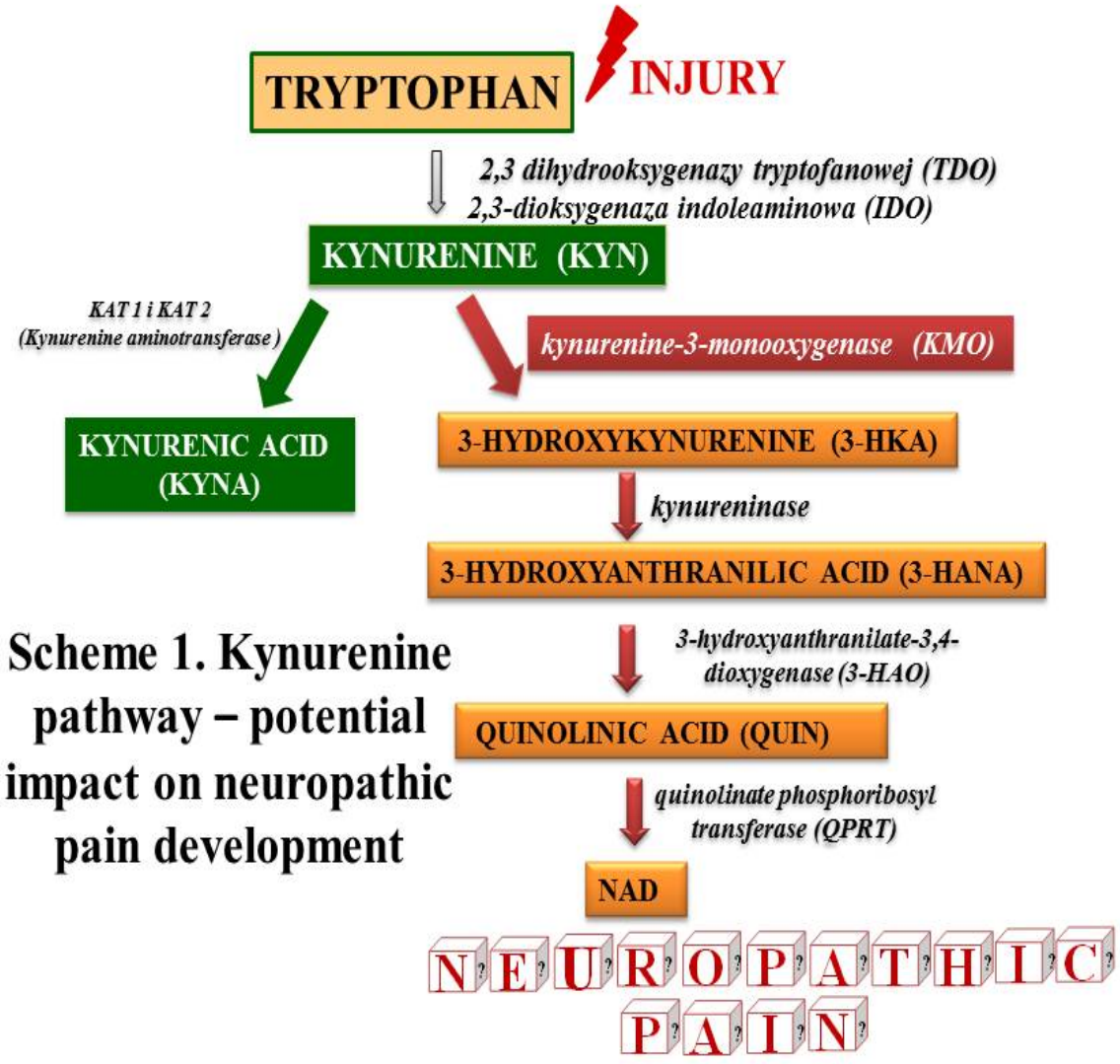
Pain acts as a warning and as a defense. It is a signal of a potential danger. In order to minimize the effects of tissue damage it triggers responses of the organism. But there are certain situations when pain ceases to act as a warning signal and becomes a source of great suffering. Such phenomenon is well-known as a chronic pain in its various forms: lasting after a surgery, after an injury in spite of tissue healing, or being accompanied by chronic illnesses such as cancer, diabetes or osteoarthritis. In such cases we face a situation when pain sensations lose their warning-defensive character and pain in itself becomes a disease and requires comprehensive and multi-dimensional proceedings. This leads to lowering a health-related quality of people's lives. Moreover, it adversely affects their emotional, physical and social functioning, burdens health care systems, and at the same time generates high social and economic costs. Neuropathic pain caused by a primary injury or dysfunction in the peripheral or central nervous system is a tremendous therapeutic challenge. Only in some cases it is possible to achieve a significant relief. Yet, in the vast majority of patients, pain is resistant to analgesic treatment and involves experiencing various side effects or intolerance of drugs used in neuropathic pain treatments. Despite numerous studies being conducted throughout the world and undoubted advances in medicine this very pain remains serious therapeutic problem. Therefore, it is so important to study the mechanisms of pain and to discover new targets for its therapy with the use of models since it will help to carry out the analysis of changes that occur as a result of a damage. In our Laboratory we have been using the model of chronic constriction injury (CCI) (the Bennett's Model) for many years. Studies involving gene expression profiling and mass spectrometry suggest that neuropathic pain is associated with a strong activation of certain neuronal genes, as well as genes associated with immune cell responses including microglial activation. Recent studies published in *Molecular Pain* in 2014 enabled us to identify the genes whose expression changes after a nerve injury. One of these genes - KMO (kynurenine 3-monooxygenase - kynurenine pathway enzyme) has become the subject of further research which is also the main theme of this project. Past published data suggest that this enzyme plays a vital role in pathology of many autoimmune and neurodegenerative disorders, however its role in neuropathic pain is still unknown.
The key point in the grant will be the investigation of what exact effect on the changes in the activity of the enzymes and products of kynurenine pathway has a damage that initiates neuropathic pain. As our previous studies have shown, the activity of one of the enzymes of kynurenine pathway - KMO - escalates in neuropathic pain. It seems that other enzymes of the pathway, may have a significant impact on nociception processes and therefore we want to make an attempt to modulate them (Scheme 1).

