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FACULDADE DE MEDICINA

Ana Paula Silveira Leite

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Tese apresentada à Faculdade de Medicina,Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de Botucatu, como requisito para obtenção do título de Doutora em Cirurgia e Medicina Translacional.

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Palavras-chave: Biopolímero de fibrina; Junção neuromuscular; Lesão nervosa periférica.

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"Nada na vida deve ser temido, somente compreendido. Agora é hora de compreender mais para temer menos."

(Marie Curie)

RESUMO

As lesões nervosas periféricas podem causar interrupção do impulso nervoso, e mesmo após intervenção cirúrgica é comum que a recuperação funcional não seja completa, nesse sentido é importante investigar a resposta das junções neuromusculares (JNMs), responsáveis pela interação entre o axônio motor e sua musculatura alvo. Na busca por novas terapias que potencializem a regeneração, se destacam os tubos ou conduítes, que podem ser de variados materiais e fornecem um conduto para o crescimento axonal. Combinado a esses tubos o uso de adjuvantes pode ser acrescido, dentre eles destaca-se o biopolímero de fibrina (FB) com possível fator protetor e trófico no ambiente da lesão. Assim, o objetivo deste trabalho foi avaliar a regeneração das Junções Neuromusculares (JNMs) e proteínas associadas, após lesão nervosa periférica, seguida de técnica de tubulização e uso do FB. Foram utilizados 52 ratos *Wistar* machos adultos, distribuídos em 4 grupos experimentais: Controle Sham (S), Controle Desnervado (D); Tubulização (PCL) e Tubulização + Biopolímero de Fibrina (FB). No grupo S, foi realizada apenas incisão, localização e debridação do nervo isquiático. Nos Grupos D, PCL e FB foram realizados modelos de lesão completa, com gap de 8 mm nos grupos PCL e FB. Após neurotmese no grupo D, os cotos foram invertidos e fixados a tela subcutânea. Nos grupos PCL e FB, após a lesão, os cotos nervosos foram inseridos e fixados na prótese tubular de policaprolactona (PCL) de 12mm com fio de sutura 10-0. No grupo FB foi adicionado após a fixação dos cotos ao tubo 500μl do FB. Antes da realização dos procedimentos cirúrgicos, e quinzenalmente, foram realizadas análises no Catwalk em todos os animais. Noventa dias após o procedimento cirúrgico, os animais foram pesados, os músculos sóleos e nervos isquiáticos direitos foram coletados, fixados e submetidos as seguintes análises: morfológica e morfométrica dos receptores de acetilcolina (AChRs) através de microscopia confocal de varredura a laser; Análise morfológica e morfométrica do nervo isquiático; Quantificação proteica de células de Schwann, agrina, LRP4, MuSK , rapsina, MMP3, MyoD, Miogenina, MURF1 e Atrogina-1 e dos AChRs: alfa, gama e épsilon. A análise morfológica e morfométrica do nervo isquiático compactua com a reconexão dos cotos nervosos observada nos grupos PCL e FB, onde para a maioria dos parâmetros houve equivalência dos valores entre os grupos, exceto pela área dos axônios do grupo PCL, os quais foram menores que os do grupo S. Na função motora, não houve alteração entre os grupos no que se refere ao índice funcional do fibular, ainda que em outros parâmetros como área da pegada e contato máximo com a plataforma, os grupos experimentais não apresentaram valores similares aos valores do grupo S. Na análise dos AChRs, a morfologia, os valores do índice de compactação, da área dos AChRs e da placa motora do grupo FB foram os únicos similares ao grupo S. Pode-se concluir considerando os resultados obtidos que o uso do FB associado a tubulização promoveu uma estabilização dos AChRs, potencializando a regeneração neuromuscular.

