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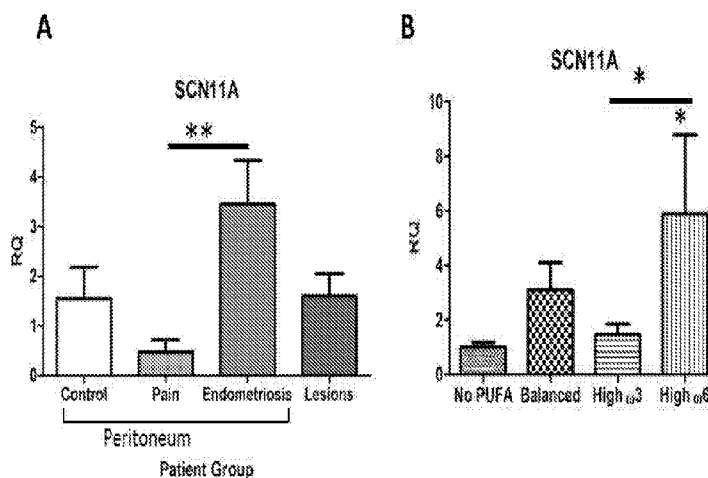
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(54) Title: NOVEL TREATMENT FOR ENDOMETRIOSIS

FIGURE 1



(57) Abstract: Described herein are new treatments for the pain associated with endometriosis. More particularly, there is provided a novel combination of at least one SCN11A antagonist and at least one SCN11A expression level modifier for use in the treatment of endometriosis.

NOVEL TREATMENT FOR ENDOMETRIOSIS

FIELD OF THE INVENTION

The present invention relates to a novel therapy for the treatment of endometriosis comprising the combination of a SCN11A antagonist and a SCN11A expression level
5 modifier. In particular the invention provides the combination of omega-3 fatty acid and menthol for the treatment of endometriosis.

BACKGROUND OF THE INVENTION

Endometriosis is a painful and distressing chronic inflammatory and proliferative disease affecting up to 1 in 10 women of childbearing age, in which cells which in normal
10 development would line the uterus, the endometrial cells, and thereby naturally detach and leave the body as part of the menstrual cycle, in fact grow outside the lining of the uterus womb. Whilst this abnormal growth of endometrial tissue is common on the ovaries and fallopian tubes, it can occur on other organs in the pelvic and bladder regions. During the menstrual cycle, such abnormal endometrial tissue outside of the uterus has no natural way
15 to exit the body and the sufferer will commonly experience pain. However, the long term damage to the ovaries and fallopian tubes, as well as fertility issues associated with endometriosis and the fact that there is no cure, mean that a diagnosis of endometriosis can be very distressing, especially for women wishing to have children.

As there is no treatment or cure for endometriosis *per se*, presently symptoms associated
20 with endometriosis are managed by a variety of different approaches. Pain relief is the most common treatment, and is routinely treated using over the counter analgesics / pain relieving medicaments. Alternatively, reduction of oestrogen levels, via hormone treatment is used to provide symptomatic relief. However this is not usually a long-term approach. Particularly where potential fertility is an issue, varying degrees of invasive treatments to remove areas
25 of endometriosis can be employed via endometrial ablation, laparoscopy, laparotomy or in extreme cases hysterectomy. Although the nature of this condition is such that it can return even post-surgery.

Thus there is a high unmet medical need for effective therapies which can deliver improvements in either the management of the symptoms associated with endometriosis,
30 and/or the underlying chronic condition itself.

The search for a potential therapy for the treatment of endometriosis has been long-hindered by the lack of clarity in relation to the aetiology of the disease. We have now found that

SCN11A has a potentially prominent role in the search for an effective treatment for the pain associated with endometriosis.

SCN11A, also known as Nav1.9 is a voltage-gated sodium ion channel protein which in humans is encoded by the SCN11A gene. Mutations in the SCN11A gene have been associated with loss of pain perception in man (Nature Genetics 45 1319 2013). We have now found that that SCN11A has a prominent role in endometriosis, and in particular we have found that SCN11A is overexpressed in the peritoneum of women with endometriosis compared to women that have pain but no endometriosis. We have also demonstrated that omega-3 fatty acids down regulate gene expression of SCN11A in rat dorsal root ganglia neurons.

In brief we have found that there is an increased expression of SCN11A in peritoneal tissue of women with endometriosis and without wishing to be bound to any particular theory we believe that a combination therapy based on a SCN11A expression level modifier and a SCN11A antagonist would provide a potentially highly effective treatment for endometriosis-associated pain.

Although various ion channels have been reported as being associated with pain, and in particular it has been proposed by Martinez and Melgar, European Journal of Pain 12 (2008) 934-944, that there may be a link between voltage-gated sodium ion channels, such as SCN11A (Na_v 1.9 protein) and pain, prior to the present invention there had been no suggestion of the implication of SCN11A in the treatment of endometriosis.

Although the analgesic properties of menthol are well established, having been used for the delivery of pain relief since Greek times, and it has more recently been proposed that at least some of these analgesic properties may be mediated by voltage-gated sodium channels, Gaudio *et al.*, Pain, 153 (2012) 4730484, prior to the present invention there has been no suggestion that menthol may be of particular utility in the potential treatment of endometriosis.

The potential impact of omega-3 fatty acids on endometriosis has been reported, Netsu *et al.*, Fertility and Sterility, Vol 90, Suppl 2, October 2008. However, prior to the present invention there has been no suggestion that these effects are mediated by SCN11A or the potential impact of fatty acids on the pain associated with endometriosis.

SUMMARY OF THE INVENTION

According therefore to an aspect of the present invention there is provided a novel therapy for the treatment of endometriosis comprising the combination of a SCN11A antagonist and a SCN11A expression level modifier.

5 According to an aspect of the present invention there is provided a novel therapy for the treatment of endometriosis comprising the combination of omega-3 fatty acid and menthol.

According to a yet further aspect of the present invention there is provided a novel therapy for the treatment of endometriosis comprising one or more agents which inhibit the expression levels and/or activity of SCN11A.

10 According to a still further aspect, the present invention provides a novel therapy for the treatment of dysmenorrhea comprising one or more agents which inhibit the expression levels and/or activity of SCN11A, and in particular the combination of a SCN11A antagonist and a SCN11A expression level modifier.

15 According to further aspects there are provided pharmaceutical compositions of the above-noted therapies and their medical utility, particularly for the treatment of pain associated with endometriosis.

