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ORIGINALS

Partial Rat Kidney Resection Using Autologous Fibrinogen Thrombin Adhesive System

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Summary. The Fibrinogen Thrombin Adhesive System (FTAS) enables local haemostasis to occur in parenchymatous organs without tissue damage. The aim of this study was to investigate the degradation of FTAS and the process of wound healing after partial kidney resection in rats using FTAS for induction of local haemostasis.

In 28 rats partial kidney resection was performed bilaterally. Haemostasis was achieved with Fibronogen Thrombin Adhesive System. Four experimental groups were formed. Group A (n = 3): Haemostasis with unlabelled FTAS, subcutaneous injection of 0.1 ml = 60 µ Ci $Na^{125}I$. Group B (n = 3): Haemostasis with unlabelled FTAS, subcutaneous injection of 0.1 ml = 60 µ Ci ^{125}I FTAS. Group C (n = 6): Haemostasis with ^{125}I labelled FTAS. Group D (n = 16): treated like Group C. In Groups A - C ^{125}I-elimination in 24 h urine samples was determined with a gamma-scintillation counter. Pairs of animals in Group D were killed after 2, 6, 12 and 24 h and 3, 7, 14 and 21 days. Kidneys were examined under the light and electron microscope and by autoradiography. In animals of Groups B and C two peaks of ^{125}I excretion were observed: one peak within the first 48 h postoperatively which corresponded to the amount of free iodine injected with FTAS (FTAS contains 15% free iodine); a second peak after 120 h which was most probably due to the degradation of FTAS. Fibrinolysis was not observed. FTAS was resorbed mainly by macrophages. The time course of wound healing paralleled that of physiological fibrinogen concentration. Renal parenchymal damage was not observed.

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Key words: Kidney-resection, Fibrinogen, Wound-repair, ^{125}I-elimination.

INTRODUCTION

When performing operations on the renal parenchyma, reliable haemostasis is required but conventional suturing techniques secure haemostasis only by means of compression of the parenchyma, which necessarily interferes with kidney function (13, 14). The adhesive properties of acrylic monomers (4) in renal surgery were found to be insufficient (11). Among the disadvantages of the Acrylic-adhesive systems are the requirements for a bloodless field, a short period of renal ischaemia, inflammation, foreign body reaction and necrosis. The use of fibrin adhesives is a new method of potential clinical use in bringing about total haemostasis in parenchymatous wounds.

The Fibrinogen Thrombin Adhesive System (FTAS) allows local haemostasis in parenchymatous organs without tissue damage (12, 13). In this study we have examined the degradation of FTAS during healing after partial kidney resection in rats, using FTAS for production of local haemostasis. We followed the fate of the autologous fibrin clot histologically and by monitoring the redistribution of ^{125}Iodinated fibrin fragments.

MATERIALS AND METHODS

Fibrin Thrombin Adhesive System (FTAS)

FTAS consists of the following components:

1. Unlabelled Fibrinogen-Cryo-Precipitate (FCP) of Rats (IMMUNO, Vienna; 70-80% clottable protein) and ^{125}I-conjugated Fibrinogen-

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Cryo-Precipitate (8), 0.08 Ci/mg FCP, about 15% free iodine.

2. Thrombin ("Topostasin"^{R}, ROCHE) dissolved in 2.5 ml distilled water, 0.5 ml 10% calcium-gluconicum, 0.5 ml = 10000 KIE Aprotinin (Trasylol^{R}, BAYER) and 0.5 ml = 0.2 mg Epsilon Aminocapronic Acid (KABI).

FTAS was applied on a supporting collagen fleece (DISPERGER, Vienna), placed on the resection wound (13) and lightly pressed digitally on the resection area for 60 seconds. 28 male albino rats were used (Wistar SPF breed, average weight of 350 gs). The animals were kept in single cages and fed with Tagger^{R} complete food and water ad libitum. In order to achieve complete blockage of iodine absorption into the thyriod gland all animals were given 25 drops of Lugol's solution (ÖAB 9, solutio jodi aquosi) in 40 ml drinking water 5 days before starting the experiment. Under diethyl ether anaesthesia the kidneys were exposed through lumbar incisions, bilateral lower partial kidney resections were performed and haemostasis of the parenchymatous wound was achieved with FTAS as described above. 20% of the renal parenchyma was removed. Clamping of the renal vascular pedicle was avoided.

The animals were divided into 4 experimental groups:

Group A (n = 3): bilateral partial kidney resection, haemostasis with unlabelled FTAS, subcutaneous injection of 0.1 ml = 60 µ Ci Na^{125}I (AMERSHAM, IMS. 1 P ^{125}I Na Thiosulfate).

