Cytotherapy, 2016; 18: 760–770

International Society for Cellular Therapy
ISCT

NEURAL STEM CELLS

Extracorporeal shockwave treatment: A novel tool to improve Schwann cell isolation and culture

CHRISTINA M.A.P. SCHUH[1,2], DAVID HERCHER[1,2], MICHAELA STAINER[1,2], RUDOLF HOPF[1,2], ANDREAS H. TEUSCHL[2,3], ROBERT SCHMIDHAMMER[1,2] & HEINZ REDL[1,2]

[1]*AUVA Research Center, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria,* [2]*Austrian Cluster for Tissue Regeneration, Vienna, Austria, and* [3]*Department of Biochemical Engineering, University of Applied Sciences Technikum Wien, Vienna, Austria*

Abstract

Background aims. As new approaches for peripheral nerve regeneration are sought, there is an increasing demand for native Schwann cells for *in vitro* testing and/or reimplantation. Extracorporeal shockwave treatment (ESWT) is an emergent technology in the field of regenerative medicine that has also recently been shown to improve peripheral nerve regeneration. *Methods.* In this study, we elucidate the effects of ESWT on Schwann cell isolation and culture. Rat sciatic nerves were dissected and treated with ESWT, and Schwann cells were isolated and cultured for 15 passages. *Results.* Single treatment of the whole nerve *ex vivo* led to significantly increased extracellular adenosinetriphosphate as an immediate consequence, and subsequently a number of effects on the culture were observed, starting with a significantly increased Schwann cell yield after isolation. In the ESWT group, the quality of culture, reflected in consistently higher purity (S100b, morphology), proliferation rate (5-bromo-2-deoxyuridine, population doublings per passage) and expression of regenerative phenotype-associated markers (P75, glial fibrillary acidic protein, c-Jun), was significantly improved. In contrast, the control group exhibited progressively senescent behavior, reflected in a decrease of proliferation, loss of specific markers and increase in $P16^{INK4A}$ expression. *Conclusions.* ESWT has beneficial effects on Schwann cell isolation and culture.

Key Words: *extracorporeal shockwave treatment, peripheral nerve regeneration, Schwann cells*

Background

Peripheral nerve lesions occur with an incidence of approximately 300 000 cases annually in Europe, representing a frequent cause of hospitalization and displaying a major burden to patients and social health care [1].

Although the peripheral nerve system has a remarkable regenerative potential, regeneration over nerve gaps or over long distances (e.g., after proximal lesions) presents several difficulties. In this regard, nerve autografts are the gold standard to treat peripheral nerve injuries with tissue loss but often do not result in a satisfactory outcome [2]. In particular, long-distance gaps or severe injuries affecting several nerves push autografting to its limits regarding the availability of donor material. Alternatives to facilitate nerve regeneration, such as artificial nerve guidance tubes or other types of scaffolds or application of neurotrophic substances, are sought. Some of these approaches are currently used in clinical nerve repair, although there is an ongoing debate concerning their appropriate use, effectiveness and side effects [3,4]. One of the major reasons for the unsatisfactory outcome after repair of long-distance gaps is the limited proliferative capacity of Schwann cells [5]. Schwann cells play a key role in peripheral nerve regeneration: they participate in the removal of myelin and axonal remnants, start proliferation and align to build the so-called bands of Büngner [6]. After the axon has

Correspondence: **Christina M.A.P. Schuh**, PhD, AUVA Research Center, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Donaueschingenstraße 13, A-1200 Vienna, Austria. E-mail: christina.schuh@trauma.lbg.ac.at

(Received 18 January 2016; accepted 5 March 2016)

ISSN 1465-3249 Copyright © 2016 International Society for Cellular Therapy. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcyt.2016.03.002

elongated along the bands of Büngner, the Schwann cells start to remyelinate the newly formed axon to complete the regenerative process.

A novel strategy to improve the functional outcome of peripheral nerve regeneration is the therapy of injured nerves with extracorporeal shockwave treatment (ESWT). ESWT has its origin in the field of urology, in which it is used to destroy kidney stones [7], but it has also been proven to be an effective therapeutic tool in the field of regenerative medicine. In preclinical and clinical trials, beneficial effects have been reported in treatment of various medical indications such as non-union fractures [8–10], ischemia-induced tissue necrosis [11], or chronic wounds [12,13]. The shockwave generated is a sonic pulse and is characterized by an initial rise, reaching a positive peak of up to 100 MPa within 10 ns, followed by a negative amplitude of up to −10 MPa and a total life cycle of less than 10 µs. Biological responses are thought to be triggered by the high initial pressure, followed by a tensile force and the resulting mechanical stimulation [14].