DETAILED DESCRIPTION

20 The gene SCN11A is also known as NaN, SNS-2, NAV1.9, and SCN12A. SCN11A encodes Nav1.9, a voltage-gated sodium channel alpha subunit, type XI. Nav1.9 shares 50% identity with most of the other Nav isoforms, but does not, based on phylogeny, belong to a new Na channel subfamily. Nav1.9 is best known for its high expression levels in peripheral sensory neurons and is thought to carry the NaN current, a persistent and tetrodotoxin (TTX)-resistant voltage-gated Na current, and as indicated herein the involvement of Nav 1.9 in pain has been proposed.

25 A SCN11A antagonist as defined herein means an agent capable of nullifying, or otherwise reducing the impact of SCN11A and examples of SCN11A antagonists suitable for use herein are independently selected from: menthol; mibefradil; inorganic I(Ca) blockers; and mixtures or combinations thereof.

30 As proposed herein and as discussed in relation to the results detailed hereinafter, there are advantages for the combination of at least one SCN11A expression level modulator and at

least one SCN11A antagonist. The analgesic effect of an SCN11A expression level modulator can be augmented by co-administration with the SCN11A antagonist menthol.

Accordingly the present invention provides a novel therapy for the treatment of endometriosis comprising the combination of menthol and a SCN11A expression level
5 modifier.

SCN11A expression level modifiers, or modulators, can also be called SCN11A down-regulating agents. Suitable SCN11A expression level modifying agents for use herein are omega-3 fatty acids.

The analgesic effect of an omega-3 fatty acid, an SCN11A expression level modulator can
10 be potentiated (increased) by co-administration with the SCN11A antagonist menthol.

Accordingly the present invention provides a novel therapy for the treatment of endometriosis-associated pain comprising the combination of menthol and omega-3 fatty acid.

Omega-3 fatty acids are also known as ω -3 fatty acids or *n*-3 fatty acids, and the term refers
15 to a group of three fats: ALA from plant oils; and EPA and DHA which are commonly found in marine oils.

Alpha (α) linolenic acid (ALA), *cis, cis, cis* -9,12,15-octadecatrienoic acid, is also known as (9Z,12Z,15Z)-9,12,15-Octadecatrienoic acid, Industrene 120 and, in the nomenclature of fatty acids, 18:3 (*n*-3). In common with many essential fatty acids, ALA is abundant in
20 nature, with plant oils, specifically seed oils, and most notably chia (chia sage), perilla (shiso), flaxseed (linseed oil), rapeseed (canola) and soybeans being the richest sources, and kiwifruit (Chinese gooseberry), lingonberry (cowberry), camelina (camelina), puslane (portulaca), sea buckthorn (seaberry) and hemp (cannabis) less so, with alternative sources being the thylakoid membranes in the leaves of pea leaves (*pisum sativum*). Synthetic ALA
25 is commercially available.

Eicosapentaenoic acid (EPA), (5Z,8Z,11Z,14Z,17Z)-5,8,11,14,17-icosapentaenoic acid, is also known as timnodonic acid, and 20:5(*n*-3). Whilst common sources of EPA in dietary terms are oily fish or fish oils such as cod liver, herring, mackerel, salmon, menhaden, and sardine as well as some edible seaweeds. EPA itself is produced from algae, and is
30 available commercially via microalgae production.

Docosahexaenoic acid (DHA), *cis, cis, cis-, cis---*docosa-4,7,10,13,16,19-hexa-enoic acid or (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid, is also known as

Doconexent, cervonic acid and 22:6(n-3). The richest natural sources of DHA are cold-water oceanic fish oils. DHA can be commercially produced from *Crypthecodinium cohnii* and *Schizochytrium* microalgae.

Suitable omega-3 fatty acids for use herein are independently selected from: ALA; EPA; DHA; and mixtures and combinations thereof. Preferred herein are omega-3 fatty acids derivable from fish oils, and in particular EPA, DHA and mixtures thereof.

Accordingly the present invention provides a novel therapy for the treatment of endometriosis comprising the combination of a SCN11A antagonist and one or more fish oils. More particularly the present invention provides a novel therapy for the treatment of endometriosis comprising the combination of menthol and one or more omega-3 fatty acids derivable from fish oils, and in particular EPA, DHA and mixtures thereof.

Exemplary omega-3 fatty acids for use herein include: commercially available purified EPA and/or commercially available DHA such as are available from Swanson Health Products, Fargo, ND, USA; commercially available EPA and/or commercially available DHA such as Max Epa, Omega-3, Salmon Oil, or Superepa; commercially available mixtures of EPA and DHA such as are available from Omacor for example Omacor fish oils having EPA (465mg) and DHA (375mg).

The relative ratios of SCN11A antagonist : SCN11A expression level modifier suitable for use in the combinations according to the invention are between about: 10 : 1 to about 1 : 10; 5 : 1 to about 1 : 5; and about 2 : 1 to about 1 : 2, wherein the relative ratios are based on the total weight percent (wt. %) of antagonist(s) : modifier(s).

Within the SCN11A expression level modifier ratio, where a mixture of omega-3 fatty acids are present the relative amounts of EPA and DHA are between about 0.1% to about 99.9%. In other words the SCN11A expression modifier (100%) includes from 0.1% to 99.9% EPA and from about 99.9% to about 0.1% of DHA, wherein the relative percentages are based on weight percent (wt. %) of each material, and wherein the additive percent levels of EPA and DHA present equate to 100%. For the avoidance of doubt, where only EPA or DHA are present then the relative amount of each is 100%.

Thus the present invention provides a novel therapy for the treatment of endometriosis-associated pain comprising the combination of menthol and one or more omega-3 fatty acids derivable from fish oils wherein the ratios of menthol : omega-3 fatty acids are between about: 10 : 1 to about 1 : 10 and wherein the omega-3 fatty acids consists essentially of from 0.1% to 99.9% EPA and from about 99.9% to about 0.1% of DHA.

Thus the present invention provides a novel therapy for the treatment of endometriosis-associated pain comprising the combination of menthol and one or more omega-3 fatty acids derivable from fish oils wherein the ratios of menthol : omega-3 fatty acids are between about: 10 : 1 to about 1 : 10 and wherein the omega-3 fatty acids consists essentially of EPA or DHA.

