The Effect of Botulinum Toxin Type A after Intradermal Injection on the Surgical wound in Pig model

P. Darapong1*, S. Durongpongtorn1, P. Brikschavana1, T. Kaewamatawong2, W. Prasertsom3

1Department of Veterinary Surgery, 2Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand 3Royal Thai College of Obstetrician and Gynecology
*Corresponding author: Panadda.Dar@student.chula.ac.th

Keywords: Botulinum Toxin Type A, Intradermal, Surgical wound, Porcine

Introduction
Botulinum toxin (BTX) is a potent neuromodulator that temporarily relaxes muscles and improve wound healing (1). The effect of Botulinum Toxin Type A (BTX-A) on wound healing are currently under investigation. Initial studies concentrated on its paralytic effect and tension exertion on a wound contributing to scar elevation and the formation of hypertrophic or keloid scars. (2) As a secondary effect, inflammatory influences during the early healing phase are reduced. (3) The effect of intradermal BTX-A on wound healing has yet to be determine. For these reasons, the present study attempts to study about the effect of intradermal BTX-A injection around the surgical wound in pig model.

Material and Methods
A 30 kg mixed-breed female pig was prepared for the ID injection of BTX-A using the anesthetic protocol as follow: The pig was sedated with xylazine HCl (1 mg/kg), morphine sulphate (0.5 mg/kg) and a mixture of tiletamine and zolazepam (Zoletil™, Virbac animal health Inc.) (2 mg/kg) intramuscularly, then inducted with propofol (4 mg/kg) intravenously and maintained with isoflurane 1-3%. Both sides of the dorsal region of pig were divided into 10 areas. (Fig. 1) 10 circular surgical wounds diameter of 2 cm. were created, five of them were randomly selected and injected with BTX-A intradermal. BTX-A (0.05 ml) was injected around the wound edge with 5 mm distance away from the wound. Another 5 wounds was injected normal saline (0.05 ml) with intradermal injection as controls. Wound healing was observed and the measured of the lengths of the major axis and the minor axis of the wound. The calculation of wound area was performed using the formula modified from Kantor and Margolis.(4) 2.5 x 2.5 cm. full-thickness skin biopsies using a sterile 15 mm disposal surgical blade were harvested at 3, 7, 14, 21, 28 days after BTX-A injection, respectively.

Results and Discussion
Assessment of the wound size:
All the wounds were covered with scab 7 days post operatively. From day 14-28, both control and BTX-A treated groups showed progressive decrease in size of the wound scab. There was no significant difference between two groups neither formation nor disappearance of the scabs. There were no significant differences of wound size between two groups. (Fig. 2, 3)

Figure 1 Surgical wound preparation before injection.

Figure 2 Wound appearance between control (A) and BTX-A (B) treated groups at day 7,14,21and 28.
Figure 3  Wound size between control and BTX-A treated groups at day 7, 14, 21 and 28

Figure 4  Wound from control group at day 3, neutrophils (arrows) and necrosis area (N)

Figure 5  Granulation tissue from control (A) at day 7: connective tissue arrangement between control (B) and BTX-A treated groups (C)

Figure 6  Re-epithelization (arrows) of wound from BTX-A treated group at day 14.

Histopathology:
Day 3: Both control and BTX-A treated groups found debris and exudate cover on wound area with inflammatory cells, mainly neutrophils, infiltration around, the wound edge. Necrosis of adjacent area and hemorrhage were also observed. (Fig. 4)
Day 7: Both control and BTX-A treated groups showed similar lesion as follow: Granulation tissue consisted of fibroblast and fibrocytes infiltration, and neovascularization were observed in the wound area. Hemorrhage and inflammatory cells including neutrophil, lymphocyte and macrophage were also found in the granulation area. Debris and exudate still covered on the top of wound area. However, the arrangement of the fibroblast and fibrocyte in the granulation areas of BTX-A group was more orderly than controls. (Fig. 5)
Day 14-28: Both control and treated groups showed gradually progression of wound healing process including re-epithelization on the top of wound, less number of inflammatory reactions, less neovascularization and less hemorrhage. (Fig. 6)

In this study, we concluded that BTX-A may be advantage in the early stages of wound healing as evidence of more orderly arrangement of fibroblast and fibrocyte in the granulation area when compare to the controls. However, in the rest of the observation time showed no difference of the progression of wound healing between the control and BTX-A treated groups.

Acknowledgements
This research was supported by Graduate School Thesis Grant 2016, Chulalongkorn University, Thailand
References