ABSTRACT

Peripheral nerve injuries may cause interruption of the nerve impulse, and even after surgical intervention it is common that functional recovery is not complete. In this sense it is important to investigate the response of neuromuscular junctions (NMJs), responsible for the interaction between the motor axon and its target muscle. In the search for new therapies that enhance regeneration, tubes or conduits, which can be made of various materials and provide a conduit for axonal growth, stand out. In combination with these tubes, the use of adjuvants may be added; among them, fibrin biopolymer (FB) stands out as a possible protective and trophic factor in the injury environment. Thus, the objective of this work was to evaluate the regeneration of Neuromuscular Junctions (NMJs) and associated proteins, after peripheral nerve injury, followed by tubulization technique and use of FB. We used 52 adults male Wistar rats, distributed in 4 experimental groups: Sham Control (S), Denervated Control (D); Tubulization (PCL) and Tubulization + Fibrin Biopolymer (FB). In Group S, only incision, localization and debridement of the ischiatic nerve was performed. In Groups D, PCL and FB, complete injury models were performed, with an 8 mm gap in the PCL and FB groups. After neurotmesis in Group D, the stumps were inverted and fixed to subcutaneous mesh. In the PCL and FB groups, after injury, the nerve stumps were inserted and fixed to the 12-mm polycaprolactone (PCL) tubular prosthesis with 10-0 suture points. In the FB group, 500µl of FB was added after fixing the stumps to the tube. Before the surgical procedures were performed, and every fifteen days, Catwalk analyses were performed on all animals. Ninety days after the surgical procedure, the animals were weighed, the soleus muscles and right ischiatic nerves were collected, fixed and subjected to the following analyses: morphological and morphometric analysis of acetylcholine receptors (AChRs) by laser scanning confocal microscopy; morphological and morphometric analysis of the ischiatic nerve; protein quantification of alpha, gamma and epsilon AChRs, Schwann cells, agrin, LRP4, MuSK, rapsin, MMP3, MyoD, Myogenin, MURF1 and Atrogin-1. The morphological and morphometric analysis of the ischiatic nerve agrees with the reconnection of the nerve stumps observed in the PCL and FB groups, where for most parameters there was equivalence of values among the groups, except for the area of the axons of the PCL group, which were smaller than those of the S group. There was no alteration in motor function (fibular funcional index) among the groups, although in other parameters such as the print area and max contact area, the experimental groups did not reach the values of group S. In the analysis of the AChRs, the morphology, the values of the compactness index, the area of the AChRs and the endplate of the FB group were similar to the S group, different from the other groups. In addition, the FB showed higher values of S100 protein, and epsilon subunit, when compared to group S and all other groups respectively. It can be concluded considering the results obtained that the use of FB associated with tubulization promoted a stabilization of the AChRs, potentiating neuromuscular regeneration.

Sumário

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Lista de Abreviaturas

ACh- Acetilcolina
AChE – Acetilcolinesterase
AChR – Receptores de acetilcolina
BF- Biopolímero de Fibrina
Ca^{2+} - Cálcio
CEVAP- Centro de Estudos de Venenos e Animais Peçonhentos
CEUA- Comissão de Ética no Uso de Animais
D- Grupo Desnervado
Dok-7 – Proteína citoplasmática derivada da quinase 7
EPL- *Print lengh* lado lesionado
ETS- *Toe spread* lado lesionado
EUA- Estados Unidos da América
FMB- Faculdade de Medicina de Botucatu
g- Gramas
GAPDH- Gliceraldeído-3-fosfato desidrogenase
GDNF- Fator neurotrófico derivado de linhagem de célula glia
IFF- Índice Funcional do Fibular
JNM- Junção Neuromuscular
Kg- Quilogramas
Lrp4 - Receptor lipoprotéico relacionado a proteína 4
mg- Miligramas
µL- Microlitros
µm- Micrômetros
MMP3- Metaloproteinase 3 da matriz
MRF4- *Muscle regulatory factor 4*
MuSK- Receptor tirosina quinase músculo-específico
MyF5- *Myogenic factor 5*
MyOD- *Myoblast determination* protein *1*
NGF- Fator de crescimento neural
NPL- *Print lengh* lado normal
NTS- *Toe spread* lado normal

PBS- Tampão fosfato-salino

PCL- Policaprolactona/ grupo tubulização a base de policaprolactona

PLA- Poliácido lático

RIPA- *Radioimmunoprecipitation assay buffer*

S- Grupo Sham

SNARE- *Soluble N-ethylmale-imide-sensitive factor-attachment protein receptors*

UNESP- Universidade Estadual Paulista “Júlio de Mesquita Filho”

UNICAMP- Universidade Estadual de Campinas

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