According to a still further aspect, the present invention provides a novel therapy for the treatment of dysmenorrhea comprising one or more agents which inhibit the expression levels and/or activity of SCN11A, and in particular the combination of a SCN11A antagonist and a SCN11A expression level modifier.

Suitable SCN11A expression level modifying agents for such use are omega-3 fatty acids, as defined hereinbefore, and in particular EPA and/or DHA as defined and in the relative levels as defined hereinbefore.

Suitable SCN11A antagonists for such use are as defined hereinbefore is in particular menthol.

Menthol may be utilised either alone or within a preparation containing peppermint oil. Peppermint oil contains between 30% to 55% menthol, a commercially available source of menthol in a peppermint oil based preparation is Colpermin® available from McNeil Healthcare.

In general terms dysmenorrhea is related to pain in women of childbearing age. Primary dysmenorrhea is often referred to as period pain, and is generally associated with painful periods, and is most often experienced by younger women from their teenage years until the end of their 20s.

Secondary dysmenorrhea is often referred to as period pain due to an underlying cause. Pain during the menstrual cycle can be caused by problems in the womb or pelvis, such as for example endometriosis.

The present invention additionally provides a novel therapy for the treatment of pain associated with secondary dysmenorrhea comprising one or more agents which inhibit the expression levels and/or activity of SCN11A, and in particular the combination of a SCN11A antagonist and a SCN11A expression level modifier.

FORMULATIONS AND THERAPEUTIC METHODS

The invention provides formulations for use in the novel therapy according to the present invention, in any of the forms detailed herein, and particularly at least one SCN11A antagonist and at least one SCN11A expression level modifier formulated for pharmaceutical and/or nutraceutical use and optionally further comprising a pharmaceutically and/or nutraceutical acceptable diluent, excipient and/or carrier.

The invention therefore additionally includes pharmaceutical formulations which may include, in addition to the therapeutic compositions as defined hereinbefore, which include at least one SCN11A antagonist and at least one SCN11A expression level modifier, a pharmaceutically or nutraceutically acceptable diluent, excipient and/or carrier. Such formulations may be used in the methods of the disclosure. Additionally or alternatively, pharmaceutical or nutraceutically formulations may include a buffer, stabiliser and/or other material well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the at least one SCN11A antagonist and at least one SCN11A expression level modifier. The precise nature of the carrier or other material will depend on the route of administration, which may be any suitable route.

For the provision of relief from pain associated with Endometriosis, the oral route is preferred.

Alternative routes which may be used are independently selected from: local (topical administration); parenteral administration and particularly by infusion or injection (with or without a needle); subcutaneous injection; intravenous injection; infusion. Other routes of administration which may be used include administration by inhalation or intranasal administration.

The formulations of the invention may be administered rectally or vaginally, for example, in the form of a suppository, pessary, or enema. Cocoa butter is a traditional suppository base, but various alternatives may be used as appropriate.

The formulations may be to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release. A preferred formulation herein is an oral modified release formulation.

According to a further aspect the invention provides a pharmaceutical formulation for oral delivery comprising one or more SCN11A antagonist, one or more SCN11A expression level modifier and a pharmaceutically acceptable diluent, excipient and/or carrier wherein said formulation is formulated for modified release.

According to a further aspect the invention provides for co-administration via oral delivery of a pharmaceutical formulation formulated for immediate or modified release comprising one or more SCN11A antagonist, and a pharmaceutical formulation formulated for modified release comprising one or more SCN11A expression level modifier wherein each formulation independently additionally includes a pharmaceutically acceptable diluent, excipient and/or carrier.

DOSAGE

Amounts effective for therapeutic use, which may be a prophylactic use, will depend upon the severity of the disease and the general state of the patient's health. Therapeutically effective amounts of at least one SCN11A antagonist and at least one SCN11A expression level modifier are those which provide either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer.

Single or multiple administrations of the pharmaceutical or nutraceutical formulations of the invention may be administered depending on the dosage and frequency as required and tolerated by the patient. In any event, the formulation should provide a sufficient quantity of at least one SCN11A antagonist and at least one SCN11A expression level modifier as disclosed herein to effectively treat the patient, bearing in mind though that it may not be possible to achieve effective treatment in every instance. The dosage can be administered once but may be applied periodically until either a therapeutic result is achieved or until side effects warrant discontinuation of treatment. The dose may be sufficient to treat or ameliorate symptoms or signs of disease without producing unacceptable toxicity to the patient.

Suitable daily dose ranges for the at least one SCN11A antagonist are: from about 15mg to about 200mg per day; from about 20mg to about 150mg per day; from about 30mg to about 100mg per day; wherein said doses may be provided as a single daily dose, or dosed periodically during the day.

According to a further aspect menthol is provided at: from about 15mg to about 200mg per day; from about 20mg to about 150mg per day; from about 30mg to about 100mg per day; wherein said doses may be provided as a single daily dose, or dosed periodically during the day.

Suitable daily dose ranges for the at least one SCN11A expression level modifier are: from about 1.5mg to about 2000mg per day; from about 2mg to about 1500mg per day; from about 3mg to about 1000mg per day; wherein said doses may be provided as a single daily dose, or dosed periodically during the day.

According to a further aspect EPA and/or DHA is provided at: from about 1.5mg to about 2000mg per day; from about 2mg to about 1500mg per day; from about 3mg to about 1000mg per day; wherein said doses may be provided as a single daily dose, or dosed periodically during the day.

- 5 For the avoidance of doubt any specific dosage of a pharmaceutical formulation comprising at least one SCN11A expression level modifier, for example a fish oil, such as EPA and/or DHA, and at least one SCN11A antagonist, for example menthol, as defined hereinbefore may be provided in a single dosage form, such as a single tablet, or in a multiple dosage format, such as two or more tablets in accordance with the needs of the particular patient.
- 10 Where the at least one SCN11A antagonist and the at least one SCN11A antagonist are co-administered as separate pharmaceutical formulations as detailed hereinbefore then each may be independently provided in a single dosage form, such as a single tablet, or in a multiple dosage format, such as two or more tablets in accordance with the needs of the particular patient.
- 15 The novel and inventive treatment in accordance with the present invention may also be provided in combination with one or more of any of the pre-existing medicaments which are known to have potential for the amelioration of the symptoms of endometriosis, such as for example currently prescribed medicaments for pain relief and/or hormonal treatment.

