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Neurosensory changes in a human model of endothelin-1 induced pain: A behavioral study

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Abstract

Although pain is a frequent feature in patients with cancer, its etiology is still poorly understood. In recent years, endothelin-1 (ET-1) has become a major target molecule in the etiology of cancer pain. In this randomised, double-blind study the effects of intradermal injection of ET-1 on spontaneous pain, temperature perception and sensation of punctate stimulation were evaluated. Thirty-five subjects were randomised to receive either placebo or one of four concentrations of ET-1 (ranging from 10^{-10} to 10^{-6} M). Besides assessment of spontaneous pain, three neurosensory testings were performed: (1) cold and warm sensation, (2) cold and heat pain, and (3) punctate stimulation using a von Frey monofilament. ET-1 produced a dose-dependent flare zone that was absent after placebo injection. Subjects reported a short-lasting spontaneous pain upon administration of the highest concentrations of ET-1. Injection of ET-1 induced a long-lasting and dose-dependent punctate hyperalgesia in an area around the injection site (secondary hyperalgesia). Thermal testing revealed a short period of hypoesthesia to non-noxious warm and cold stimuli after some doses of ET-1. In addition to the mechanical hyperalgesia, intradermal injection of ET-1 almost instantaneously induced a state of cold hyperalgesia outlasting the study period (120 min). No development of heat hyperalgesia was observed. The observed psychophysical characteristics of this new model of ET-1 induced nociception indicate its potential as a human experimental model for cancer pain.

Keywords: Endothelin-1; Intradermal injection; Cancer pain; Human

Pain is a frequent and disabling consequence of (metastatic) cancer syndromes in humans [7]. Our understanding of the basic mechanisms that underlie the development of pain associated with malignancy is limited, but may involve mediator-dependent signalling by tumor cells. Tumor cells are known to secrete a variety of different substances, many of which are potential algogens [10,18]. One of these mediators is endothelin-1 (ET-1). This 21-residue peptide, known mainly for its potent vasoconstrictor effects, is secreted in high concentrations by several cancer cell lines, such as metastatic prostate and breast cancer cells [22,27,32]. Increasing data suggest that multiple functions of the endothelin axis have associations with mitogenesis, apoptosis inhibition, and angiogenesis [17,34].

In addition, a role for this peptide in nociception is also envisaged and as such ET-1 is known to induce pain or overt nociception in animals and humans [13,14,29]. ET-1 also seems to trigger the development of hyperalgesia to noxious chemical, mechanical and thermal stimuli [8,25,26,28]. A crucial point for supporting the ET-1 role in nociception was the demonstration that ET-1 induced pain does not derive from its vasoconstrictor effect [13]. Furthermore, in vivo and in vitro neurophysiological studies have shown that the nociceptive actions of ET-1 are most likely mediated by direct excitation of nociceptive primary afferents [16,36]. New findings suggest that activation of ET_A receptors leads to the onset of nociceptive behaviors in animal pain models [20], whereas ET_B receptors seem to play a role in communicating an inhibitory signal.

When injected into human skin, ET-1 causes a potent, long-lasting vasoconstriction at the site of the administration, surrounded by a profound, wide-spread vasodilatation [6,11,23]. Previous studies with ET-1 in humans have primarily focused on its vasoconstrictive effects. Nociceptive changes were hereby considered as side effects and never the main focus of interest. Injection of ET-1 into the brachial artery induced severe muscular pain and prolonged, touch-evoked allodynia [12]. Another study reported a burning pruritus after intradermal injection of ET-1 [19].

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Aiming to further characterize the role of ET-1 in human nociception, the present study was designed to elucidate whether intradermal injection of minute doses of ET-1 altered the perception of different sensory modalities. In addition, dose-responsiveness and time course of these sensory alterations were investigated to infer mechanisms of action of ET-1. To our understanding this is the first report targeting the ET-1-induced sensory alterations in humans.

Experiments were performed on the volar aspect of the right forearm of 35 healthy male volunteers (mean age 26 ± 4 years, range 21–38). Subjects were asked to refrain from food and caffeine-containing drinks for at least 4 h prior to their participation in the study in order to prevent any interfering vasoconstrictive effects from these substances. All experiments were performed in the supine position in a room at a constant temperature and a relative humidity of $30.2 \pm 1.4\%$. None of the subjects were on any medication. The aim of the study and the nature of the testing were explained to the subjects. The local Ethics Committee of the Antwerp University Hospital approved the study. All volunteers gave written informed consent to participate in the study, which was performed according to the Declaration of Helsinki.

