Srinagarind Med J 1993; 8(1) 20-26

Experimental Studies on the Use of K1 K8 Raw Thai Silk in Skin Wound Suture

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การศึกษาทดลองใช้ไหมไทยดิบจากสายพันธุ์ K1 K8 เย็บแผลที่ผิวหนัง

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เราใช้ไหมดิบขนาด 210–240 ดีเนียร์ ซึ่งได้จากหนอนไหมสายพันธุ์ K1 K8 และทำการเลี้ยงที่ขอนแก่น มาศึกษาทดลองเย็บแผลผ่าตัดที่เส้นแนวกลางหลังและที่เส้นแนวกลางท้องของหนู สำหรับกลุ่มเปรียบเทียบนั้นเราใช้ Mersilk W775, Ethicon เย็บแผล แผลผ่าตัดของทั้งสองกลุ่มจะเหมือนกัน แล้วเลี้ยงหนูทั้งของกลุ่มไว้ในห้องที่ปราศจากเชื้อโรคและจัดเวลาให้แสงและความมืดอย่างละ 12 ชั่วโมงต่อวัน เลี้ยงด้วยอาหารมาตรฐานสำหรับหนูทดลอง เราทำการศึกษาทดลองที่คณะแพทย์ชาริ่งครอสและเวสท์มินสเตอร์ ประเทศอังกฤษ สำหรับกลุ่มที่มีแผลที่แนวเส้นกลางหลังเราเก็บไว้ 7 วัน กลุ่มที่มีแผลที่แนวเส้นกลางท้องเราเลี้ยงไว้ 10 วัน หลังจากผ่าตัดแล้วเราสังเกตสัตว์ทดลองอย่างใกล้ชิด เมื่อครบกำหนด 7 วัน และ 10 วันแล้วเราฆ่าสัตว์และนำเอาเนื้อเยื่อแผลนั้น มาทำการศึกษาด้วยวิธีทางจุลกายวิภาคศาสตร์ เราพบว่าแผลที่เย็บด้วยไหมดิบสายพันธุ์ K1 K8 ของไทยนั้น มีขบวนการหายของแผลคล้ายกับที่เย็บแผลด้วยไหม Mersilk ซึ่งมีขายในท้องตลาด แต่ไหมดิบดังกล่าว

จะใช้ยากเพราะไม่ได้ย้อมสีให้มองเห็นง่าย และผิวหยาบของไหมดิบนั้นทำให้เย็บแผลได้ไม่สวย กล่าวโดยสรุปได้ว่า เราไม่พบผลร้ายหรือปฏิกิริยาที่ไม่พึงประสงค์เกิดขึ้นที่แผลสัตว์ทดลองที่เย็บด้วยไหมดิบสายพันธุ์ K1 K8 เลย พบแต่ว่าแผลหายได้อย่างที่ควรจะหาย และการเจริญซ่อมแซมเนื้อเยื่อก็จัดว่าอยู่ในระดับที่ดี เราแนะนำว่าควรจะศึกษาถึงความเหนียว และการอยู่ทนนานในเนื้อเยื่อของไหมไทยดิบสายพันธุ์ K1 K8 อีกต่อไป

Abstract

The raw silk of 210-240 deniers from K1 K8 silk worms reared in Khon Kaen was used as a suturing material for the middorsal and midventral surgical wounds of the rats. The identically-made skin wounds of the control group were sutured by Mersilk W775, Ethicon. Both groups of animals were kept pathogen-free in the 12:12 hr. dark and light room and maintained on a standard rodent diet at Charing Cross and Westminster Medical School. The experimental animals were kept 7 days (middorsal wounds) and 10 days for the group simulated the laparotomy wounds. We observed the animals after operation very closely. The wound tissues were processed through the histopathological techniques for microscopic study. We found that skin wounds sutured with K1 K8 Thai silk healed in a similar manner to those treated with commercially available silk sutrue (Mersilk) but the former presented difficulties in manipulation on the tissue background during operation. The rough texture of the raw silk did not facilitate as neat a suture as the commercial silk. In conclusion we have seen no adverse reaction but the wounds healed readily and the high level of regeneration was achieved. The tensile strength and the durability of the K1 K8 raw Thai silk Should be further investigated.