Such prescribed medicaments for pain relief include for example: non-steroidal anti-inflammatory drugs (NSAIDS) such as Ibuprofen, Voltarol and Ponstan (mefenamic acid);
 20 paracetamol; pain modifiers including tricyclic anti-depressants such as Amitriptyline; glucocorticoids, opioids, anticonvulsants, antidepressants, Na⁺ or Ca²⁺ channel blockers, inhibitors of TRPV1 channel function.

Such prescribed hormonal based medicaments include for example: the combined oral
 25 contraceptive pill ("the Pill"); Mirena Coil; progestogen-containing drugs such as for example Medroxyprogesterone (Provera), Norethisterone (Primolut), Dydrogesterone (Duphaston) and Depo-Provera; GnRH analogues such as for example Leuprorelin (Prostap), Goserelin (Zoladex), Nafarelin (Synarel), Buserelin (Suprecur) and Triptorelin (Decapeptyl); synthetic androgens (testosterone) such as for example Danazol and Gestrinone (Dimetrioise).

- 30 An approach including both current medicaments for relief of symptoms in conjunction with a pharmaceutical or nutraceutical formulation including least one SCN11a antagonist and at least one SCN11A expression level modifier in accordance with the present invention may be particularly suited to subjects who are experiencing chronic pain from a pre-existing

diagnosis of endometriosis and whom are already receiving medication for symptom management.

KIT-OF-PARTS

Inasmuch as it may be desirable to administer a combination of different active compounds, for example, for the purpose of treating a particular disease or condition, it is within the scope of the present invention that two or more pharmaceutical compositions, at least one of which contains a combination in accordance with the invention, may conveniently be combined in the form of a kit suitable for coadministration of such compositions.

Thus the kit of the invention comprises two or more separate pharmaceutical or nutraceutical compositions, at least one of which contains a pharmaceutical or nutraceutical composition of at least one SCN11A antagonist and at least one SCN11A expression level modifier in accordance with the invention, and means for separately retaining said compositions, such as a container, divided bottle, or divided foil packet. An example of such a kit is the familiar blister pack used for the packaging of tablets, capsules and the like.

The kit of the invention is particularly suitable for administering different dosage forms, for example, oral and parenteral, for administering the separate compositions at different dosage intervals, or for titrating the separate compositions against one another. To assist compliance, the kit typically comprises directions for administration and may be provided with a so-called memory aid.

For the avoidance of doubt, references herein to "treatment" include references to curative, palliative and prophylactic treatment.

EXPERIMENTAL METHODS

Human tissues

Peritoneal endometriotic lesion biopsies, taken from the Pouch of Douglas (EL; n=21) and peritoneal biopsies from an adjacent site (EP; n=12) were obtained from women who were undergoing laparoscopic surgery for the treatment of endometriosis associated pain.

Peritoneal biopsies (PP; n=11) were collected from women undergoing explorative laparoscopic surgery for pelvic pain but with confirmed absence of endometriosis.

Peritoneal biopsies were also collected from women with no pain or endometriosis who were undergoing surgical sterilisation via laparoscopy (Control; n=4).

The women were aged 18-45 years.

The biopsy samples were taken during different stages of the menstrual cycle from women who had not been taking exogenous hormones at least 3 months prior to surgery. Ethical approval for this study was obtained from the Lothian Research Ethics Committee (LREC 11/AL/0376). All samples were collected after informed consent was obtained. Biopsies were collected into RNAlater, which is commercially available from Applied Biosystems, Warrington, UK, for qRT-PCR.

Mouse model of endometriosis

The methodology for the mouse model of endometriosis utilised herein is in accordance with that detailed in Greaves *et. al.*, The American Journal of Pathology, Vol. 1894, No. 7, July 2014, pp1931-1939, the disclosures of which are incorporated herein by reference. In brief, menstruation was induced in adult donor mice (~8 weeks of age) using a protocol developed by the applicant (Figure 1A upper timeline). In brief, ovariectomized mice (day 1) were primed with subcutaneous (sc) injections of 100ng estradiol-17 β (E2, days 7-9), treated with progesterone (P4; Sigma) delivered via a SILASTIC implant (DOW CORNING, USA) from days 13-19 and injected with 5 ng E2 in sesame oil on days 13, 14 and 15. Decidualization was induced within one uterine horn using 20 μ l oil (day 15) and endometrial tissue in the process of being shed from the decidualized horn recovered from mice culled on day 19, 4h after P4 withdrawal (removal of pellet) by opening the horn longitudinally in a Petri dish and scraping the tissue away from the myometrial layer using a scalpel (Figure 1, B and C). The tissue mass was suspended in 0.2 ml PBS and passed through a 19g needle before being injected intra-peritoneally (i.p.) into anesthetized recipient mice (~8 weeks of age) which had been previously ovariectomized and implanted with an E2 (Sigma) SILASTIC implant (Figure 1A lower timeline). Tissue from one decidualized donor horn was used to inoculate each recipient mouse (approx. 40 mg tissue/ 0.2 ml PBS per mouse). Three weeks following i.p. injection recipient mice were culled, photographs taken with lesions carefully dissected from surrounding tissue and either fixed in 4% normal buffered formaldehyde for histological analysis or placed in RNA Save (GENEFLOW, UK) for RNA extraction.

A pilot experiment was conducted using two groups of mice (n=4 each group) with experimentally induced endometriosis; following lesion establishment for 2 weeks mice received 5% dietary intake of EPA which was extracted from Omacor® fish oil capsules or control sesame oil. Omacor® are FDA approved and widely used by women, and can therefore be easily transferred to a clinical trial. Pain associated behavior was recorded on consecutive days for 1 week during hours of darkness for periods of ten minutes with mice in observation boxes. The pain score was based on numerical assessment of periods spent licking the abdomen and over-grooming.

Cell culture and PUFA treatment

Dorsal root ganglia (DRG) were dissected from the spinal cords of E15.5 Sprague-Dawley rat embryos. The DRGs were dissociated using a solution of: 30 µg/ml of Papain (commercially available from Worthington, Lakewood, NJ, USA); 0.24 mg/ml of L-Cysteine (commercially available from Sigma, Dorset, UK; and 40 µg/ml of DNase1 (also available from Sigma, UK) in minimal essential medium (MEM) (commercially available from Sigma, UK).

