A NOVEL PHARMACOLOGICAL APPROACH FOR THE TREATMENT OF NEUROPATHIC PAIN: TOLL-LIKE RECEPTOR 4 ANTAGONISTS AS MODULATORS OF ACTIVATED MICROGLIA

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Recent findings suggested that activated microglia strongly contribute to the maintenance of neuropathic pain. Among the receptors expressed on activated microglia in the spinal cord there are the Toll-like receptors 4 (TLR4) whose stimulation cause production and release of inflammatory mediators including pro-inflammatory cytokines, glutamate, prostaglandins and nitric oxide from glial cells. These agents are then capable of further enhancing glial activation and production of inflammatory mediators that sensitise dorsal horn neurones, thereby contributing to neuropathic pain. On the basis of the above quoted evidence, we have developed new molecules with TLR4 antagonistic activity to be employed in vivo to study whether the modulation of microglial activation can represent a novel therapeutic target for the treatment of neuropathic pain First we have characterized the pharmacological properties of such new molecules as selective antagonists of the TLR4 receptor monitoring the activation of NF-kB (the final step after the stimulation of TLR4) in HEK293 cell line stably transfected with human TLR4. The data indicate that the compounds are able to inhibit the NF-kB activation induced by LPS in HEKTLR4+ cells, while no effect was observed in the same cells transfected with human TLR9 receptor and stimulated with the selective agonist (CpG). The most potent molecule has been then used in vivo, employing the well characterized animal model of neuropathic pain: the chronic constriction injury of the sciatic nerve in mice. The drug was administered i.p. (5 mg/kg) once a day for one week starting the day after the sciatic nerve lesion. This therapeutic regimen evoked a relief of both thermal hyperalgesia and mechanical allodynia in neuropathic mice. The antihyperalgesic effect was accompanied by a decrease in NF-kB activation in the nuclear extracts from spinal cord (L4-L6 segment) so suggesting that the compound reduce the production of pronociceptive and proinflammatory mediators inhibiting the activation of the transcription factor responsible for their expression via TLR4 blockade. The pharmacological effect was absent when the compound was administered to TLR4 knockout animals, so showing the specificity of the action. Furthermore, the demonstration that the TLR4 knockout mice developed neuropathic pain with an hyperalgesia smaller than that observed in wild type strongly support the idea of a key role of TLR4 receptors in the pathogenesis of neuropathic pain. The findings strongly suggest that the inhibition of microglia activation through TLR4 antagonists may represent a new strategy to treat neuropathic pain.