 1 °C/s) used in the two studies. This could at least partly be explained by the differentiated nociceptor responses to skin heatings with different rates of temperature increase [10]. Low rates (>0.9 °C/s) of skin heating are mainly activating C-fibers whereas high rates (<0.5 °C/s) are predominantly activating Aδ-fibers [11].

The secondary hyperalgesic response evoked by topical capsaicin is a central phenomenon, and hence it could be hypothesised that centrally expressed MCRs might play a role in the manifestation of central pain modulation [4,12]. The subtype MC4R is known to be involved in the modulation of hyperalgesia and pain and MC1R to have an anti-inflammatory effect via the attenuation of pro-inflammatory cytokine production [13]. Therefore, a mutation in the MCRs could possibly lead to a difference in the expression of experimentally induced hyperalgesia and inflammatory reactions. The observed smaller secondary pin-prick hyperalgesic area in the red haired females could indicate the central role of MCRs in development or maintenance of hyperalgesia. The difference observed between red haired and non-red haired females may have implications for pain management regimens as compounds interacting with sensitisation such as NMDA-antagonists or alpha-2-delta-ligands may exert different types of action in people with MC1R mutation.

4.2. Methodology

Mutation of MC1R almost exclusively leads to red hair but Mogil et al. showed that subjects who where red haired and who had only one variant allele did possess functional MC1Rs. This was interpreted as the hair color could be due to other genes than the MC1R [4]. A limitation of the present study was that no genotyping analysis was performed to confirm that the redheads were carriers of two variant MC1R alleles. However, Liem et al. performed a study comparing redheads with dark haired volunteers, in which they based their division on phenotype and were able to show a difference in pain perception between the two groups [2].

5. Conclusion

The present study showed that red haired females were less sensitive to capsaicin induced cutaneous pin-prick hyperalgesia compared with blond/dark haired females.

6. Implications

The clinical implications of these experimental findings are difficult to extrapolate, but they may be important for efficacy of the management regimens applied in red haired versus blond/dark haired females with chronic pain with sensitisation.

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