Effect of photosensitization reaction on myelinated or unmyelinated nerve

Haruka TAKAHASHI1, Risa HAMADA1, Emiyu OGAWA1, Tsunenori, ARAI1,2
1School of Fundamental Science and Technology, Graduate School of Science and Technology, Keio University
2Department of Applied Physics and Physico-Informatics, Faculty of Science and Technology, Keio University

Abstract: To investigate nerve injury by a photosensitization reaction ex vivo, we observed an uptake of talaporfin sodium into crayfish nerve and porcine phrenic nerve, and measured electrophysiological conduction velocity of the crayfish nerve during the reaction. We found the drug uptake of inside the perineurium was lower than that of outside in porcine phrenic nerve. The crayfish nerve was immersed into 20 µg/ml talaporfin sodium for 15 min and irradiated by a 663 nm laser light with 120 mW/cm². Since we found the measured conduction velocity was decreased increasing the irradiation time, the nerve might not be resistant to the photosensitization reaction at atmospheric oxygen environment. It was reported that the phrenic nerve was intact when an electro blockade using photosensitization reaction was performed in vivo animal experiment. Low uptake of talaporfin sodium inside the perineurium and low oxygen partial pressure of nerve might be the mechanism to preserve phrenic nerve in vivo animal experiment.

Keywords: photosensitization reaction, talaporfin sodium, phrenic nerve, oxygen partial pressure

1. Introduction
The purpose of this study is to investigate nerve injury by the photosensitization reaction with talaporfin sodium ex vivo. Phrenic nerve paralysis is major side effect on current atrial fibrillation ablation using radiofrequency energy [1]. Phrenic nerve was kept its electrophysiological ability and its histopathology. It was reported that the phrenic nerve was intact electrophysiological and histologically during/after an electro blockade using the photosensitization reaction in vivo animal experiment [2,3]. There are no reports for nerve injury by the photosensitization reaction with talaporfin sodium ex vivo experiment, although in vitro study using schwann cells reported that low uptake of talaporfin sodium and low oxygen partial pressure of nerve might be the mechanism to preserve phrenic nerve in vivo experiment [4]. To study nerve injury by the photosensitization reaction ex vivo, we observed an uptake of talaporfin sodium into crayfish nerve and porcine phrenic nerve. In the case of crayfish nerve experiment we investigated influence of the reaction on electrophysiological conduction velocity.

2. Material and Method
2.1 Talaporfin sodium uptake
A healthy extracted crayfish ventral nerve cord and an extracted porcine phrenic nerve were immersed into 20 µg/ml talaporfin sodium for 0-240 min. These nerves were sliced into 50 µm thickness by a cryostat (C150OMS, Leica, Japan) to make a sample. A Fluorescence microscope (FSX100, OLYMPUS, Japan) was used to get an image of the sample. In the case of crayfish nerve, the mean fluorescence brightness inside the nerve area was analyzed by an image processor ImageJ (NIH, USA). In the case of porcine phrenic nerve, the mean fluorescence brightnesses inside and outside the perineurium were analyzed by ImageJ, respectively.

2.2 Nerve injury by the photosensitization reaction under atmospheric oxygen environment
A scheme of cross sectional structure of electrophysiological measurement chamber is shown Fig. 1. The spacing between the electrodes made of stainless wire in the chamber was 2.0 mm and the electrode diameter was 0.55 mmΦ. A pair of electrodes was employed to stimulate crayfish nerve. A stimulation voltage was 1-5 V and a stimulation pulse width was 0.1-0.5 ms. Conducted action potentials were recorded using a biological amplifier (DAM50, World Precision Instruments, USA) with a data acquisition system (Powerlab, AD Instruments, UK).

The healthy extracted crayfish ventral nerve cord was immersed into 20 µg/ml talaporfin sodium for 15 min in the chamber. The nerve was irradiated by 663 nm laser light with 120 mW/cm². The difference of conduction time of action potential between up portion of the irradiation area and down portion of the area was recorded. Action potential was measured for each irradiation. The maximum total irradiation time was set to 65 s. The conduction velocity was calculated from the conduction time.

3. Results
3.1 Talaporfin sodium uptake
Figure 2, 3 show fluorescence images of the crayfish nerve and porcine phrenic nerve cross section with a drug immersion time of 240 min, respectively. The mean fluorescence brightness of the image increased depending on the immersion time and saturated around the immersion time of 120 min. We found that the drug uptake of inside the perineurium was lower than that of outside in porcine phrenic nerve. Since there are nerve fibers directly related to electrical conduction inside the perineurium,
we consider that low uptake of talaporfin sodium inside the perineurium might be one of the nerve preservation mechanism in vivo animal experiment.

Fig. 2 Fluorescence image of the crayfish nerve cross section shown the drug uptake (talaporfin sodium immersion time 240 min)

Fig. 3 Fluorescence image of the porcine phrenic nerve cross section shown the drug uptake (talaporfin sodium immersion time 240 min)

3.2 Nerve injury by the photosensitization reaction under atmospheric oxygen environment

Figure 4 shows the waveform before the irradiation. Figure 5 shows the dependence of the conduction velocity on the irradiation time. The conduction velocity was decreased increasing irradiation time, and finally action potential of after the irradiation area was disappeared. From these results, we consider that the crayfish nerve might not be resistant to photosensitization reactions at atmospheric oxygen environment.

Fig. 4 Action potential waveform before irradiation

Fig. 5 Typical photosensitization reaction effect on conduction velocity

4. Conclusion

We found that the drug uptake of inside the perineurium was lower than that of outside in porcine phrenic nerve. We also found that the crayfish nerve might not be resistant to the photosensitization reaction at atmospheric oxygen environment. From these results, low uptake of talaporfin sodium inside the perineurium and low oxygen partial pressure of nerve might be the mechanism to preserve phrenic nerve in vivo animal experiment.

Reference