

The characterization and assessment of immunological response after utilization of epineural grafts in prevention of posttraumatic neuroma formation in peripheral nervous system

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Summary

The peripheral nervous system has significant regenerative capacity as compared to the central nervous system. Interruption of peripheral nerves continuity elicit multivariate cascade of events. Within few minutes after injury Schwann cells and surrounding tissues release numerous proinflammatory cytokines which initiate infiltration of immunocompetent cells from blood vessels, among which the most numerous are macrophages. Their role is to secrete neurotrophic factors inducing axonal regrowth towards distal stump. In case of the absence of distal nerve stump or if the distance between proximal and distal nerve stump is too long and lack of appropriate scaffold to provide direction for growing axons, it may occur chaotic hypertrophy of nerve and connective tissue subsequently forming traumatic neuroma. These structures are responsible for generation of neuropathic pain which significantly reduce quality of life of patients after extensive surgery or limb amputation.

Many different methods were applied for treatment of neuroma, but none of these techniques is effective enough to be a standard in clinical application. Pharmacotherapy is the first choice of treatment in case of neuropathic pain of different origin. The most important limitation of long-term drug treatment is oppressive side effects occurrence and often the lack of desired effects. Moreover, when the neuroma is diagnosed, surgical removal may be executed, but after this procedure there is still the same risk of neuroma formation at the free distal stump. Facing described difficulties the isolation of nerve stump from the proregenerative impulses from surrounding environment during surgical procedure of amputation by different capping techniques may constitute an appropriate solution in preventing of neuroma formation. For this purpose implantation of nerve stump inside a vein or muscle are widely used. Despite promising results availability of veins as well as muscles in surrounding tissues frequently may be limited, thus this technique is not dedicated for many patients. To avoid this kind of problems transplants of soft tissues of various origin and synthetic materials are extensively studied and perform promising results in inhibition of neuroma formation. However, these materials present

some limitation as in case of soft tissues the use is restricted by major histocompatibility complex that force clinicists to implant mainly autologous transplants. Whereas synthetic materials have unlimited availability, they are impermeable for blood vessels and other cells and as a foreign material for the body may induce inflammatory response.

The perfect solution to avoid presented problems is implementation of biological material that is immunologically neutral. Such requirements may be fulfilled by the epineurium. Epineurium is the outermost layer of connective tissue surrounding nerve fascicles in peripheral nervous system. It mostly consists of type I and III collagen, thus it can be used on the free proximal nerve stump as cap (epineural jacket) which will prevent neuroma formation by providing a barrier and isolation from impulses stimulating neuroma outgrowth. Up to date the structure and function of epineurium were thoroughly described; however, there is the lack of studies about its immunological, neurogenic and angiogenic properties. This knowledge may constitute a very important source of information from the transplantation perspective.

To meet presented requirements the first aim of this thesis was to characterize the biology of epineurium by comparing immunological, neurogenic and angiogenic properties of epineural sheaths collected from ilioinguinal nerves taken from deceased organ donors with epineurium from sciatic nerves taken from limbs amputated due to critical limb ischemia. The results obtained from this stage of research provided the knowledge about optimal source of epineurium and allow to start next experiments where the efficacy of epineurium in prevention of neuroma formation were tested. The epineural jacket were applied on the proximal stump of transected rat's sciatic nerve in isogenic (rat's epineurium) and xenogenic (human's epineurium) transplantation model. Moreover, the immunological response to transplant was assessed by analysis of the presence of immunocompetent cells in operated area after surgical procedure. The influence of immunological and neurotrophic factors on neuroma formation and neurophatic pain generation was evaluated as well by comparing the results after xenogenic epineural transplant in rats with functional thymus with athymic rats deprived of the thymus function.

After the first step of experiment it was established that epineurium obtained from ilioinguinal nerves taken from deceased organ donors presented reduced number of T lymphocytes, decreased expression of HLA class II antigens on infiltrating cells and more numerous blood vessels comparing to epineurium collected from amputated limbs. Less immunogenic and higher proangiogenic properties of epineurium from organ donors proved that this material constitutes a safer tool and may serve as biologic material in transplant and

regenerative medicine. Thus, this source of epineurium was used in the next stage of this experiment.

Results obtained in second stage of research revealed no characteristic signs of neuroma formation in any of groups where epineural jacket was applied, compared to group with nerve defect without epineural protection where neuroma was developed as confirmed by behavioral tests and by histology. Thus, epineurium effectively inhibit neuroma formation in peripheral nervous system. The features of inflammation were observed in the operated area in rats with isogenic and xenogenic epineural transplants; however, there were no neuroma outgrowth. Persistent inflammation in animals with functional thymus and transplanted epineurium induce and maintain neuropathic pain that impair sensory and motor function of operated limb but less intense than development of neuroma. These observations suggest low immunogenicity of epineurium isolated from peripheral nerves presumably due to the presence of blood vessels and tissue-resident scattered cells like macrophages, fibroblasts, mast cells and single lymphocytes. Importantly, this biological feature of epineurium has no influence on efficacy of this material in preventing neuroma formation in peripheral nervous system. This was proved in the group of athymic rats, where reduced function of the immune system resulted in response to chemical, physical and thermic stimulation at the end of follow-up period approximately equal to unoperated contralateral limb.