INTRODUCTION

Silk has a long and fascinating history extending over more than 3000 years when the industry became established in China and countries of the far east. Later, spun silk yarns and fine silk clothes became available in Mediterranean countries through trade routes passing through India and Persia. Excavations in Egypt have shown that silk was used at the time of Cleopatra. Although evidence of Egyptian silk is seen in the mummies from tombs of Abu Simbel, it is unclear as to when silk first became available in that region (Lubec *et al,* 1993).

Nowadays, in addition to its widespread use in fine clothes prepared by techniques developed over many centuries, silk thread has acquired an important role in surgery. We do not know when silk was first used in surgery, but with modern technology and improved refining methods, high quality braided silk threads have become well-established in wound suture. Products like Mersilk (Ethicon, Edinburgh, Scotland) are available in different gauges and welded to precision cutting needles, to provide the surgeon with an efficient and effective means of suture. Silk sutures are preferred by some surgeons because they do not biodegrade, are hypo-allergenic, and they achieve a maximal healing response.

It will be appreciated that in the physical and chemical processes involved in the preparation of commercial silk threads for surgical suture, many extraneous substances other than the silk protein are present in the finished product. These will include dye-stuffs, stabilising

substances and maybe metal ions. Adverse reactions to such substances may contra-indicate their use in certain situations.

It was of interest to examine the suitability of a natural or "raw" silk fiber in skin wound healing, and to compare its efficacy in skin wound healing with a commercially available silk suture. Such a study is aesthetically and financially relevant in the University of Khon Kaen Medical School where a long-standing interest exists in the use of local silk and in the development of the industry (Srung-Boonmee, personal communication). The present study was designed using a genetically defined and environmentally controlled experimental animal model which has been employed previously in the evaluation of the interaction of intrinsic and environmental factors in skin wound healing (Lansdown, 1991, 1992).

MATERIALS AND METHODS

Silk Thread:

The raw silk thread used in the present studies was kindly donated by the silk industry close to the city of Khon Kaen, where it is traditionally used in the "Mud-Mee" handicraft industry. The untreated threads ranged in length from 1000-1500 m/cocoon and were produced by the K1 K8 silkworms fed on mulberry leaves. When reeled, the silk threads measured 210-240 deniers.

Silk threads were prepared for experimental surgery by trimming the fibres to 1 m in length and then threading them as single or double strands through 5 cm straight needles (Surgicraft, Redditch, Worcestershire, England). These were then packed in surgical greaseproof paper and sterilised by autoclaving for 15 minutes. Preliminary studies showed that this procedure did not adversely influence the tensile strength of the thread or its knotting capacity.

Experimental Models:

Inbred Wistar rats weighing 150-180 g were employed in all segments of this study. The animals were reared under full barrier maintained specified-pathogen-free (SPF) conditions at the Charing Cross and Westminster Medical School. They were maintained in polypropylene cages under controlled environmental conditions of 22+/ – 2℃ and 55 + 5% relative humidity. Light periods of 12 hours were followed by 12 hours of darkness. Animals were allowed free access at all times to a standard rodent diet (CRM pellets, Special Diet Services, Witham, Esses, England) and tap water. Animal husbandry was in accordance with that specified under the Animal (Scientific Procedures) Act 1986.

