Satellite cell transplantation in delayed reinnervation of the canine larynx.

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BACKGROUND

Chronic vocal fold paralysis can be experimentally restored by selective reinnervation of the abductor and adductor muscles. Does satellite cell implantation improve trophicity and reinnervation of laryngeal atrophic mucles? The aim of the present study was to answer this question.

1. MATERIAL AND METHODS

Nine female Beagle dogs were separated in two groups and three controls :

-group A: (n=3) denervated and reinnervated
-group B: (n=3) denervated, reinnervated and implantation of cell culture
-CC: (n=1) denervated and implantation of cell culture
-CD: (n=1) denervated
-CH: (n=1) healthy control

Institutional guidelines of Rouen School of Medicine University for animal experiments were followed as formulated by French law. Under general anesthesia, eight dogs underwent a right vocal fold paralysis by right vagus nerve resection. Nine months after denervation, six dogs (group A and groupB) underwent a selective unilateral reinnervation with a phrenic nerve root for the posterior cricoarytenoid (PCA) muscle and the thyrohyoid branch of the hypoglossal nerve for the thyroarytenoid (TA) muscle[4]. Few days before, under general anethesia, a skeletal muscle biopsy was explanted from sartorius muscle of dogs in groups B and CC. The satellite cells were expanded in culture for seven days. The cultures were labelled with chloromethylbensamide-DiI (cmDiI) and bromodeoxyuridine (BrdU). The autologous culture was implanted in the PCA and in the TA muscles during the surgery of reinnervation in dogs in groups B and CC.

After a six month delay, videolaryngoscopy and electromyography of the laryngeal muscles was carried out under slight general anesthesia. Then, the dogs underwent a cervicotomy: identification of the nerve anastomosis, electrical stimulation of the nerves. The animals were euthanized at completion of the study and specimens of muscles were removed for histological analysis. The PCA and TA muscle tissue was processed for the following histochemical reactions: ATPase pH=9,4, 4,63, 4,35, NADH tetrazolium reductase, Hematoxylin-eosin, immunochemistry for BrdU and revelation of cmDiI immunofluorescent staining.

2. RESULTS

Videolaryngoscopy and electromyography of the laryngeal muscles provided evidence of functional rehabilitation in two dogs in Group A and one dog in Group B. Laryngeal muscles of the animals which received cell cultures demonstrated an important inflammation with major signs of atrophy and fibrosis. BrdU and cmDiI were not identified on histological muscle slides.

3. DISCUSSION

Of the three dogs in group A, two demonstrated functional reinnervation. The inspiratory nature of the phrenic nerve makes it an ideal source for reinnervation of the PCA muscle. Moreover the thyrohyoid branch of the hypoglossal nerve has an expiratory and phonatory activity[3]. The muscles wich received cell culture showed atrophy and fibrosis, wich suggest that the transplantation of cell culture induced inflammatory response as described in other studies[1, 5]. We failed finding cell culture markers because they were probably died. Beauchamp reported the majority of the grafted cells die and only 1% remained four days after grafting[1]. Cell culture did not improve the selective reinnervation of the laryngeal atrophic muscles in our present study. The cells were implanted during the reinnervation surgery, when the muscles

were atrophic and denervated. The results would perhaps have been better if cell transplantation was done long after reinnervation surgery, at the time of neuromuscular junction recovery.

4. CONCLUSION

Selective reinnervation can restore long term vocal cord paralysis. Satellite cell culture implantation don't provide functional or trophic improvement, even in the non reinnervated larynx.
Future direction could be:

1. To test a non cultured skeletal muscle cells to avoid the cell culture expansion and perhaps the inflammatory response[2].
2. To culture a population of long-time proliferating cells wich improved the efficiency of muscle regeneration[5].
3. To inject the cells at the time where axonal growing is achevied.

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5. REFERENCES

[1] Beauchamp JR, Morgan JE, Pagel CN, Partridge TA. *Dynamics of myoblast transplantation reveal a discrete minority of precursors with stem cell-like properties as the myogenic source.* J Cell Biol.1999; 144: 1113-1122.
[2] Borenstein N, Bruneval P, Hekmati M, Bovin C, Behr L, Pinset C, Laborde F, Montarras D. *Noncultured, autologous skeletal muscle cells can sucessfully engraft into ovine myocardium.* Circulation 2003; 107: 3088-3092.
[3] Crumley RL. *Selective reinnervation of vocal cord adductors in unilateral vocal cord paralysis*. Ann Otol Rhinol Laryngol 1984; 93: 351-356.
[4] Marie JP, Lacoume Y, Magnier P, Dehesdin D, Andrieu Guitrancourt J. *Selective bilateral motor reinnervation of the canine larynx.* 4th European Congress of Otorhinolaryngology, Head and Neck Surgery. May 13-18/2000, Berlin, Germany. Laryngo-Rhino-Otologie, 2000,79, S188-9.
[5] Qu-Petersen Z, Deasy B, Jankowski R, Ikezawa M, Cummins J, Pruchnic R, Mytinger J, Cao B, Gates C, Wernig A, Huard J. *Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration*. J Cell Biol 2002; 157: 851-864.