Recently, Hausner et al. [15] showed a novel approach of accelerating regeneration after peripheral nerve injury, bridged with an autologous nerve graft. After dissecting and bridging the sciatic nerve of a Sprague-Dawley rat, extracorporeal shockwaves were applied at the site of injury. Six weeks after surgery, animals of the ESWT group exhibited a significantly improved functional recovery relative to controls. On the basis of this study, we investigated *in vitro* Schwann cell behavior after ESWT treatment with focus on their regenerative capacity.

Methods

Shockwave treatment of nerve tissue and Schwann cell isolation

All animals were euthanized according to established protocols, which were approved by the City Government of Vienna, Austria, in accordance with the Austrian Law and Guide for the Care and Use of Laboratory Animals as defined by the National Institutes of Health. Animals and treatment/control groups were randomly chosen and analyzed without pre- or post-selection of the respective nerves or cultures.

For *ex vivo* shockwave treatment an unfocused electro-hydraulic device was used (Dermagold 100, MTS Medical. The applicator was attached to a water bath as described in other studies [16–18], ensuring direct contact to the pre-warmed (37°C) water, allowing reproducible physical propagation and application of shockwaves *in vitro*. Sciatic nerves of adult male Sprague-Dawley rats were dissected, and each nerve was transferred into a 15-mL conical centrifuge tube (PAA Laboratories) containing phosphate buffered saline (PBS; PAA Laboratories) pre-chilled on ice. Nerves were kept on ice until further use but not longer than 1 h. For ESWT application, tubes were placed 5 cm in front of the applicator inside the water container. Subsequently, unfocused shockwaves were applied using the parameters chosen according to previous experiments [15] to maximize the effect of the ESWT treatment, while minimizing possible negative effects: 300 pulses at an energy level of 0.10 mJ/mm^2 with a frequency of 3 Hz. The corresponding second nerve from the same animal served as control and was placed in a water bath (37°C) for the time of treatment to avoid the creation of artifacts due to different sample treatments.

After ESWT treatment, Schwann cells were isolated from the treated and non-treated sciatic nerve tissues according to a method adapted from Kaewkhaw et al. [19]. Briefly, the epineurium was removed and nerves were weighed on a fine scale to assess nerve wet weight (Sartorius). Nerves were subsequently strained and minced. Nerve fragments were incubated with 0.05% collagenase (Sigma-Aldrich) for 1 h at 37°C and then filtered through a 40-µm cell strainer and centrifuged at 400*g* for 6 min. After washing the cell pellet with Dulbecco's Modified Eagle Medium (DMEM; PAA Laboratories) containing 10% fetal calf serum (FCS; PAA Laboratories), the pellet was resuspended in DMEM-D-valine (PAA Laboratories), supplemented with 10% FCS, 2 mmol/L L-glutamine (PAA, Austria), 1% antibiotics (PAA Laboratories), N_2 supplement (Invitrogen), 10 µg/mL bovine pituitary extract (Sigma-Aldrich), 5 µmol/L forskolin (Sigma-Aldrich). This medium is subsequently referred to as "Schwann cell medium." Cell suspension was seeded on six-well plates (PAA Laboratories) coated with poly-L-lysin (Sigma-Aldrich) and laminin (Sigma-Aldrich).

Cell culture and experimental setup

Cells were subcultured for the first time after 19 days, to establish a proliferative phenotype and keep them in a proliferative state. Schwann cell medium was added on day 5 after isolation (1 mL) and was partially (50%) changed on days 9, 13 and 17. Subsequent splitting of cells was performed for 15 passages as follows: cells were detached with a cell scraper, centrifuged at 1200 rpm for 5 min and seeded at a density of 4×10^4 cells/cm^2 on plates previously coated with poly-L-lysin. Residual cells were used for flow cytometric analysis, 5-bromo-2-deoxyuridine uptake (BrdU) assay and protein isolation. Medium was partially (50%) changed every third day, and cells were split every sixth day.

Download English Version:

https://daneshyari.com/en/article/2171169

Download Persian Version:

https://daneshyari.com/article/2171169

Daneshyari.com