Two surgical models were used in the present experiments. The first of these involved whole thickness wounds in the mid-dorsal skin of young adult animals. Groups of 5 rats were anaesthetised using pentabarbital sodium (Sagatal, RMB Animal Health, Dagenham Essex, England). (Experience has shown that 0.3 ml of a 50 : 50 dilution of Sagatal in sterile water or saline produces full anaesthesia for approximately 30 minutes.) The back skin was shaved with electric clippers and the area sterilised using 70% ethyl alcohol. A single 15 mm full thickness skin wound was made using a 22a scalpel blade. The wound was sutured at three equidistant sites with single thread raw Thai silk. (A comparable number off control rats were sutured with Mersilk W775, Ethicon). Animals were allowed to recover consciousness using a warm pad where necessary, and returned to their cages for continued observation. One group of five silk-sutured rats was euthanised using carbon dioxide asphyxiation after 72 hours, and a second group terminated after 7 days.

In the second series of experiments, wounds were made in the abdomen to simulate laparotomy. Under full anaesthesia (using 0.4 ml dilute Sagatal to provide increased anaesthesia), wound sites were prepared as above. A 10-15 mm full thickness skin wound was made

surgically in the mid-ventral line posterior to the xiphisternum, with a comparable length incision through the body wall musculature. Care was taken not to domage peritoneal membranes. To improve the tensile strength of the silk, double strand sutures were used to close body wall musculature. Single or double strand sutures were used to close the skin. Groups of five animals of each experimental and control group were euthanised at 72 hours and at 10 days.

Throughout the experiments, wound sites were examined daily for evidence of haemorrhage, infection, ruptured sutures or adverse reactions. Hair regrowth, healing patterns and suture loss were noted. At the termination of experiments, wound sites were excised, preserved in phosphate buffered 10% formalin, and then prepared for histological examination. Haematoxylin and eosin stained sections were adequate for evaluation of the sequential stages in the healing process, and in the detection of adverse reactions due to the silk fibres.

RESULTS

Dorsal Skin Wounds:

In the 24 hours following surgery, these wounds remained clean and showed minimal haemorrhage or other reaction (Fig 1). Local oedema was minimal and erythema was limited to the wound margin. In most animals the suture remained in place for the entire 7-day observation period. By this stage, the wound showed an advanced healing pattern with the suture line only marginally perceptible.

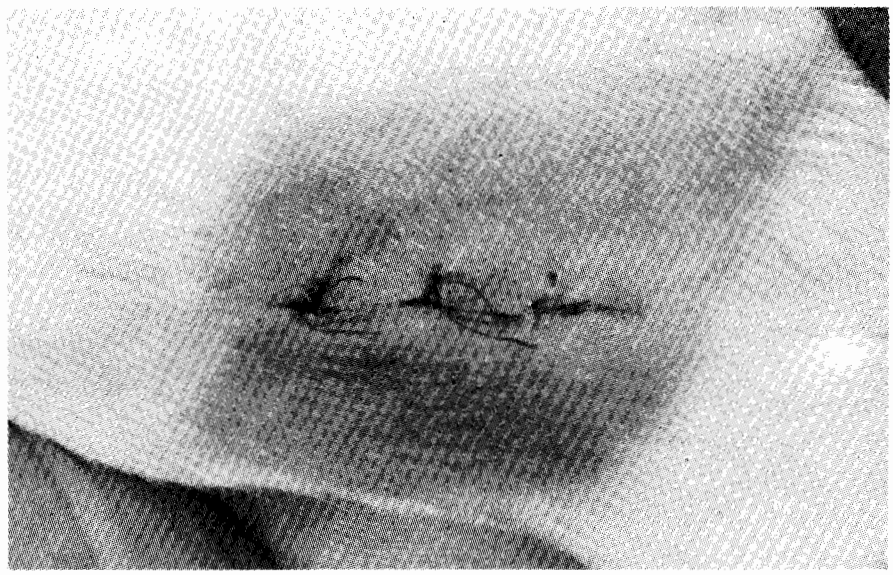

Figure 1 Dorsal skin wound in a young adult Wistar rat after 72 hours. Sutures of raw Thai silk. x2.5.