A randomized, double-blinded, placebo-controlled design methodology was applied. Volunteer subjects were randomized to one of five experimental groups ($n = 7$ for each group), receiving either placebo or one of the following concentrations of ET-1: 10^{-6}, 10^{-7}, 10^{-8} and 10^{-10} M. After rating of spontaneous pain, three neurosensory tests were performed: (a) warm and cold sensation, (b) heat and cold pain, and (c) punctate stimulation. The same order of the stimuli was used in all subjects: punctate stimulation, cool, warm, cold pain and heat pain. This order was chosen because it ranges from the lowest stimulus to the highest stimulus. Following the reading of a standard script of instructions, baseline neurosensory testing was performed on the volar aspect of the subject's right forearm (half-way between the wrist and antecubital fossa). The skin was prepped with an alcohol pad and 1 min later 40 μl of one of four concentrations of ET-1 or 40 μl of 0.9% saline placebo was injected intradermally using a micro-volume syringe mounted with a 25-gauge needle (SGE, Australia). To ensure proper intradermal injection, the surface of the skin was stretched and the tip of the needle was inserted, bevel upwards, almost parallel to the skin surface. ET-1 solution was then slowly injected into the uppermost layer of the skin, inducing a raised papule with 'peau d'orange' appearance. Solutions of ET-1 (Clinalfa®, Merck Biosciences AG, Switzerland) were prepared by dilution with 0.9% sterile pyrogen-free saline solution (PBS) to give final concentrations of 10^{-6}, 10^{-7}, 10^{-8} and 10^{-10} M. An equivalent of normal sterile saline in identical vials was prepared for placebo testing. The doses of ET-1 chosen for this experiment represent the lower end of those applied in previous studies on the vasoconstrictive effects of ET-1 and were considered safe for administration to volunteers with minimal risk of side effects and unblinding the study. Pain rating and neurosensory testing was performed before and at 1, 10, 30, 60 and 120 min after ET-1 or placebo injection. To minimize external influences, all testing was performed by the same investigator (GH) and took place at the same time of day. All subjects and the examiner were blinded with respect to administration of ET-1 or saline. The code was broken after data entry and analysis.

At the beginning of each testing session the subjects were asked to report any sensation of pain. Volunteers rated the intensity of spontaneous pain induced by ET-1 using a visual analogue scale (VAS) 10 cm in length and anchored by word descriptors at each end (left-hand end: 'no pain' and on the right-hand end: 'worst imaginable pain'). Subjects marked on the line the point that they felt represented their current state of nociception.

Development of secondary hyperalgesia was assessed by punctate mechanical stimulation with a von Frey monofilament applied at 90° to the skin surface (bending force of 254.9 mN, Stoelting Co., USA). This von Frey probe, which causes only a slight discomfort sensation in normal skin, was applied along a line that marked the edge of the visual flare. The subjects were instructed to report the occurrence of a definite change in sensation during this stimulation, often to a more intense stinging with a prolonged after-sensation. The hyperalgesic area was defined as the skin region in which punctate stimulation produced a definite change in the quality of the sensation described by the subjects as 'painful', 'burning', 'tenderness', 'more intense pricking', 'more unpleasant' (from high to low intensity). No numerical scale was used; subjects were asked to describe the qualitative perception of von Frey hair stimulation in the presence or absence of ET-1 treatment to confirm that the descriptors mentioned above were reported after treatment only. These response codes have been used previously by other investigators in order to monitor development of hyperalgesia in humans [5,35].

Perception thresholds for thermal sensation (warm, cold, cold pain and heat pain) were measured by a thermotest device (Medoc TSA 2001, Ramat Yishay, Israel) prior to and after ET-1 application and placebo. A thermode of Peltier elements measuring 32 mm × 32 mm was applied exactly in the flare area surrounding the ET-1 injection site. The temperature of the thermode could either rise or fall depending on the direction and the intensity of the current flow through the Peltier device. The methods of limits was used by applying ramp stimuli with a velocity of 1 °C/s starting from 32 °C (baseline temperature). Warm and cold detection thresholds, cold pain thresholds, and heat pain thresholds (in that order) were recorded. By pressing a button, subjects indicated when the respective thresholds were reached. Thresholds were calculated as the average of four (cold and warm sensation) or three (cold and heat pain) successive measurements with a random interstimulus interval of 3–8 s. All measurements of a given perception were completed before testing the next perception. To protect the skin from possible thermal injury, the increase of the thermode temperature was limited to 50.5 °C. Limit for decreasing temperatures was 0 °C.

Prior sample size calculation for differences in spontaneous pain and sensitivity to punctate stimulation, revealed the necessity of a sample size of 7 (double sided population analysis with alpha 0.05 and power 0.9). To compare the data of spontaneous pain measurement and thermal testing, two-way repeated measures ANOVA (two-way RM-ANOVA) was performed, and a