Therapeutic potential of paracrine factors and secretome-based treatment in spinal cord injury

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Injuries to the spinal cord, mainly affecting young and active patients, are often associated with irreversible neurological deficits with limited therapeutic options, thereby causing major individual and socioeconomic burden [1-2]. Following the initial trauma, a second hit caused by the endogenous response to spinal cord injuries (SCI) aggravates damage to the spinal cord further deteriorating the neurologic outcome. This second phase of SCI is characterized by a multi-layered and complex combination of cellular and molecular pathways [3, 4]. Aside from its undesirable characteristics this secondary cascade offers numerous potential targets for therapeutic intervention [5]. Although substantial progress was made describing involved mechanisms and despite a large numbers of pre-clinical and clinical trials investigating novel treatment strategies, a satisfying treatment option for patients affected by SCI remains to be discovered [6]. Recent studies showed that mechanisms involved in the secondary cascade after SCI promote damage progression, but are also pivotal for initiating spinal cord repair and containing inflammation. Some pathways and mechanisms were shown to be capable of exerting both beneficial and unfavorable characteristics depending on the timing of activation [7-9]. This peculiarity serves as one explanation for repeated failures of novel therapeutics in pre-clinical and clinical studies [10, 11].

Particularly the inflammatory response to SCI displays multifaceted properties. On the one hand, inflammation after SCI is thought to drive on-going tissue damage via oxidative stress, enzymatic degradation, disruption of the blood-brain barrier and disturbance of central nervous system homeostasis [12, 13]. Especially recent studies demonstrated a
The central role of macrophages orchestrating the immunological response to SCI. Depending on their origin and their polarization, macrophages were shown to be pivotal for recovery and even regeneration after SCI [8, 12, 14].

In recent years, research of our group focused on utilization of conditioned medium as a source of paracrine factors suitable for therapeutic application. Progress made in the field of stem cell therapy and insights in involved modes of action triggered the idea of using conditioned medium therapeutically. In the beginning of this century, stem cells were under investigation as potential treatment for myocardial infarction and its regenerative capacity [15, 16]. A set of clinical studies reported promising short-term results although implanted stem cells failed to migrate to the infarcted myocardium and were found in the spleen and liver shortly after administration. It was also shown that transplanted stem cells in large part even undergo apoptosis prior transplantation. Furthermore, therapeutic effects were observed as early as 72 hours after application [17-19]. Taken together, these findings suggested mechanisms involved other than cardiomyogenic transdifferentiation and cellular replacement. Gnecchi and his co-workers postulated in 2005 that paracrine factors released by implanted cells are responsible for observed therapeutic capacity. His group was also able to show that increased secretory capacity, for example via the induction of survival genes, is capable of augmenting the therapeutic effect [17, 18, 20].

Peripheral blood mononuclear cells (PBMCs) represent an easy accessible and heterogeneous cell population. Previous published data showed that conditioned medium derived from either PBMCs or stem cells contains comparable amounts of secreted factors [21]. We were interested in the therapeutic capacity of secretory and paracrine factors produced by PBMCs. To increase secretory capacity our group decided to induce apoptosis in PBMCs with lethal gamma irradiation prior 24 hours of cultivation. Subsequent
centrifugation allowed further processing of the cell-free supernatant or secretome, also referred to as conditioned medium. Several pre-clinical models were used to investigate the therapeutic potential of this secretome derived from apoptotic PBMCs (ApoSec, patent number: WO2010/070105, WO2010/079086) [22-28]. Therapeutic efficacy was shown to be a synergistic effect caused by modulation of multiple molecular pathways, many of which are also known to be involved in secondary cascades after SCI. Recent studies by our group aimed to describe the unique composition of ApoSec in detail. Aside from secreted proteins, we found oxidized lipids, microvesicles and exosomes. Within these vesicles, either actively secreted or a by-product of apoptotic breakdown, high contents of specific RNA, proteins and lipids were observed [29, 30].

In a previous study, we intended to investigate whether this secretome is capable of exhibiting therapeutic capacity in SCI [31]. The following findings lead to the idea and initiation of this pre-clinical SCI trial. Firstly, ApoSec was shown to up-regulate pathways associated with cell survival and inhibition of apoptosis in vitro and in vivo [26-29]. Secondly, the secretome of apoptotic PBMCs improves microvascular flow via inhibition of thrombocyte activation and vasodilation and features angiogenic characteristics [24, 28]. Thirdly, ApoSec was shown to be capable of modulating the inflammatory response in vitro and in vivo [25]. And finally, many factors present in the secretome were successfully tested as single-factor treatment in various pre-clinical SCI studies [27, 30].