Histological sections taken transversely across the wound site 72 hours after surgery showed characteristic regenerative changes. Degenerative tissue and eschar formation marked the wound, and granulation tissue was present in the incision site with macrophage infiltration and local vascular dilatation. Chronic inflammatory changes were not extensive and limited to the wound margin. Evidence of regenerative activity was evident in the epidermis adjacent to the incision site, and some acanthosis was identified. Silk Threads were identified in several sections (Fig 2). They exhibited no obvious evidence of biodegradation and did not appear to be associated with increased lymphoid or other inflammatory

cells. After 7 days, the wound site was re-epithelialised, with the new epidermis showing a modest acanthosis and immature keratinocyte population. In the dermis, the wound site was marked by usual fibrous scar tissue. Some vascular dilatation persisted but in general, inflammatory changes were minimal. Where silk fibres were identified, they were not associated with any obvious adverse reaction.

Macroscopic and histological changes seen in the control rats in which the wounds had been sutured with Mersilk, were essentially similar to those described above. The sequence and timing of the wound healing was also similar.

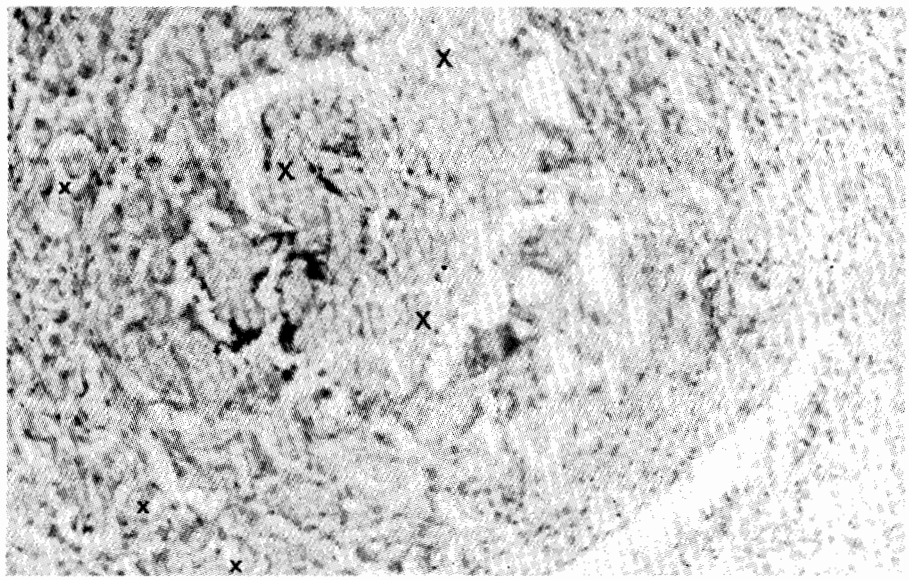

Figure 2 Transverse section of silk thread (xxx xxx) used to suture a dorsal skin wound in a Wistar rat. Note the low level of inflammation or fibrotic response. x10 objective.

Abdominal Skin Wounds:

Wound healing profiles in the abdominal skin were very similar to those noted above in the dorsal skin wounds. No obvious adverse reaction to the silk sutures were seen and wound closure was seen after 10 days (Fig 3). In some cases mild eschar formation persisted for about 3-4 days. Not unexpectedly, Some sutures were exteriorised naturally or otherwise lost during the healing process, but wounds did not open or become infected. Modest hair regrowth was seen in the observation period. (Although advantage was taken in some experiments of using double strand Thai silk to improve the tensile strength of sutures, all abdominal skin wounds sutured with double or single strands healed normally.)