The dissociated DRGs were plated in 200 µl drops (with approximately 100,000 soma per well) using a pipette, onto Poly-D-Lysine at levels of from 5 to 10 µg/ ml (obtained from Sigma, UK;) and Matrigel (obtained from BD Biosciences, Oxford, UK) at a ratio of 1:40 diluted in sterile water) coated coverslips in M1 media (high glucose Dulbecco's Modified Eagle Medium (DMEM; obtained from Gibco), supplemented with a solution of 0.01% Penicillin-Streptomycin (Pen/Strep), 10% fetal calf serum (FCS) and 0.1 µg/ml nerve growth factor (NGF obtained from Serotec, Oxford, UK) and maintained at 37°C in an atmosphere containing 7.5 % CO₂.

After 24h hours the wells were then carefully flooded with 2ml M1, using a pipette supplemented with the anti-mitotic FdU (10 µM, obtained from Sigma, UK) and the media was changed every 2 days until contaminating astrocytes were eliminated (≤ 6 days; confirmed by microscopic visualisation). At least 24 hours prior to running experiments the medium was changed from a supplemented M1 medium, to a phenol red free medium supplemented with 10% fatty acid free bovine serum albumin (BSA).

The dissociated DRGs were then treated with either: vehicle control; a balanced PUFA formulation; a formulation of high Omega-3; or high Omega-6 PUFA. The relative compositions of these are illustrated in Table 1.

The treatments were performed in triplicate, and three separate experiments performed. After an incubation of 24h the DRGs were then harvested for RNA extraction.

Table 1

	Mg			
Mg/250ml	Control	Balanced	High Omega3	High Omega6
Palmitic acid (mg)	0	5.2	5.2	5.2
Oleic (mg)	0	5.1	5.1	5.1
Linoleic (mg)	0	2	2	2
Alpha Linoleic (mg)	0	0.1	0.1	0.1
Arachidonic (mg)	0	0.6	0	1.2
Eicosapentanoic (mg)	0	0.6	1.2	0
Ethanol final volume (μl)	1	1	1	1

5 RNA extraction and cDNA synthesis

Total RNA was extracted from mouse and human tissues by homogenisation in TRI Reagent, which is a monophasic solution of phenol and guanidinium isothiocyanate (a single-step RNA isolation reagent available from Sigma-Aldrich), followed by chloroform phase separation. The top phase (lysate) was then processed using standard methodology by use of an RNAeasy Kit (available from QIAGEN, Crawley, UK). Total RNA from cells was extracted using Buffer RLT (a lysis buffer) and an RNAeasy Kit (both available from QIAGEN). Complementary DNA was synthesized using SuperScript®VILO™ enzyme and reaction mix (both available from Invitrogen, Paisley, UK).

Quantitative real time PCR

Real-time PCR reactions were performed using the Roche Universal ProbeLibrary (available from Roche Applied Science, West Sussex, UK) and Express qPCR Supermix (from Invitrogen, Paisley) on a 7900 Fast Real-Time PCR with 18S as the endogenous control. Primer sequences: Human SCN11A; (F) acctgagcctgaacaacagg, (R) ttgaactctctggctcgtg, Rat SCN11A; (F) cagaggacgatgcctctaaaa,m (R) ttctgggacagtcgtttggt.

20 Statistics

Statistical analysis was carried out using a D'Agostino & Pearson omnibus normality test followed by a one way analysis of variance with a Newman Keuls post-hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, using GraphPad Prism 5 software.

The invention will now be described, by way of illustration only, with reference to the following examples and figures accompanying the specification.

Brief Description of the Figures

Figure 1: shows two charts A and B, wherein chart A indicates the expression levels of SCN11A in the peritoneal tissue of different groups of women where the vertical axis is the level of SCN11A expressed as relative quantification (RQ) compared to a control sample, and the horizontal axis indicates the test groups, and chart B indicates the expression levels of SCN11A in dorsal root ganglia where the vertical axis is the level of SCN11A expressed and the horizontal axis indicates the test groups. Statistical analysis was performed using a one way analysis of variance (ANOVA) followed by a Newman Keuls post-comparison test. ** $p < 0.01$.

Figure 2: shows various aspects of a murine model of endometriosis. A is a schematic of experimentally induced endometriosis in a mouse model. B is an illustration of an H & E stain of a typical lesion recovered from the peritoneum of experimental mice. C is an illustration of dual immunofluorescence for the endothelial cell marker CD31 and the pan neuronal marker PGP9.5 indicating high levels of vascularisation in lesions and readily detectable small diameter nerve fibres. In the subsection indicated by the dotted line which has been expanded the arrows are pointing to the neuronal marker PGP9.5. In this expended subsection an area of immunofluorescence for the endothelial cell marker CD31 is clearly indicated around the third arrow. D indicates the increased levels of pain associated behaviour ($p < 0.001$) exhibited by mice with experimentally induced endometriosis ($n = 6$), compared to mice without any manipulation (control; $n = 3$) and mice which have been ovariectomised followed by E2 supplementation (OVX+E2; $n = 3$). Pain scores are based on a numerical assessment of periods spent licking the abdomen and excessive grooming during a ten minute window in observation boxes during hours of darkness. E is an illustration of the results of the QPCR analysis of EP2. F is an illustration of the results of the QPCR analysis of COX1. G is an illustration of the results of the QPCR analysis of COX2 in the uterus (NU; $n = 6$) and peritoneum (NP; $n = 9$) from cycling mice and the peritoneum (EP; $n = 6$) and lesions (EL; $n = 8$) from mice with endometriosis. RQ: Relative quantification. *** $p < 0.001$.

Figure 3: shows two charts A and B, wherein chart A indicates the pain associated behavior in control endometriosis mice compared to endometriosis mice which have been supplemented with Omacor® (highly purified omega-3 acid ethyl esters), and chart B indicates the serum PGE2 levels in control endometriosis mice compared to endometriosis mice which have been supplemented with Omacor. N=4 for each group.

EXPERIMENTAL RESULTS

EXAMPLE 1: Expression of SCN11A in peritoneal tissue of different groups of women

To determine whether there was a difference in the level of SCN11A expressed in the peritoneal tissue or lesions of women with endometriosis, versus the peritoneum of healthy women without pain or endometriosis (control), or women with chronic pelvic pain but no demonstrable underlying pathology we tested 4 different groups of women. The composition of these groups was:

Control: Peritoneum from women with no pain and no endometriosis undergoing a surgical sterilisation via laparoscopy (n=4).

