Title of project: Investigating immune-modulatory actions of Nociceptin/Orphanin FQ (N/OFQ) system in peripheral human blood

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Background:
There is a body of evidence supporting that clinical and recreational use of opioids can modulate the innate and adaptive immune function with some degree of controversy regarding the exact mechanisms of such effect.[1] Opioid receptors are classified as MOP (μ, mu), DOP (δ, delta) and KOP (κ, kappa) or classical naloxone sensitive receptors and NOP (receptor for nociceptin/orphanin FQ [N/OFQ]) which is naloxone insensitive.[2] Opioids in current medical practice act mainly as agonists on classical opioid receptors (MOP, KOP, DOP).[1] It has been suggested that opioid induced immune modulation may occur via direct action on immune cells themselves. Several previous studies suggested the expression of classical opioid receptors on peripheral blood mononuclear cells (PBMC's)[1,3] ;However the presence of opioid receptors in peripheral individual immune cells was evaluated by our group in series of experiments[4-6] failing to detect MOP, KOP, and DOP receptors but identified NOP receptor on immune cells and peripheral whole human blood. PCR NOP mRNA transcripts detected by our group were strongly expressed and consistent leading to the conclusion that NOP functioning receptor protein is present in peripheral human blood.

There is a relative lack of functional information and the significance of NOP receptor on immune cells, their involvement in immune modulation/regulation, and whether or not the N/OFQ-NOP system can be a potential future immune modulatory therapeutic route.

We conducted series of experiments studying mRNA expression classic and non-classic opioid receptors in peripheral human blood and effects of different opioid and N/OFQ agonists/antagonists on peripheral immune cells migration in vitro (native from volunteers and cell line based).

Methods:
This investigation was conducted according to the University of Leicester approval for Anaesthesia, ICU, Pain, and Cardiovascular volunteer research. We included 10 healthy volunteers from our academic department (4 females and 6 males) who all gave their written informed consent and had mean age of 40.6 years (range 32-54), mean weight of 86.4 Kg (range 55-110) and mean height of 174.9 cm (range 153-196). 10-30mls of venous blood was collected into EDTA containing monovette® tubes using 21G needle from anticubital fossa veins. Whole blood was divided in individual 2ml screw top micro centrifuge sterile tubes under Class 2 microbiological safety cabinet conditions at room temperature. Samples were incubated for 24 hours (overnight) at 37.0°C and 5.1kpa carbon dioxide humidified air environment at atmospheric pressure after the addition of drugs and reagents. Positive control samples were prepared utilising Chinese Hamster ovary cells (CHO) expressing MOP, KOP, DOP, and NOP receptors.

Blood was incubated overnight in variable conditions (plain control, culture media, Lipopolysaccharide, Morphine, Fentanyl, and Peptidoglycan) in different combinations to study mRNA opioid receptors expression.

We performed whole blood RNA extraction using RiboPure™-Blood kit (AM1928) and protocol by Ambion® (life technologies). RNA clean-up and cDNA reverse transcription was performed using commercially available kits and protocols (Ambion® and High-capacity cDNA reverse transcription kit (Applied Biosystems® – life technologies™ respectively)

Real time quantitative Polymerase chain reaction qPCR was conducted using Applied Biosystem® StepOne™ System and protocol. Genetic material quantification was performed using NanoDrop™ 2000 UV-Vis Spectrophotometer from Thermo Scientific®. Reverse transcription, thermal incubation and thermo cycling PCR reactions were performed using eppendrof® Mastercycler® equipment

We used Gel electrophoresis method and DNA sequencing to test for DOP receptors mRNA due to the false positive results and limitations of qPCR DOP probes in detecting genomic DNA rather than mRNA.

Peripheral blood polymorph-nuclear cells (PMN) were isolated using gradient method (Axis shield Polymorphprep®) and quantified using a haemocytometer counting chamber. 3micron cell culture Trans well cell migration assay inserts were used for functional cell analysis. PMN cells were then incubated for two hours in different doses of NOP agonists/antagonists (Nociceptin, Ro64-6198 (non-peptide agonist), UFP-101 (peptide antagonist/partial agonist) SB612111 (non-peptide antagonist) at clinically relevant concentrations (picomolar –nanomolar range)) in various combinations to study their effect on cell migration. fMLP was utilised to encourage PMN's chemotaxis before cell collection and quantification after the 2 hours incubation period. Morphine was added to some wells to further study the lack of functional classical opioid receptor agonist effects.

Results:
Peripheral human whole blood expressed no classical opioid receptors (MOP, KOP, DOP) mRNA (and by inference receptor protein) despite overnight incubation with variable opioid receptor agonists and in-vitro sepsis models. NOP receptors mRNA was expresses in all samples with inconsistent effects of in vitro sepsis on their expression.

Nociceptin (endogenous NOP receptor peptide agonist) caused significant inhibition of trans-well PMN cell migration with Ec_{50} of 8.2 nM. Morphine caused no significant difference in PMN cell migration.

Conclusion:
Peripheral human blood expresses no classical opioid receptors which indicate that direct peripheral immune-modulatory opioid effects on immune cells unlikely. Nociceptin/orphanin FQ system is both expressed and causes functional alteration of PMN cell migration (and y inference function) which may be of future therapeutic potential.

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Publication:
This work is part of an MD project with the University of Leicester and will be submitted for publication in the British Journal of Anaesthesia.

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