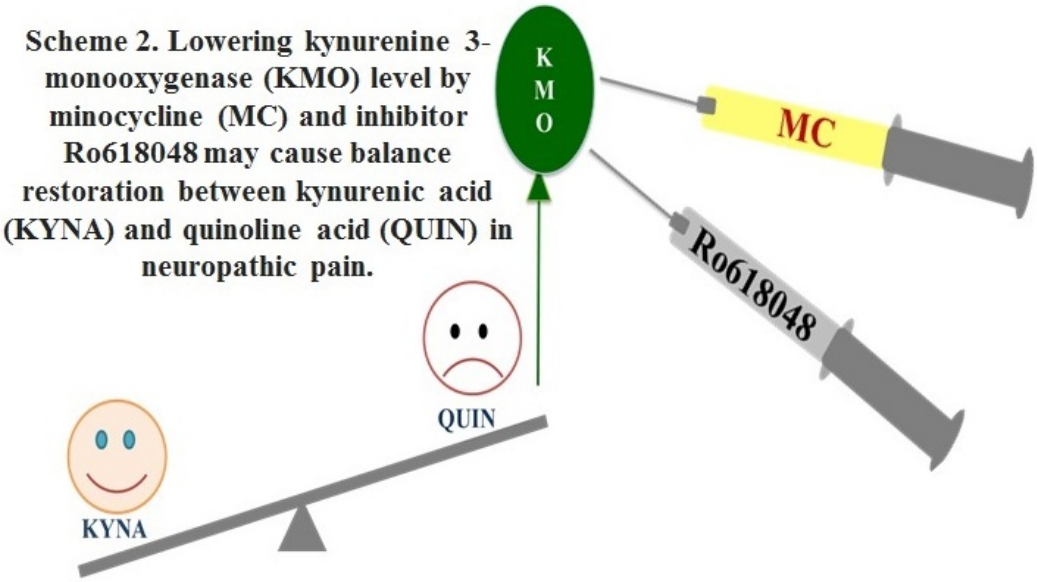


Scheme 1. Kynurenine pathway – potential impact on neuropathic pain development

Within the framework of the research tasks set out in the grant we are planning to investigate whether KMO enzyme plays a key role in the activation of one of the two pathways: neuroprotective and neurotoxic. The inhibition of enzyme activity in neurotoxic pathway may lead to the advantage of neuroprotective pathway and may be one of the ways of protecting neurons from injuries. We assume that the strengthening of neuroprotective pathway through increasing the amount of kynurenic acid secretion can produce analgesic effects. An important objective of the project is marking a cellular source of the products or/and enzymes of kynurenine pathway, what is planned to be checked in one of the stages of our research concerning primary culture of microglia

and astroglia. Subsequently to determining the cellular source of selected products or enzymes of kynurenine pathway we are planning to use microglia inhibitors or/and astrocytes inhibitors. In this task we wish to get the answer whether the inhibition of microglia and astroglia activation after administrations of inhibitors - minocycline and fluorocitrate respectively, affects nociception processes. Parallel to behavioral studies, biochemical tests will be carried out in order to determine whether inhibiting of a selected enzyme changes the levels of enzymes and products important for a particular pathway. Our hypothesis assumes that if the activation of kynurenic acid occurs in astrocytes, it can induce neuroprotective and analgesic effects. By contrast, increasing KMO enzyme activity may lead to the production of toxic products of the pathway (e.g. quinolinic acid) what may result in the increase of pain sensations. The suppression of KMO activation through the administrations of an inhibitor (e.g. Ro618048) or minocycline may demonstrate neuroprotective effects (Scheme 2).

It will shield neurons and in the future it might have enormous therapeutic relevance. The idea of our project is to investigate whether pharmacological modulation of the changes in the activation of neurotoxic and neuroprotective kynurenine pathway can inhibit pain development. In the project we are also planning biochemical studies on the changes of proteins activity and/or mRNA level after the modulation of kynurenine pathway. The results will allow us to identify the changes of a broad spectrum of tryptophan metabolites belonging to kynurenine pathway and will supplement fundamental knowledge about functioning of the organism under normal and pathological conditions. Few, preliminary studies concerning the possibilities of kynurenine pathway modifications in neurodegenerative disorders raise great hopes for their clinical use in the future. We do hope that they will also apply in neuropathy.



Scheme 2. Lowering kynurenine 3-monooxygenase (KMO) level by minocycline (MC) and inhibitor Ro618048 may cause balance restoration between kynurenic acid (KYNA) and quinoline acid (QUIN) in neuropathic pain.

The results of the research tasks outlined in this project will supplement the existing knowledge about the mechanisms responsible for the development and sustainability of neuropathic pain symptoms and therapeutic problems associated with this problem. It appears that the pharmacological modulation of kynurenine pathway might provide satisfactory therapeutic effects in the future. Therefore, we would like to make an attempt and identify imperative stages of kynurenine pathway in terms of pain treatment. We have hope that the obtained results will be the basis for testing pharmacological modulations of these changes and exploring their impact on neuropathic pain development. The results will also allow us to recognize behaviors of a wide spectrum of tryptophan metabolites belonging to kynurenine pathway.

Today it is still too early to determine whether the use of inhibitors or stimulators of this pathway may be important in diagnostics or therapy. Therefore, preliminary studies, which are the basic idea of this project, are crucial. For this reason, the projects going to define the mechanisms of chronic pain development and on the other hand it is going to seek new targets for its therapy. This is a highly vital issue since complaints accompanying pain significantly reduce the quality of patients 'lives and the possibilities of their daily functioning. According to various literature data the efficacy of neuropathic pain treatment, despite systematic introductions of new techniques and drugs and ongoing searches for more effective treatment options, is still unsatisfactory.