We utilized a commonly used contusion injury model in rats to investigate the potential therapeutic capacity of ApoSec in this setting. We used human PBMCs for cultivation and production of secretome used in all experiments. This production process meets good manufacturing practice (GMP) guidelines and was recently approved by the Austrian authorities for the use in human trials. Secretome treatment was administered systemically in all experiments. Animals in the treatment group experienced improved recovery of hind limb motor function compared to control group [31]. This finding was confirmed histologically with treated animals experiencing smaller spinal cord cavity formation, damped axonal damage and higher vascular density around the lesion [31]. To further dissect involved mechanisms, we went back to in-vitro and ex-vivo experiments. ApoSec was able to induce angiogenesis not only in aortic rings, but also in spinal cord tissue ex-vivo, which was confirmed immunohistologically using confocal microscopy [31]. Aside from neovascularization, previously described inhibition of micro-vascular obstruction via inhibition of thrombocyte activation and vasodilation might also be involved in observed improved vascularity in treated animals [24, 31]. To assess possible involved immunologic mechanisms further investigation revealed higher levels of CD68 expression with concomitant lower expression levels of inducible NO-synthase (iNOS) 3 days after injury in animals treated with ApoSec. A higher number of CD68 expressing cells proximate to the injury site induced by secretome treatment was already described in an acute myocardial infarction model and supports this finding [27]. In contrast, 28 days after injury histology of treated animals revealed lower levels of CD68 expression. Taken together, we interpreted these findings as increased presence of macrophages triggered by systemic secretome treatment. Lower expression level of iNOS despite the increased number of cells potentially capable of expressing iNOS is suggestive of an immunosuppressive polarization of migrating macrophages. This increased presence of macrophages seems to cause an accelerated clearance of immunologic activity [31]. Especially in the light of recently published research these findings add an interesting mode of action to secretome therapy. Macrophages were shown to be essential for recovery after injuries to the spinal cord. Even regeneration of peripheral nerves was reported to be dependent on the presence of macrophages and their guidance along vascular structures [8, 32].

Injuries to the spinal cord induce an endogenous response comprising numerous cellular and molecular pathways [5]. Therefore, it seems unlikely that targeting single molecules exhibits sufficient therapeutic potential. Accordingly, therapy based on single factors failed to deliver satisfying results in a multitude of pre-clinical and clinical studies. Even negative effects of single-molecule treatment were reported, which was reversible when a combination of factors was administered [33, 34]. With negative long-term results and a growing list of reported complications, cortisol treatment regimens are current topic of controversial discussion [35]. With data indicating that the immune response not only aggravates damage to the spinal cord but also is capable of driving recovery and regeneration, global immunosuppression is very unlikely to provide desired therapeutic effects. Therefore, we postulate that only a multi-layered therapy approach is sufficient to modulate secondary mechanisms involved in progression and aggravation of damage to spinal observed after injuries to the spinal cord [31]. Other groups demonstrated efficacy of conditioned medium obtained from mesenchymal stem cells (MSCs) further supporting our findings [36, 37]. PBMCs as cellular basis for secretome treatment provide important advantages compared to utilizing MSCs. It is readily available via simple peripheral venous blood withdrawal and is currently discarded during the production of erythrocyte concentrates. ApoSec, as we used it in these experiments, is produced under conditions adherent to GMP guidelines and
approved for human trials by the Austrian authorities. This approved secretome derived from human PBMCs was shown to be effective in a rodent model of SCI warranting initiation of first clinical trials in SCI patients. Further pre-clinical studies with the purpose of delineating molecular and cellular mechanisms involved in secretome-based treatment with an emphasis on modulation of macrophages and their response are planned. Furthermore, a clinical phase I study investigating safety of autologous ApoSec has been initiated recently by our group (“Marsyas 1”, EudraCT Nr.: 2013-000756-17, ClinicalTrials.gov Identifier: NCT02284360).

Conflicting interests

The authors have declared that no competing interests exist.

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H.J.A. is a shareholder of APOSIENCE AG, which owns the right to commercialize PBMC secretome for therapeutic use. The Medical University of Vienna has claimed financial interest (patent number: EP2201954, WO2010070105-A1, filed 18 December 2008).

References


