Main topic

Long-term histological results following glutaraldehyde cross-linked collagen injection into the suburothelial space of the mini-pig bladder

Peter Frey¹, Enrico Curschellas², and Max Kaeslin³

¹ Department of Paediatric Surgery, University Children’s Hospital, Basel, Switzerland, ² Institute of Pathology, University of Basel, Switzerland, ³ Department of Experimental Surgery, University Children’s Hospital, Basel, Switzerland

Abstract. Endoscopic correction of vesicoureteric reflux by subureteric injection has become an accepted method of treatment in selected cases. Its efficacy has been demonstrated in the treatment of well over 1,000 ureters. However, there are doubts as to the long-term safety of the commonly injected substance Teflon. Glutaraldehyde cross-linked bovine collagen, a biodegradable substance, was suggested as an alternative material for injection. The aim of this study was to evaluate the histological long-term behavior of glutaraldehyde cross-linked bovine collagen injected into the mini-pig bladder. One hundred and eight deposits of cross-linked collagen, each containing 0.2 ml (n = 36) or 0.6 ml (n = 72) collagen paste, were injected into the suburothelial space of the bladders of 24 mini-pigs. The histological behavior of the implants was studied at monthly intervals over a period of 12 months. After only 2 months a marked invasion of host fibroblasts and formation of endogenous type I and III collagen could be observed. Over 12 months the invasion of fibroblasts and the formation of new collagen increased dramatically. No migration of collagen particles into the local lymphatic system could be observed. A new staining technique (solophenyl red 3BL) was introduced to selectively demonstrate type I and III collagen fibres. In addition, the efficacy of the subureteric collagen injections could be demonstrated by successfully treating reflux that was iatrogenically induced in the mini-pig bladders.

Key words: Histology – Suburothelial-subureteric injection – Collagen – Mini-pig – Vesicoureteric reflux

Introduction

Endoscopic correction of primary and secondary vesicoureteric reflux by subureteric injection of Teflon paste has become an accepted method of treatment in selected cases. The efficacy of the technique was originally shown in animal experiments as well as in humans [21, 25].

In recent years the subureteric injection technique has been used to cure well over 1,000 refluxing ureters in children.

In 1970, Stone et al. [30] described granuloma formation at the site of injection and local migration of Teflon particles. Mittleman and Marraccini [18] first reported granuloma formation in the lung after periurethral Teflon injection for incontinence. Malizia et al. [15, 16] and Rames and Aaronson [26] demonstrated migration of Teflon particles into distant organs such as the lungs and brain after subureteric and periurethral injection of Teflon paste. These findings have induced doubts as to the long-term safety of Teflon injection in humans. As a result, alternative substances for injection such as autologous fat tissue and blood [13] and collagen preparations have been evaluated. Glutaraldehyde cross-linked bovine collagen paste (Zyplast, Collagen Corporation, Palo Alto, California, USA) seems to be an adequate alternative to Teflon. Cross-linked collagen has been widely used for soft-tissue augmentation in dermatology and plastic surgery [6, 10, 31] and to treat paralytic vocal cords [7] and gastroesophageal reflux [20].

In urology collagen has been injected periurethrally to treat incontinence [29] and subureterically [8, 9, 14, 19, 23] to treat vesicoureteric reflux. Several authors studied the histological behaviour of collagen after injection into the skin, subcutaneous space, and larynx of experimental animals and humans [2, 7, 12]. They all described very mild or absent perifocal inflammatory reactions, invasion of the implant by fibroblasts, and endogenous collagen formation. However, little has been published about the histological long-term fate of cross-linked collagen after urological application. Canning et al. [3] studied the histological behavior of cross-linked collagen injected submuously into rabbit bladders over a period of 6 months. They described a perifocal fibrous tissue reaction with very limited inflammatory reaction and no granuloma formation.

The aim of this study was to evaluate the histological long-term behavior of glutaraldehyde cross-linked bovine
Fig. 1. Four marked collagen deposits (0.6 ml Zyplast) injected into the submucosal space of the operatively opened mini-pig bladder. Note the excellent vascularisation of the bladder mucosa.

Fig. 2. Histological examination of a bovine collagen implant excised 12 months after injection (sulphophenyl red 3BL, x 40). Note newly formed collagen fibres (arrows) (Reduced to 85%)

Fig. 3. Histological examination of a bovine collagen implant excised 12 months after injection (sulphophenyl red 3BL under polarized light, x 40). Note newly formed collagen fibres (arrows) (Reduced to 85%)

Fig. 4. Histological examination of a bovine collagen implant excised 12 months after injection (sulphophenyl red 3BL under polarized light, x 80). Note fine, bright yellow-green collagen type III fibres (arrow) as well as thicker orange type I fibres (double arrow) (Reduced to 85%)