Group B (n = 3): bilateral partial resection, haemostasis with unlabelled FTAS, subcutaneous injection of 0.1 ml = 60 µ Ci ^{125}I FTAS.

Group C (n = 16): bilateral partial kidney resection, haemostasis with ^{125}I FTAS.

Group D (n = 16): bilateral partial kidney resection, haemostasis with ^{125}I FTAS.

The ^{125}I-elimination in a 24 h urine sample from animals in Groups A - C was measured by a gamma-scintillation counter daily up to the 10th postoperative day.

For morphological studies 2 animals of Group D underwent laparotomy 2, 6, 12 and 24 h and 3, 7, 14 and 21 days after surgery. The kidneys which had been partially resected were perfused with Hanks' solution to remove all intra-renal blood and then perfused for 10 min with 2.5% glutaraldehyde in 0.1 molar cacodylate-buffer (pH 7.4) (9). The tissue samples were embedded in Epon 812 and 1 µ sections were stained with 1% toluidine blue. For autoradiography Kodak Nuclear Track-Emulsion was applied to the sections, exposure time was 28 days at 4°C. Ultra-thin sections were examined in an EM9S electron microscope.

Serum creatinine and BUN were determined photometrically on the 3rd and 10th post-operative days.

RESULTS

General

No animal died immediately after operation or within the period of observation. Three animals developed a unilateral wedge-shaped, ischaemic renal infarction. Parenchymatous destruction to a maximum depth of 3 to 20 tubular lumina could be found in all other kidneys. In 2 cases a stone was found in the renal pelvis. Diffractometric X-ray analysis showed the stone composition to be calcium oxalate monohydrate. No animal developed uraemia.

Dynamics of ^{125} Iodinated FTAS and ^{125}I Na Thiosulfate

Mean values and standard deviations of the ^{125}I excretion in 24 hours urine samples indicated in percentage of the ^{125}I total excretion during the 10-day observation time for animals of Group A - C are shown in Fig. 1.

Group A (Subcutaneous Injection of 60µ Ci Na ^{125}I). ^{125}I excretion was maximal on the 2nd post-operative day (50.5±8.4%) and an exponential decrease of ^{125}I-elimination occurred after this time. By the third post-operative day 80% of the measured total dose had been eliminated. ^{125}I-

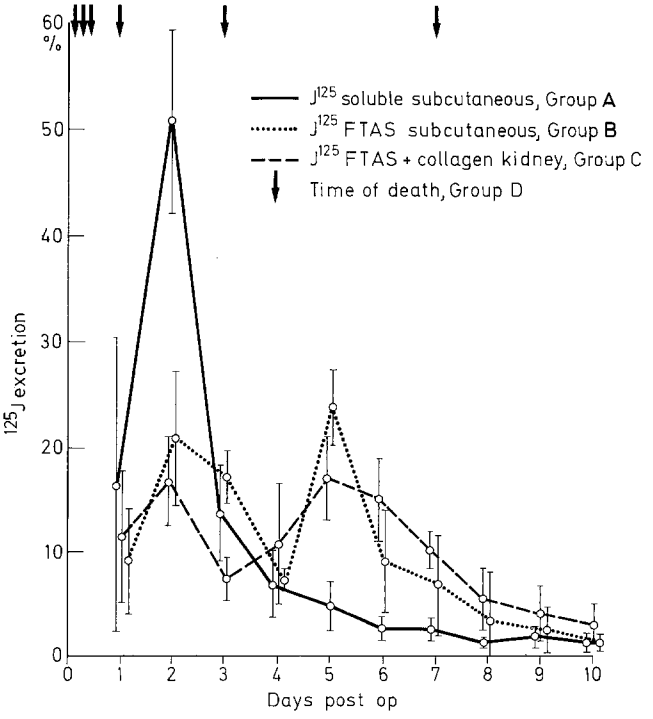

Fig. 1. Mean values and standard deviation of ^{125}I-excretion in 24 h total urine, indicated in % of total excretion per 10 days

elimination on the 10th post-operative day was 1.5±0.75%.

Group B (Subcutaneous Injection of 0.1 ml (Containing Approximately 75 mg Protein) = 60 µ Ci FTAS). Maximal ^{125}I-excretion was on the 2nd (20.6±6.2%) and on the 5th postoperative days (23.6±3.5%). The least ^{125}I-elimination occurred during the 4th post-operative day (7±1.3%). A slow decrease of ^{125}I-elimination occurred from 5th post-operative day onwards. ^{125}I-excretion on 10th post-operative day was 1.1±0.6% of the measured total dose.

