● PERSPECTIVE

Diabetes, its impact on peripheral nerve regeneration: lessons from pre-clinical rat models towards nerve repair and reconstruction

The global number of patients with type 1 and type 2 diabetes is about to increase substantially in the coming decades. The reasons for this are two-fold, at first there is actual increase in incidence of type 1 diabetes and at second there is global increase in living expectancy and a high prevalence of patients with type 2 diabetes among the elderly. The diabetic condition is affiliated with reduced peripheral nervous system maintenance, such as peripheral neuropathies (Juster-Switlyk and Smith, 2016) that may be more common in men.

Additionally, not only in young adults, but also in the active elderly population, there is a high incidence of peripheral nerve functional impairment and injuries of which with a transected or lacerated nerve trunk need urgent surgical intervention, such as repair or reconstruction. Therefore, either a tension-free end-to-end repair with sutures or, in case of additional nerve tissue loss, a reconstructive nerve grafting procedure to bridge the gap between the transected nerve ends is necessary.

The gold standard grafting procedure for acute and delayed nerve gap reconstruction is the interposition of autologous nerve grafts, harvested from a less important nerve (Siqueira and Martins, 2017). The use of processed nerve allografts, such as AxoGen® Nerve Allograft, has been proposed as an alternative to the use of autologous nerve grafts for reconstruction. The clinical use of processed allografts is, however, less widespread in Europe than in the USA, where also comprehensive clinical data have been collected (RANGER study, Cho et al., 2012). Alternative approaches for nerve reconstruction further include the use of autologous muscle-in-vein grafts, which are of an autologous vein segment filled with autologous muscle fibers (Geuna et al., 2014). The use of muscle-in-vein grafts is to the best of our knowledge mostly performed in specific cases of acute reconstruction of digital nerve injuries. The experimental use of muscle-in-vein grafts in the rat sciatic nerve critical gap lengths (15 mm) model for delayed reconstruction (45 days after injury) does fail to induce a considerable rate of functional recovery (unpressed). Muscle-in-vein grafts have thereby demonstrated a decreased efficacy in comparison to the second alternative approach of using bioartificial nerve guides for the gap reconstruction (unpressed). Bioartificial nerve guides comprise off-the-shelf solutions to bridge peripheral nerve gaps (Tian et al., 2015). Nerve guides made out of polyglycolic acid, poly-D,L-lactide acid and poly-ε-caprolactone copolymers, or collagen have received clinical approval as NeuroTube®, Neurolac®, and NeuraGen® Nerve Guides, respectively, but are currently not approved for clinical use in long distance reconstruction (> 2.6 cm, Tian et al., 2015).

For successful peripheral nerve regeneration, which finally leads to recovered function of the distal sensory and muscular targets, the timely formation of a regenerative matrix between the separated nerve ends is crucial to allow proper formation of a vascularized nerve trunk with Schwann cell columns (Bands of Büngner) and axonal outgrowth (Haastert-Talini, 2017). When the conditions of general health impairment by diabetes, or even evident peripheral neuropathy, and a peripheral nerve transection or laceration injury coincidence, the patients' perspective for sufficient outcome of nerve repair or reconstruction is expected to be lowered. Therefore, we have recently performed several experimental studies investigat-

ing differences between healthy and diabetic rats during the initial phase after nerve repair or reconstruction (six days, three weeks, six weeks). Wild type rats were compared to diabetic Goto-Kakizaki (GK) rats, with moderately increased and human type 2 diabetes relevant blood glucose levels. Repair or reconstruction approaches investigated were comprised of (I) acute end-to-end repair with sutures (Stenberg and Dahlin, 2014), (II) acute autologous nerve grafting (Stenberg et al., 2016), (III) acute nerve bridging with a chitosan nerve guide, addressing both a moderate gap length of 10 mm (Stenberg et al., 2016) and a critical distance of 15 mm (Meyer et al., 2016), as well as (IV) also the 45 days delayed critical length nerve gap reconstruction with chitosan nerve guides (Stenberg et al., 2017). Furthermore, for acute nerve reconstruction, two types of chitosan guides were compared-hollow ones (hCNGs) and those separated into two longitudinal chambers by an introduced chitosan film (CFeCNGs) (Meyer et al., 2016). To estimate the prospects for regeneration outcome, the quality of the initial regeneration process has been evaluated by analyzing the regenerative matrix formation, length of axonal outgrowth, and activation and apoptosis of Schwann cells at different distances from the repaired or reconstructed proximal nerve end.

Generally, one may expect that the regeneration process is more efficient in healthy than in diabetic individuals. Studying the selected parameters six days after nerve repair by end-to-end sutures, we indeed found the nerve regeneration process (length of axonal outgrowth) to be better in healthy than in diabetic GK rats. The difference was more obvious for female rats. Interestingly, male rats, with higher blood glucose levels than female rats in both, the healthy and the diabetic condition, demonstrated an improved axonal outgrowth, although the general weight increase was similar for both sexes. The high blood glucose levels in the diabetic GK rats, resembling human type 2 diabetes, correlated with an increased axonal outgrowth, and with an increased number of activated Schwann cells in the distal nerve segment. The latter also positively correlated with the number of apoptotic Schwann cells, indicating a balance in proliferation of Schwann cells during the nerve regeneration process (Stenberg and Dahlin, 2014).

