Impact of angiotensin receptor type 1 antagonists in neuropathic pain and opioid analgesic tolerance

Ph.D. thesis

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1. Introduction

Despite the large arsenal of analgesic medications available, neuropathic pain (NP) management is not solved yet. Based on preclinical studies carried out in the last decades, angiotensin receptor type 1 (AT1) has been proposed as a potential pharmacological target in the treatment of NP. In addition, connections between the renin-angiotensin system (RAS) and opioid system regarding analgesia have been described by our research group [1] and others [2,3]. Here we present the first evidence for an enhanced antiallodynic effect and a delay in opioid analgesic tolerance following co-administration of an angiotensin receptor blocker (ARB) and morphine.

2. Objectives

The present Ph.D. work aimed to investigate:

1. the potential effect of AT1 receptor antagonists, losartan or telmisartan, in reducing allodynia in an acute treatment setting in NP.
2. to evaluate the effect of a combination of AT1 receptor antagonists and morphine in NP.
3. to assess whether co-treatment with of AT1 receptor antagonists influences morphine analgesic tolerance.
4. to assess the side effect profile of test compounds regarding motor function in doses achieving promising results *in vivo*.

In order to understand more about the molecular and pharmacological mechanisms of the interaction between the RAS and opioid system, we have carried out the following studies:

5. measuring the influence of telmisartan on changes observed in morphine-stimulated [^{35}S]GTPγS binding in the spinal cord of rats with NP after repeated morphine administration.

6. identifying the potential off-target effect of activating PPARγ for tested AT1 receptor antagonist compounds in the opioid tolerance model.
7. assessment of the degree of microglial infiltration in the spinal cords of chronically treated, opioid tolerant animals and check the effect of AT1 receptor antagonists.
8. mapping spinal neuroanatomical localisation of the target receptors (AT1 and MOR) utilising RNA Scope® *In-Situ* RNA Hybridisation
9. measuring the influence of AT1 receptor antagonists alone or in combination with morphine on CSF L-glutamate and D-serine content in neuropathic and opioid tolerant rats.

3. Methods

3.1. Mononeuropathic pain model

Following an acclimatisation period, male Wistar rats weighing 120-150 g underwent partial sciatic nerve ligation (pSNL), as described previously [4,5]. The development of mechanical allodynia as the hallmark symptom of NP was assessed using dynamic plantar aesthesiometer (DPA). Animals received acute or chronic oral treatment with ARBs losartan (50, 100 or 150 μmol/kg) or telmisartan (20, 40 or 80 μmol/kg) alone or in combination with subcutaneous morphine (10 μmol/kg).

After 10 days of chronic treatment, animals were sacrificed, and spinal cord and cerebrospinal fluid (CSF) samples were taken. Morphine-stimulated [^{35}S]-GTPγS binding assays were performed on spinal cord samples. L-glutamate and D-serine content of CSF samples was determined by capillary electrophoresis.

3.2. Morphine analgesic tolerance model

To assess opioid antinociceptive tolerance, in a separate experimental setting, male Wistar rats weighing 170-200 g were

subjected to repeated treatment with high-dose subcutaneous morphine (31,08 μmol/kg), oral ARBs (telmisartan (20 μmol/kg) or losartan (50 μmol/kg)) and their combinations. The antinociceptive efficacy of morphine was monitored using radiant-heat tail-flick test over a 10 day period. Next, CSF samples were collected to determine L-glutamate and D-serine content by capillary electrophoresis.

In another set of experiments animals received treatment in a similar manner as described above, and the selective PPARγ antagonist GW9662 (7.22 μmol/kg) or its vehicle intraperitoneally. Animals in this experiment were sacrificed after 10 days of chronic treatment and spinal cord tissue samples were collected. Subsequently, these samples were postfixed, embedded in paraffin and subjected to immunohistochemical analysis using anti-iba1 primary antibodies.

3.3. Motor function testing

The rat rotarod test was used to assess the effect of oral telmisartan (80 μmol/kg), subcutaneous morphine (10 μmol/kg), or their combination on the motor function of Wistar rats weighing 170-200 g in an acute treatment setting. High dose morphine (31.08 μmol/kg) was used as a positive control.