Group C (^{125}I FTAS for Haemostasis of Kidney-Wounds). Maximal ^{125}I-elimination occurred on 2nd (16.5±4.2%) and 5th post-operative days (16.5±3.8%). The lowest excretion rate occurred on the 3rd post-operative day (7.3±2%). A slow decrease of ^{125}I-elimination occurred after 5th post-operative day. ^{125}I-elimination on 10th post-operative day was 3±2% of the measured total dose excreted.

Radioisotope excretion did not depend on the daily volume of urine.

Histological, Electron-Microscopic and Autoradiographic Findings in Animals of Group D

2, 6 and 12 Hours After Operation. No reaction of connective tissue was observed under the light and on the electron microscope. Collagen fleece was inbibed with erythrocytes and partly lifted off the parenchymatous area by small haematomas.

24 Hours After Operation. Light and electron microscope studies showed emigration of neutrophilic granulocytes and macrophages into the interstice (Fig. 2). Autoradiography showed larger amounts of labelled fibrin at the area of adhesion.

3 Days After Operation. Cell-rich granulation tissue and infiltration of granulocytes was seen under the light microscope (Fig. 3). Marked resorption of fibrin clots by phagocytising macrophages (Figs. 4, 5) as well as capillary outgrowth was seen under the electron microscope. A high concentration of radioactively labelled FTAS was still present.

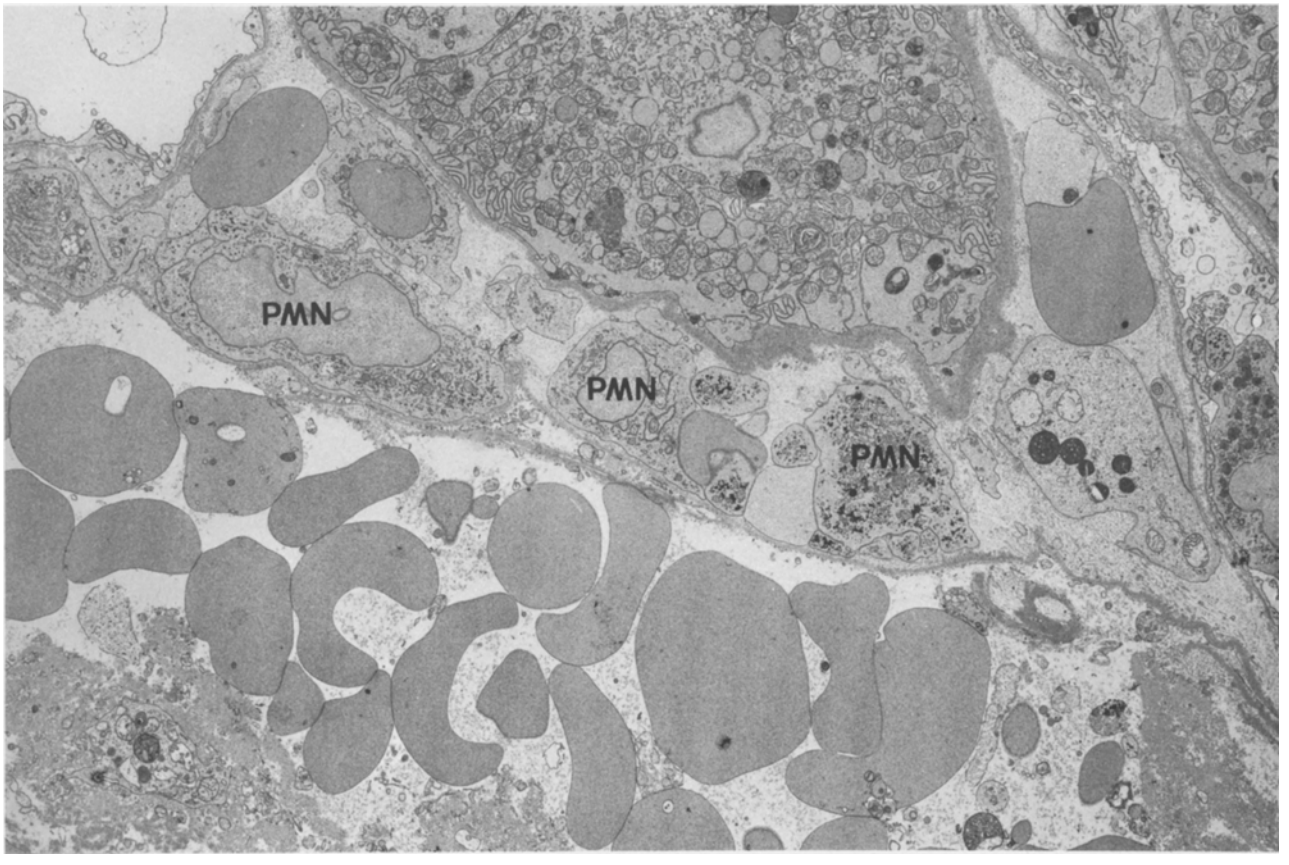

Fig. 2. Explanation see text, 24 h after operation (x 4,500)