The same parameters as above were analyzed at three weeks after acute autologous nerve grafting and chitosan nerve guide reconstruction of a moderate length 10 mm rat sciatic nerve gap. Surprisingly, a significantly extended axonal outgrowth was detected in the diabetic GK rats compared to healthy rats after both nerve reconstruction approaches. The autologous nerve graft reconstruction was superior probably due to the presence of higher numbers of activated Schwann cells. The regenerative matrix in the chitosan nerve guide was thicker in diabetic rats. In contrast to the nerve repair by end-to-end sutures, increased blood glucose levels improved the conditions for axonal outgrowth in gap-bridging nerve reconstruction approaches; a phenomenon that could be related to a regeneration process proceeding in an environment with not yet reestablished vascularization. This was especially intriguing because the association between activated and apoptotic Schwann cells and axonal outgrowth was not linear after applying the reconstruction approaches in the healthy and diabetic GK rats (Stenberg et al., 2016).

When in the next step a 15 mm critical gap length in a rat sciatic nerve defect was bridged with classic hollow (hCNGs) or two-chambered chitosan film containing (CFeCNGs) chitosan nerve guides, a substantial regenerative matrix was detected at 6 weeks after repair in wild type and diabetic GK rats, irrespective of the general health condition. We could especially demonstrate that during the phase of regenerative matrix formation, axonal regeneration was equally supported in healthy and diabetic GK rats after critical nerve defect reconstruction, particularly with CFeCNGs. Therefore, we concluded that peripheral nerve reconstruction by means of classic or enhanced chitosan nerve guides represents a promising alternative to standard approaches for nerve reconstruction also in diabetic

conditions (Meyer et al., 2016).

To go ahead in our translational research, we finally went another step further and delayed the reconstruction of a 15 mm critical gap length in rat sciatic nerve defects for 45 days. Reconstruction was then again performed using one-chambered classic hCNGs or two-chambered chitosan film enhanced CFeCNGs in healthy Wistar rats and diabetic GK rats. Our results surprisingly demonstrated that the diabetic condition did not affect the outgrowth of axons into the nerve distal to the nerve guides at 6 weeks after a delayed nerve reconstruction, although overall results were inferior to that observed after an immediate reconstruction. We concluded that the delayed reconstruction condition did itself modify the regeneration milieu in a way that compensated the differences between the regenerative capacities of healthy and diabetic peripheral nerves, where also the complex neuroprotective effect of HSP27, a neuroprotective protein expressed in the sensory neurons, may be considered (Stenberg et al., 2017).

As one reason for only minimal differences between healthy and diabetic GK rats after critical nerve defect reconstruction with CFeCNGs, *in vitro* studies provided us with indications that the chitosan film specifically promoted the formation of the regenerative matrix by an immunomodulatory effect. This effect triggered the polarization of blood derived monocytes towards the pro-regenerative M2c macrophage subtype, which has specific beneficial effects on extracellular matrix remodeling (Stenberg et al., 2017).

Taken together, depending on the type of nerve repair or reconstruction procedures analyzed, and the time point chosen for this analysis after the procedure, we found indications that increased blood glucose levels impaired regeneration after nerve end-to-end repair at 6 days, but could, against common expectations, positively influence the early regeneration process at 3 weeks after nerve gap reconstruction (Stenberg and Dahlin, 2014; Stenberg et al., 2016). On the other hand, we found even no relationship between normal and diabetic blood glucose levels at 6 weeks after acute or delayed reconstruction of an extended critical length nerve gap using an appropriate nerve guide (Meyer et al., 2016; Stenberg et al., 2017). Again, it is noteworthy that our results indicated a supported early regeneration process in diabetic GK rats in comparison to healthy rats when acutely treated with the hollow hCNGs or with the gold standard autologous nerve graft (Stenberg et al., 2016).

With regard to the translational impact of pre-clinical work performed in rat models, it has to be noted that scientific evidence provided through the research of the authors (Haastert-Talini et al., 2013) significantly contributed to the clinical approval of the Reaxon® Nerve Guide, which is mainly composed of chitosan, in January 2014. The chitosan nerve guide is in clinical use since then with no negative interference reported so far. Currently, also a clinical trial is been conducted, evaluating the hypothesis that chitosan nerve guides could provide a shielding and regeneration promoting effects also for separated nerve ends that have been coapted with tension-free sutures (Neubrech et al. 2016).

For translating our pre-clinical work in diabetic GK rats into the clinic, we have to take into account that the animal model used, clearly represents blood glucose levels relevant for human type 2 diabetes, but the animals did not yet develop evident neuropathies. This may represent some limitation for the translational impact of our results.

However, based on our experimental pre-clinical results, it may still be considered that even for diabetic patients it may be possible to use chitosan nerve guides when a peripheral nerve gap needs to be acutely bridged. Up to now, however, we have no evidence to recommend such nerve guides in a delayed nerve reconstruction approach of extended nerve gaps or when diabetic neuropathies have become evident already.

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