Gross and histopathological studies on biocasings for second intention urethral healing in dogs*

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A prescrotal urethrotomy is usually made in dogs to remove caliculi lodged caudal to os penis. Controversy exists over the closure of urethrotomy incision. Some surgeons prefer to allow urethrotomy incision to heal by second intention healing (Brown 1975, Wingfield and Rawlings 1979 and Gahring 1983). Stricture formation is a common complication after primary or secondary closure healing of urethral incisions (Brown 1975 and Stone 1984). Vikram Mohindra et al. (1996) reported the advantage of wrapping amniotic membrane around the urethrotomy incision to avoid stricture formation. An attempt was made to evaluate the efficacy of biocasings, viz. gelatin, fibrin and amniotic membrane for second intention healing in dogs.

Mongrel dogs (32) weighing 12-26 kg were premedicated with triflupromazine HCl @ of 2mg/kg b w intramuscularly 10 min prior to the general anaesthesia. 2.5% W/V solution of thiopentone sodium @ of 25 mg/kg b w intravenously was administered to induce and maintain general anaesthesia. The dogs were placed in dorsal recumbency and the urinary bladder was catheterized by using suitable size of the polyethylene tube and the catheter was left in situ by fixing it at preputial orifice. A prescrotal urethrotomy was performed in all the animals and randomly divided in to 4 groups of 8 animals each. The urethrotomy incision was left unsutured in control animals (group 1) and wrapped with gelatin, fibrin and amniotic membrane in animals of groups 2, 3 and 4 respectively. The cutaneous incision was sutured after leaving the retractor penis muscle over the urethrotomy incision with horizontal mattress pattern. The catheter was removed on third postoperative day. The biological materials were processed at the Central Leather Research Institute, Chennai, and supplied in a sealed ampoules/polyethylene packs in a required size.

Animals (2) from each group were euthanized on 7, 14, 30 and 60th postoperative day. Detailed autopsy was conducted and gross lesions at the operative site were observed. The tissue from urethrotomy site was collected and preserved in buffered normal saline. The tissues were then processed, 5μ thickness sections were cut and stained with a haematoxyline and eosin.

Gross examination of the operative site on day 7, revealed congestion and edema in all the groups. Congestion was severe in animals of group 1 (control) and group 2 (gelatin), whereas fibrin and amnion showed adherence to the operative site. Adhesions were thick in control and gelatin groups, filamentous in fibrin and thin in amnion groups. Animals of gelatin group showed presence of haematomas. Varying degrees of adhesions were noticed with the surrounding tissue at the operative site, indicating severity of inflammation. Poogrid and Wood (1986) and Vikram Mohindra et al. (1996) reported that inflammation might be due to a snugly fitting catheter, which allowed urine leakage. As the time advanced, the congestion and edema decreased gradually. On 14th day, the operative site was completely covered with fibrous tissue. Mild to moderate congestion and edema were noticed in subcutaneous tissue around the operative site. Two dogs from control and gelatin groups showed haemorrhagic foci on the urethral mucosa while, fibrin and amnion groups showed normal urethral mucosa.

On day 30 necropsy revealed mild changes at the operative site when compared to previous observation and healing at the operated site was complete. Urethral mucosa was clear without any haemorrhages in all the groups. No remnants of gelatin, fibrin and amnion material were observed. At the operative site there was mild swelling in gelatin group, whereas urethra in fibrin and amnion groups showed smooth mucosal surface. On 60th day in control and gelatin groups the urethral lumen was stenosed while fibrin and amnion groups showed patent urethral lumen. The incision site was completely sealed by the casings without any inflammatory

There are well-defined stages through which wound healing of urethra progresses. Subacute, proliferative and maturation phases result in epithelialization, synthesis of fibrous proteins and scar tissue contraction respectively (Peacock 1984). Accurate alignment of incised urethral edges is important since excessive granulation tissue formation occurs when unepithelialized do not cover the urethral wound (Bellah 1989). Inaccurate unepithelial apiposition often results in stricture and may cause complete obstruction. Scar tissue remodeling in corpora spongiosum leads to luminal stenosls over a period of months (Peacock 1984).