3.4. RNA Scope® *in-situ* mRNA hybridization

Naïve Wistar rats weighing 200-240 g were sacrificed and dorsal root ganglion (DRG), spinal cord brain tissue samples were collected. RNA Scope® *In-Situ* Hybridisation assay was performed using RNA Scope® Multiplex Fluorescent Kit v2 according to the instructions of the manufacturer. Probes specific to Agtr1a, Agtr2, Oprm1, Calca and Vglut1 mRNAs were hybridised. Punctate red/green/magenta dots were identified as the specific RNA staining signal. Cell nuclei were stained with 4’,6-diamidino-2-phenylindole (DAPI).

4. Results

4.1 Mononeuropathic pain model

Oral telmisartan (40 or 80 μmol/kg) or losartan (100 μmol/kg) produced acute antiallodynic effect. Upon chronic treatment of mononeuropathic animals the combination of subanalgesic doses of telmisartan and morphine ameliorated allodynia and resulted in a significant leftward shift in the dose-response curve of morphine in [^{35}S]GTPγS binding assay, indicating restoration of morphine potency. L-glutamate content was increased, while D-serine content was decreased significantly only by the combination of telmisartan and morphine in the CSF of chronically treated neuropathic animals.

4.2 Morphine analgesic tolerance model

Telmisartan or losartan in subanalgesic doses were able to delay morphine analgesic tolerance development. Similarly to the experiments described above, only the combination of telmisartan and morphine was able to significantly increase L-glutamate and decrease D-serine levels in the CSF of chronically treated animals.

Co-treatment with selective PPARγ antagonist GW9662 attenuated the beneficial effect of losartan or telmisartan on the development of morphine analgesic tolerance. Morphine treatment induced an increase in microglial infiltration of the spinal cord, which was reversed by co-treatment with ARBs. Co-treatment with GW9662 produced a moderate, not significant increase in the number of microglial cells for losartan and a significant increase for telmisartan.

4.3 Telmisartan and morphine combination is devoid of causing motor dysfunction in rats

Telmisartan, low-dose morphine or their combination was devoid of causing motor dysfunction. High-dose morphine treatment produced a significant disturbance in motor coordination.

4.4 OPRM1 mRNA co-localises with AGTR1A and AGTR2 mRNA at key points of pain transmission

Colocalisation of Agtr1a and Oprm1 mRNAs were found in cells in the periaqueductal gray matter, the spinal cord dorsal horn and the DRG. In the latter, these structures also highly colocalized with peptidergic neural marker Calca. Lower level of colocalisation between the Agtr2 and Oprm1 were found in all localisations described above.

5. Conclusions

1. The AT1 receptors are likely to participate in the development of neuropathic pain, as AT1 receptor antagonists, **losartan** or **telmisartan alleviate mechanical allodynia**, in **high systemic doses**, following **acute administration**.
2. The antiallodynic effect of **morphine is only achieved** in **high systemic doses**, not devoid of central side effects. This is in line with previous works.
3. Utilizing the co-operation between MOR and AT1 receptor could be a future strategy to treat neuropathic pain, as the combination of **morphine** and **telmisartan** in **subanalgesic doses** produces **significant antiallodynic effect** upon long-term administration.
4. **Chronic combination treatment** with antiallodynic capabilities was **devoid of analgesic tolerance**, the current obstacle to go on with long-term opioid treatment.
5. **Long-term** treatment with **high doses of morphine** results in the development of **opioid analgesic tolerance**, accompanied by the activation of spinal microglia. **Losartan or telmisartan rescues morphine analgesic efficacy** upon long-term treatment by restoring G_i mediated effect of morphine and inhibiting spinal microglial infiltration.

6. Beside blockade of AT1 receptors, activation of **PPARγ likely contributes to the effect of losartan and telmisartan**.
7. The co-operation between **AT1 receptors** and **MORs** is supported by their **colocalisation at key points** related to pain transmission (DRG, spinal cord dorsal horn and PAG). In the DRG, these structures also colocalise with peptidergic neuronal markers.
8. **Inhibition of NMDA receptor overactivation** by decreasing the levels of co-agonist D-serine, is likely to be involved in the beneficial effect of telmisartan.
9. Our results reveal **possible strategies of opioid dose tapering** through concomitant application of AT1 antagonists and opioid analgesics.

6. References

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