Pain: Peritoneum from women with pain and no demonstrable underlying pathology undergoing an exploratory laparoscopy (n=11).

Endometriosis: Peritoneum from women with pain and proven endometriosis (n=12).

Lesions: Peritoneal endometriotic lesions from women with endometriosis (n=21).

The level of SCN11A expression in each group was determined using the method described hereinbefore.

The relative levels of SCN11A expression from each group can be compared because the peritoneal tissue or lesion from each patient is biopsied from the same area (pouch of Douglas) which is prone to endometriosis development. All samples were run on the same plate and compared to the same calibrator and endogenous house-keeping gene.

The results of this experiment, and as illustrated in Chart A in Figure 1, show that women with endometriosis-associated pain have unique SCN11A mRNA levels.

Figure 1, Chart A illustrates the relative levels of SCN11A expressed in the peritoneal tissue of four groups of women: Control; Pain; Endometriosis; Lesions and demonstrates that an increased level of SCN11A expression only applies to the peritoneal tissue of women with endometriosis. In particular, Chart A of Figure 1 provides a representation of the results of our QPCR analysis of SCN11A in the peritoneum from women with no pain and no

endometriosis (control; n=4), women with pain but no endometriosis (pain; n=11) and from the peritoneum (endometriosis; n=12) and lesions (lesions; n=21) from women with endometriosis. RQ: Relative quantification ($p < 0.01$).

EXAMPLE 2: Effect of Omega-3 on SCN11A mRNA levels in an animal model

- 5 To determine the effect of omega-3 on SCN11A mRNA levels we carried out experiments to assess the impact of balanced, high omega-3, and high omega-6 levels on levels of SCN11A expressed in rat DRG neurons.

The results of this experiment, and as illustrated in Chart B in Figure 1, shows a reduction in expression levels of SCN11A mRNA by omega-3 in the rat spinal cord model.

- 10 In particular Chart B of Figure 1 provides a representation of the results of our QPCR analysis SCN11A in rat DRGs treated with different formulations of PUFAs (n=4/group). $p < 0.05$.

EXAMPLE 3: Pain Behavior in Mice with Experimentally-induced Endometriosis

- 15 To determine the baseline behaviour of mice with experimentally induced endometriosis versus control mice we carried out the following experiment. Mice (n=12) were divided into 3 groups; group 1 (control, n=3) received no procedures, group 2 (OVX+E2, n =3) were ovariectomised and supplemented with a subcutaneous slow release E2 (estradiol) pellet. Group 3 (endometriosis, n=6), were ovariectomised and induced with endometriosis in
20 accordance with the method detailed hereinbefore. After a 21 day period allowed for endometriosis lesion development all mice were observed on consecutive days during hours of darkness for a ten minute period in observation boxes. Pain scores were based on numerical assessment of periods spent licking the abdomen and over-grooming.

The results of this experiment, and as illustrated in Figure 2.

- 25 In particular Chart D of Figure 2 shows that mice with induced endometriosis have increased level of pain-associated behaviours.

EXAMPLE 4: Modification of Pain Behavior in Mice with Experimentally-induced Endometriosis

- 30 Mice were experimentally induced with endometriosis in accordance with the method detailed hereinbefore. To determine the impact of EPA on the pain-associated behaviours of

mice with experimentally induced endometriosis versus control mice we carried out the following experiment. Mice (n=8) with experimentally induced endometriosis were divided into two groups (n=4 each group); Group 1 (Control) sesame oil as oral gavage; and Group 2 (Omacor) received 5% dietary intake of EPA which was extracted from Omacor® fish oil capsules. Pain-associated behaviour was recorded as detailed hereinbefore.

The results of this experiment are illustrated in Figure 3.

In particular, Chart A of Figure 3 shows that mice with induced endometriosis have reduced pain behaviours when treated with EPA.

On the basis of the demonstrated results provided herein on animal models, and in particular the observed effects that mice with experimentally-induced endometriosis, as illustrated in Figure 3, Chart A, are associated with a knock-down of SCN11A, it is plausible to propose the use of EPA/omega-3 for the alleviation/mitigation of endometrial pain in women with endometriosis.

Further experiments have been carried out using the methodology as detailed hereinbefore for Example 4 and using a 1% dose of the SCN11A antagonist menthol, applied topically. These preliminary experiments have indicated that dosing with menthol has an additive effect (reduction of pain behaviours in mice with induced endometriosis).

Thus according to a further aspect there is provided a novel therapy for the treatment of endometriosis comprising the combination of SCN11A antagonist and a SCN11A expression level modifier, and in particular wherein the SCN11A antagonist is menthol and the SCN11A expression level modifier is one or more fish oils, especially EPA.

In addition to the results discussed which support the use in endometriosis and particularly the use for the treatment of pain associated with endometriosis, the data presented herein provides support for the proposition of the use of EPA/omega-3 for the alleviation/mitigation of pain associated with dysmenorrhea, particularly secondary dysmenorrhea.

In particular, the following experimental results support this proposition:

Figure 2, Charts E, F and G illustrate the unique EP2, COX-1 and COX-2 levels found in endometriosis lesions in the endometriosis mouse model;

Figure 2, Charts G and D illustrate that mice with experimentally-induced endometriosis have increased pain-associated behaviours; and

Figure 3, Chart A mice with induced endometriosis have reduced pain behaviour when treated with EPA whilst in Figure 3, Chart B mice with experimentally induced endometriosis which are treated with EPA have reduced levels of serum PGE2 (EP2) levels.

Thus according to a further aspect there is provided a novel therapy for the treatment of for the alleviation/mitigation of pain associated with dysmenorrhea, particularly secondary dysmenorrhea comprising the combination of SCN11A antagonist and a SCN11A expression level modifier, and in particular wherein the SCN11A antagonist is menthol and the SCN11A expression level modifier is one or more fish oils, an omega-3 fatty acid, or EPA.

10 Sequence Listing

SEQ ID NO 1. Human SCN11A (F) acctgagcctgaacaacagg;

SEQ ID NO 2. Human SCN11A (R) ttgaactctctggctcgtg;

SEQ ID NO 3. Rat SCN11A; (F) cagaggacgatgcctctaaaa;

SEQ ID NO 4. Rat SCN11A; (R) ttctgggacagtcgttttgt.