Histological evaluation of the abdominal wound site after 3 and 10 days revealed similar changes in the skin to those reported in the first experiment. Thus surface cell debris and scab formation with granuloma extending through the skin and abdominal muscle was seen after 3 days. By 10 days much of this reaction had subsided. Epidermal re-epithelialisation was achieved and a core of fibrous tissue marked the dermal wound site. Silk fibres were identified enclosed in a modest fibrous sheath in sections of skin and abdominal muscle. Associated inflammatory reactions were graded as mild to moderate (Fig 4). These reactions were entirely

local, surrounding tissues being entirely normal. Some perivascular lymphoid infiltrates were occasionally present in the region between the skin and the body wall musculature. In all respects, the histological changes seen in abdominal wound site in animals sutured with Thai silk, closely resembled those treated with Mersilk.

Figure 3 Silk suture in situ 10 days following use in closing a wound in the abdominal muscle. The silk thread is ensheathed connective tissue (arrowed).

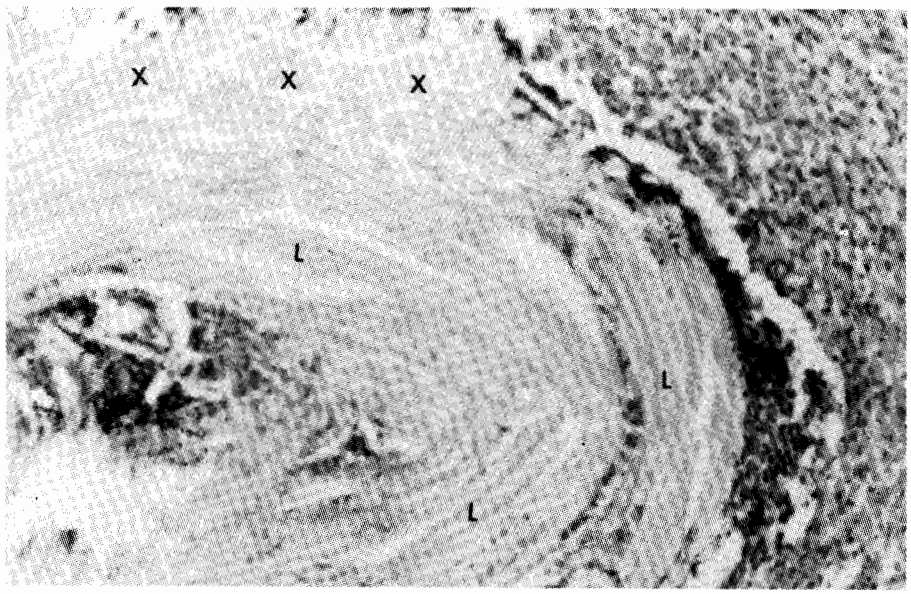

Figure 4 Section through Thai silk thread (XXX, LLL) as used to suture an abdominal wound in a Wistar rat. Note the low to moderate levels of inflammatory reaction.

DISCUSSION AND CONCLUSION

These simple experiments conducted in a well-defined experimental animal model strongly suggest that raw Thai silk is suitable for skin wound suture. With appropriate braiding to increase tensile strength, it would seem to have wider applications in dermatological surgery. From a practical view, our limited experimental studies demonstrate that the raw Thai silk does have certain disadvantages over the commercial product. Thus, the rough nature of the raw silk fibres prevents a clean suture insertion from being made. Secondly, we found that the undyed silk proved difficult to manipulate against the pale colour of the skin. Preliminary studies showed that silk fibres dyed blue were much easier to use, and are more suitable provided that the blue dye is without adverse reaction. It will be interesting to evaluate the allergenic capacities of various dyes used in silk processing.

Further studies are indicated to evaluate the tensile strength and durability of Thai silk fibres after a longer term in the body. Previous studies with silk sutures have indicated that although the fibres are non-absorbable, they are quoted as losing their tensile strength after about 60 days in vivo (Postlethwait, 1970). We have seen no evidence to suggest that silk threads evoke adverse reactions, and our study here indicates that wounds heal readily and a high level of regeneration is achieved.

ACKNOWLEDGEMENTS

We are grateful to the Royal Society for financial support enabling A B G L to visit the University of Khon Kaen Medical School. The hospitality of the University to a visiting lecturer is appreciated.

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