Histopathological studies

On seventh day, histologically there was infiltration of lymphocytes in the mucosa and submucosa of the urethra in all the groups (Fig. 1). Urethra in control and gelatin treated groups showed severe inflammatory reaction when compared to fibrin and amnion groups, while these groups showed angioblastic and fibroblastic proliferation and mild infiltration of neutrophils and macrophages (Fig. 2). Remains of biocasings were observed histologically which indicated incomplete healing process. The urethral lumen was patent. Fibroblasts oriented horizontally with collagen fibers at the site of application in fibrin excised urethra whereas amnion wrapped urethra showed urethral lumen with indication of capillary bed formation. Demodulation of the epithelium at the incision site was evident in gelatin wrapped urethra.

The sections from 14 th day revealed abundant fibrous tissue below the urethral mucosal plane in the control group whereas the fibrous tissue formation was moderate in gelatin and mild in fibrin and amnion groups. Inflammatory cells were more in control and gelatin and less in fibrin and amnion groups with severe angioblastic activity for new capillary bed formation. The epithelialization was incomplete in all the groups, however signs of epithelialization were evident (Fig. 3). The urethral lumen was patent. Cordero et al. (1991) observed fibroclastic and inflammatory action with amnion whereas Thomson and Parks (1984) reported stimulation of epitelialization by Lamin. Jodon et al. (1988) reported marked angioblastic and fibroblastic activity with mild

Figs 1-4. 1. Infiltration of Lymphocytes in the mucosa and submucosa of the urethra control group on day 7 (H&E 100×); 2. Angioblastic and fibroblastic proliferation and mild infiltration of neutrophils and macrophages, amnion group on day 7 (H&E 200×); 3. Signs of epithelialization-fibrin group on day 14 (H&E 630×); 4. Complete epithelialization with neovascularization zone. Amnion group on day 30 (H&E 200×).
infiltration with amnion. Control and gelatin treated animals showed more inflammatory cells compared to fibrin and amnion treated groups.

Most of the sections on 30th day showed complete epithelialization with neovascularized zones (Fig. 4). Fibrous tissue formation was abundant in control group moderate in gelatin and amnion groups and less in fibrin group. Mild inflammatory reaction with accumulation of neutrophils and lymphoid cells in the mucosal and sub mucosal layers was evident in control and gelatin groups. Fibrin groups showed horizontal infiltration of collagen fibers. The sections from 60th day revealed more fibrosis in control and gelatin group and less fibrosis in fibrin and amnion groups around incision site. The epithelialization was complete in all groups and there was no evidence of any inflammatory reaction and hemorrhage. The urethral lumen in control and gelatin groups was stenosed while the other groups showed patent lumen. Few sections showed focal nodular fibrosis in control and gelatin treated urethra with stenosis. Marked fibrosis and severe epithelial necrosis was noticed in urethra where urethral wall was not sutured (Weber et al. 1985, Vikram Mohindra et al. 1996). Animals of groups 3 and 4 showed less fibrosis with patent urethra. Peacock (1984) mentioned that scar tissue remodeling in peri urethral space leads to urethral stenosis.

In the present study, among the 3 biocasings used fibrin treated group showed comparatively better results followed by amnion and gelatin groups. Teh (1979) observed that, fibrin application stimulates the production of fibroblasts with increased vascularization at the grafted site. Enhanced phagocytosis, haemostatic effect and increased vascularization using fibrin were also reported (Schumacher et al. 1996). Wheaton et al. (1994) mentioned the haemostatic effect without inflammatory process and adhesions at the grafted site with fibrin application. Troensegaard and Henson (1950), Trelford and Trelford (1979) and Robson (1981) reported rapid epithelialization, pain reduction and increased vascularization properties of amnion during healing process. Though the results are in agreement with the previous reports the efficacy was lower than the fibrin groups. Orikasa and Tsuji (1970) observed early epithelialization histologically while Ganesh et al. (1994) observed faster healing process with gelatin sheet. However this group showed leakage, fistula formation and strictures indicating the ineffectiveness of the material for its usage at operative site. The biocasings proved to be satisfactory in controlling postoperative complications and to accelerate healing of urethrotomy wound in experimental dogs. Hence these can be used safely for clinical cases.

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REFERENCES