15 |

CLAIMS

1. A combination of at least one SCN11A antagonist and at least one SCN11A expression level modifier for use as a medicament.
- 5 2. A combination of at least one SCN11A antagonist and at least one SCN11A expression level modifier for use in the treatment of endometriosis.
3. A combination according to any of the preceding claims for use in the treatment of pain associated with endometriosis.
4. A combination according to any of the preceding claims wherein the at least one SCN11A antagonist is menthol.
- 10 5. A combination according to any of the preceding claims wherein the at least one SCN11A expression level modifier is an omega-3 fatty acid.
6. A combination according to any of the preceding claims wherein the at least one SCN11A expression level modifier is independently selected from: a source of EPA; a source of DHA; and/or mixtures thereof.
- 15 7. A combination according to any of the preceding claims wherein the at least one SCN11A antagonist is menthol and wherein the at least one SCN11A expression level modifier one or more fish oils.
8. A combination according to any of the preceding claims wherein the at least one SCN11A antagonist is menthol and wherein the at least one SCN11A expression level modifier is EPA and/or DHA from microalgae.
- 20 9. A combination according to any of the preceding claims wherein the SCN11A expression modifier includes from 0.1% to 99.9% EPA and from about 99.9% to about 0.1% of DHA.
10. A combination according to any of the preceding claims wherein the ratios of SCN11A antagonist : SCN11A expression level modifier(s) are between about: 10 : 1 to about 1 : 10 and wherein the SCN11a expression level modifier is a mixture of omega-3 fatty acids consisting essentially of from 0.1% to 99.9% EPA and from about 99.9% to about 0.1% of DHA, and wherein the SCN11A antagonist is menthol.
- 25 11. A combination according to any of the preceding claims wherein the ratios of SCN11A antagonist : SCN11A expression level modifier(s) are between about: 10 : 1 to about 1 : 10 and wherein the SCN11a expression level modifier is EPA or DHA.
- 30

12. A pharmaceutical formulation comprising one or more SCN11A antagonist, one or more SCN11A expression level modifier and a pharmaceutically acceptable diluent, excipient and/or carrier.
13. A pharmaceutical formulation according to Claim 11 formulated for oral delivery.
- 5 14. A nutraceutical formulation comprising one or more SCN11A antagonist, one or more SCN11A expression level modifier and a nutraceutically acceptable diluent, excipient and/or carrier.
15. A nutraceutical formulation according to Claim 13 formulated for oral delivery.
- 10 16. A combination of at least one SCN11a antagonist and at least one SCN11a expression level modifier for use in the treatment of dysmenorrhea or secondary dysmenorrhea.
17. A combination according to Claim 15 wherein the at least one SCN11A antagonist is menthol.
- 15 18. A combination according to Claim 15 or 16 wherein the at least one SCN11A expression level modifier is an omega-3 fatty acid.
19. A combination according to any of claims 15 to 17 wherein the at least one SCN11A expression level modifier is independently selected from: a source of EPA; a source of DHA; and/or mixtures thereof.
- 20 20. A combination according to any of claims 15 to 18 wherein the at least one SCN11A antagonist is menthol and wherein the at least one SCN11A expression level modifier is one or more fish oils.
21. A combination according to any of claims 15 to 19 wherein the at least one SCN11A antagonist is menthol and wherein the at least one SCN11A expression level modifier is EPA and/or DHA from microalgae.
- 25 22. A combination according to any of claims 15 to 20 wherein the SCN11A expression modifier includes from 0.1% to 99.9% EPA and from about 99.9% to about 0.1% of DHA.
- 30 23. A combination according to any of claims 15 to 21 wherein the ratios of SCN11A antagonist : SCN11A expression level modifier(s) are between about: 10 : 1 to about 1 : 10 and wherein the SCN11a expression level modifier is a mixture of omega-3

fatty acids consisting essentially of from 0.1% to 99.9% EPA and from about 99.9% to about 0.1% of DHA, and wherein the SCN11A antagonist is menthol.

24. Use of one or more agents which inhibit the expression levels and/or activity of SCN11A for the treatment of pain associated with endometriosis.

