The ultraviolet (UV) irradiation Induced Heat Hyperalgesia rat biomarker model for Pain Research

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The exposure of the skin to Ultraviolet (UV) irradiation results in a classical inflammatory reaction characterised by both erythema (flare) and hypersensitivity to noxious and non noxious stimuli (i.e. hyperalgesia and allodynia). No spontaneous pain is associated to this injury [1,2].

Cutaneous UV irradiation causes DNA damage, activates MAP kinase in keratinocytes, which leads to increased levels of pro-inflammatory cytokines, such as TNFα and IL-8, and up regulation of COX-2 [3]. Pain sensitivity is increased after thermal stimulus in the area of erythema (primary hypersensitivity).

This inflammatory reaction has been studied in both humans and rodents and represents a potential translatable pharmacology biomarker model for the assessment of efficacy of new analgesics [1, 4-6].

Methods

Animals- All procedures were carried out under the Animals (Scientific Procedures) Act, 1986. Male Sprague Dawley rats (200-300g, Charles River, UK) were housed in groups of 4 under a 12 hour light/dark cycle with food and water *ad libitum*. All experiments were carried out by an investigator blind to drug treatments.

UV Radiation Exposure

Animals were anaesthetized with a 2% isoflorane 0_2 mixture. Anesthesia was maintained via a nose cone while the plantar surface of the right paw was irradiated with 100, 200, 300 or 400mJ/cm^2 of UV irradiation. This exposure was generated by Saalmann CupCube system (Saalmann GmbH, Germany; λ=280-400 nm). The source of irradiation was adapted to encompass the plantar surface of the rats hind paw by using a shaped delivery collar (either 8mm diameter or 8x12mm oval), attached to the UV emitter (Pfizer Facilities Management and Engineering Team). The intensity of UV emitted was calibrated prior to exposure using an ABLE 1400A radiometer with SEL005/WBS320/TD filter (ABLE Instruments and Controls Ltd).

Thermal hyperalgesia

Heat hypersensitivity was assessed using the rat plantar test equipment (Ugo Basile, Italy) following a modified method as described by Hargreaves et al (1988). The plantar test consisted of three Perspex boxes (22x19x25cm) on an elevated glass table. Two rats were housed in each box, so that 6 rats could be tested simultaneously in a single apparatus, and left to acclimatize for at least 20 mins. A mobile infrared heat source was applied to the plantar surface of the hind paws. The paw withdrawal latency (PWL) was defined as the time (in seconds) taken by the rat to remove its hind paw from the heat source. The heat source was calibrated to give a response of 8-12 sec on untreated animals (105-110mW/cm^2). An automatic cut off point of 20 sec was applied to prevent tissue damage. Uninjured paw was always assessed first. Five recordings (1-2 mins intervals between recordings) from each paw, were taken during baseline recordings and expressed as a normalised mean (highest and lowest value removed). In the pharmacology test session, three recordings were collected from each paw, the average of which represents PWL for each rat.

Statistical analysis

Data are expressed as mean of 6-8 rats per group. Each treatment group was compared at each time point to vehicle-treated group using a one way ANOVA followed by a Dunnets t test, blocked for each day of treatment.

Results

The time course of development of thermal hyperalgesia was evaluated as well as the activity of a number of standard agents seen effective in chronic pain diseases.

A cohort of Sprague Dawley rats was exposed to various intensities of UV irradiation (100-400 mJ/cm^2; n=6-9 rats/group) and thermal hyperalgesia was assessed every day up to 7 days post UV application.

The development of hypersensitivity showed a significant reduction in thermal PWL from 24hrs post UV in all groups. The peak of hyperalgesia was observed at 48 hours, maintained up to 96 hours but fully resolved at 7day post the induction of erythema. Within the range of UV exposures used, 300 mJ/cm2 appear to produce the highest degree of hypersensitivity without causing tissue damages. Dry skin was indeed observed with higher intensity. No vesicles or severe skin damage was seen at any of the exposures used.

The efficacy of various standard analgesic therapies was assessed. The non selective, COX inibitor, Ibuprofen (100 and 300 mg/kg PO), the COX2 inhibitor, Valdecoxib (30mg/kg, PO), the μ-opioid agonist, oxycodone (0.3-1 mg/kg, SC) and the non selective sodium channel blocker, Mexiletine(10-30 mg/kg, SC) significantly reversed hyperalgesia at various time points post drug administration and data were successfully reproduced by two investigators in double blind study design.

Conclusion

We have demonstrated that post UV skin exposure, rats develop thermal hyperalgesia localized at the area of erythema. This hypersensitivity can be pharmacologically reversed by standard compounds seen therapeutic in inflammatory (Ibuprofen), chronic nociceptive (Oxycodone and Valdecoxib) and neuropathic pain condition (Oxycodone and Mexiletine). Most of this mechanisms have been able to reverse heat hypersensitivity in the human UV model [4,7; internal data) so that it has been proposed this model could be a useful translatable pharmacology biomarker for Pain research.

Further analysis is on going to explore whether UV model could also be a suitable model to escalate efficacious doses from rat to man.

References

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