FIGURE 1

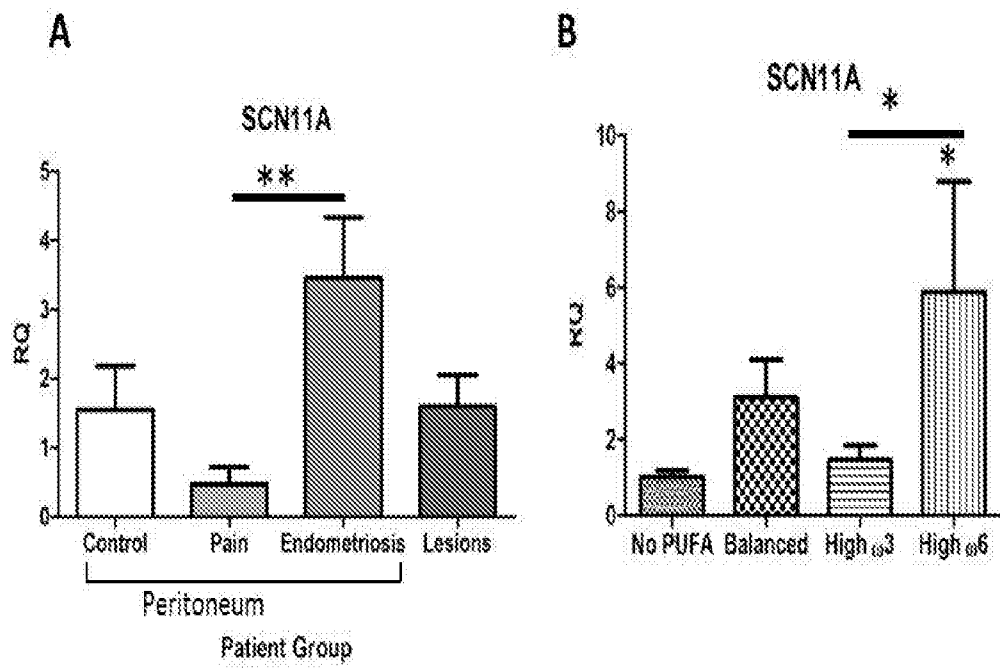


FIGURE 2

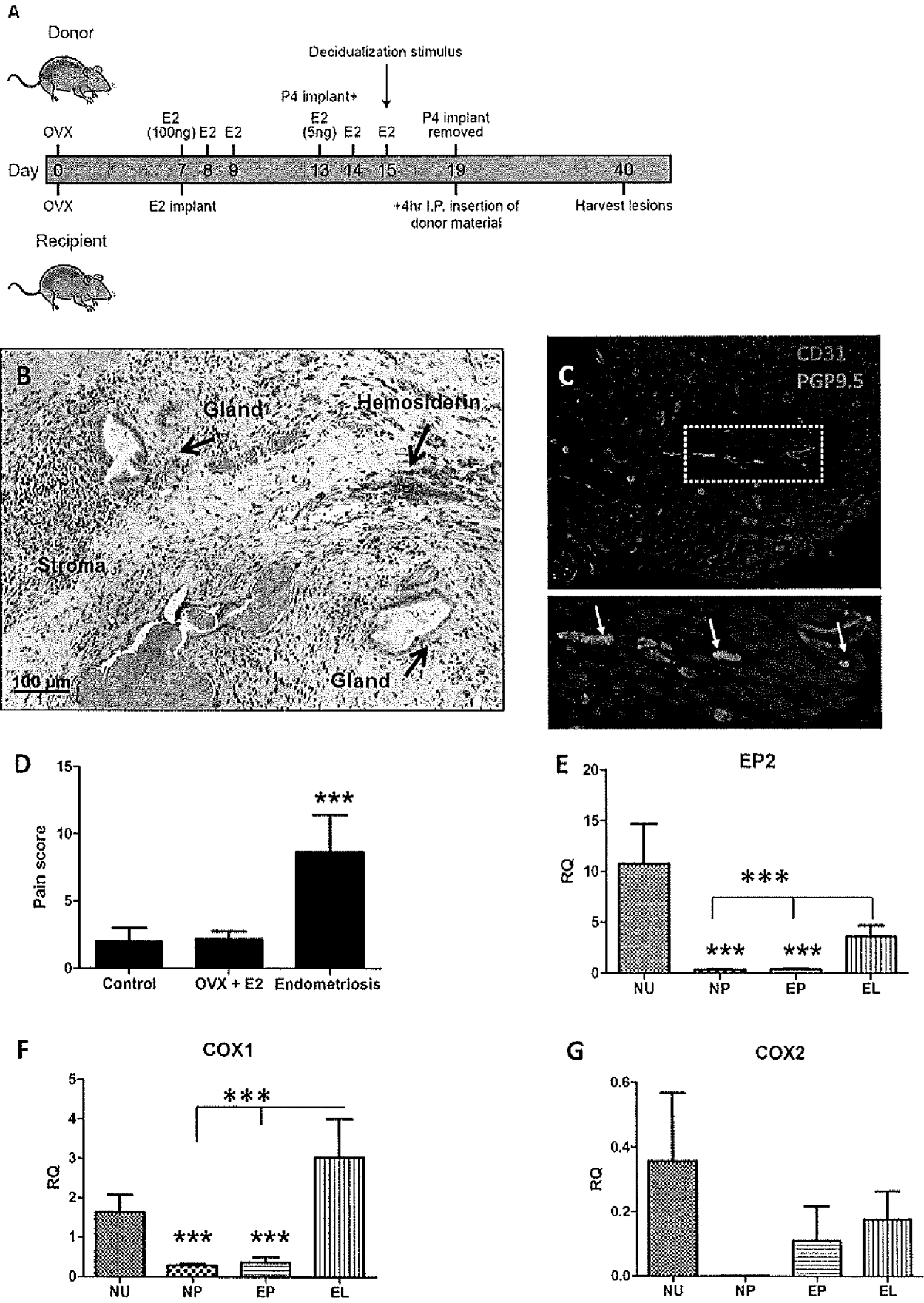
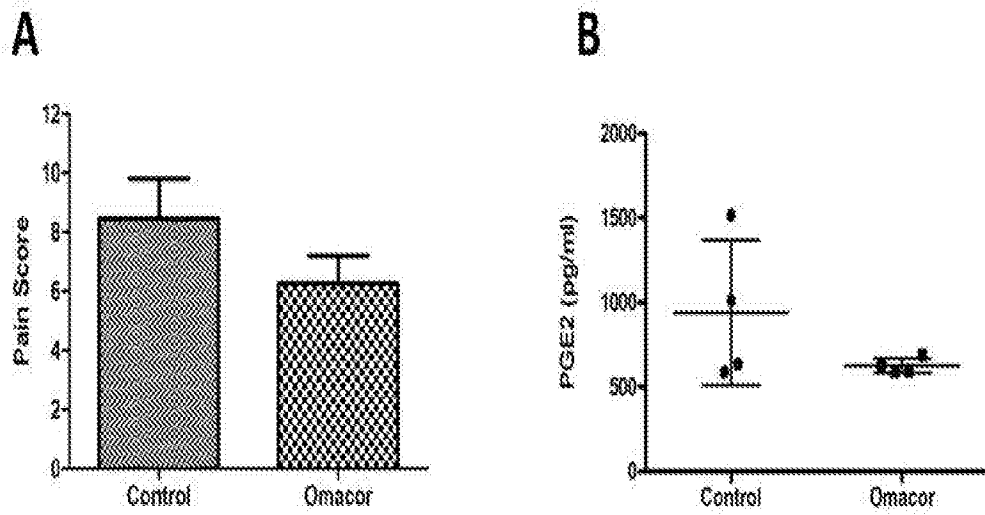


FIGURE 3



INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2015/051853

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/00 A61K31/045 A61K31/20 A61P15/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/095437 A1 (PHARMANUTRA S R L [IT]; PETRAGLIA FELICE [IT]; LUISI STEFANO [IT]; LAC) 6 August 2009 (2009-08-06)	24
Y	claims 1-16	1-24
Y	----- NETSU S ET AL: "Oral eicosapentaenoic acid supplementation as possible therapy for endometriosis", FERTILITY AND STERILITY, ELSEVIER SCIENCE INC, NEW YORK, NY, USA, vol. 90, no. 4, 1 October 2008 (2008-10-01), pages 1496-1502, XP025479669, ISSN: 0015-0282, DOI: 10.1016/J.FERTNSTERT.2007.08.014 [retrieved on 2007-12-03] cited in the application figure 1 ----- -/-	1-24

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

1 October 2015

Date of mailing of the international search report

19/10/2015

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INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2015/051853

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2015/051853

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members

International application No

PCT/GB2015/051853

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