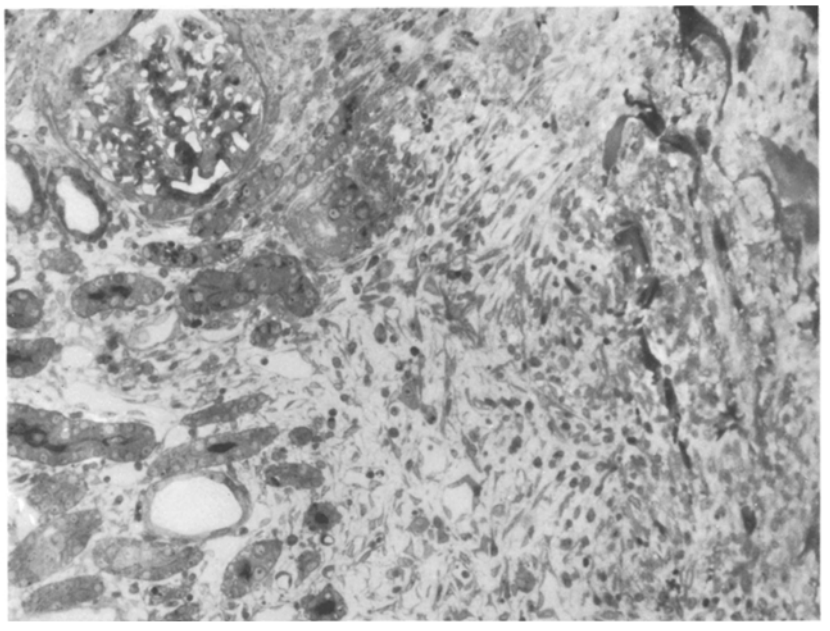

Fig. 3. Explanation see text, 3rd postoperative day (x 32)

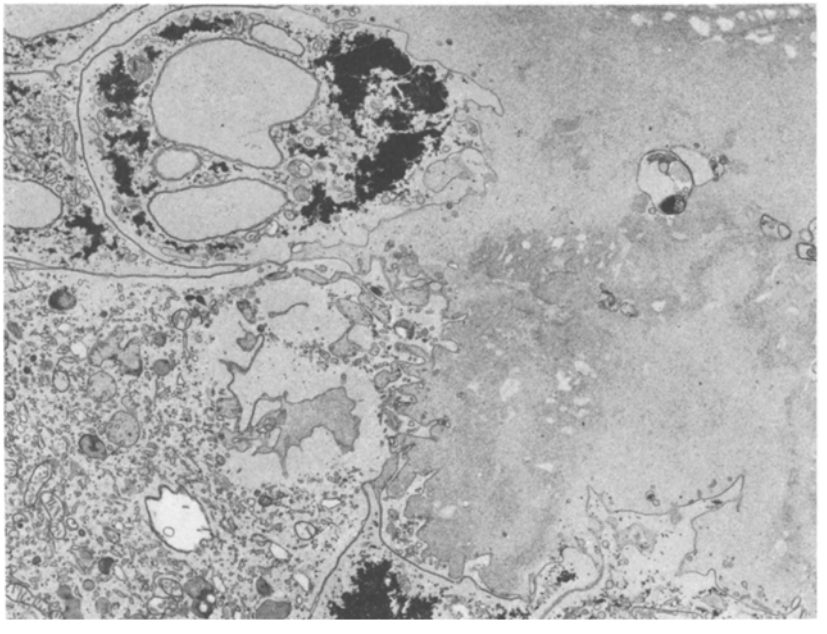

Fig. 4. FTAS-resorption by macrophages, 3rd postoperative day (x 5, 700)

7 Days After Operation. Collagen fibre appeared and isolated remnants of radioactively labelled fibrin were seen in the granulation tissue with numerous macrophages.

14 and 21 Days After Operation. Collagen-rich granulation tissue with a markedly decreased number of infiltrating cells was found. Until 14th post-operative day, radioactively labelled fibrin was found in macrophages (Fig. 6). There was decreasing infiltration of round cells.

DISCUSSION

^{125}I is mostly excreted in the urine after absorption of labelled iodine into the thyriod gland has been prevented by prior oral administration of an overdose of stable iodine. Analogous to the in vitro-examinations by Alkjaersig (1) and Dudock (5, 6) the determination of ^{125}I-elimination in urine gives information about the degradation of labelled fibrin clots.

After subcutaneous injection, ^{125}I- Na Thiosulfate was eliminated in the urine maximally on the

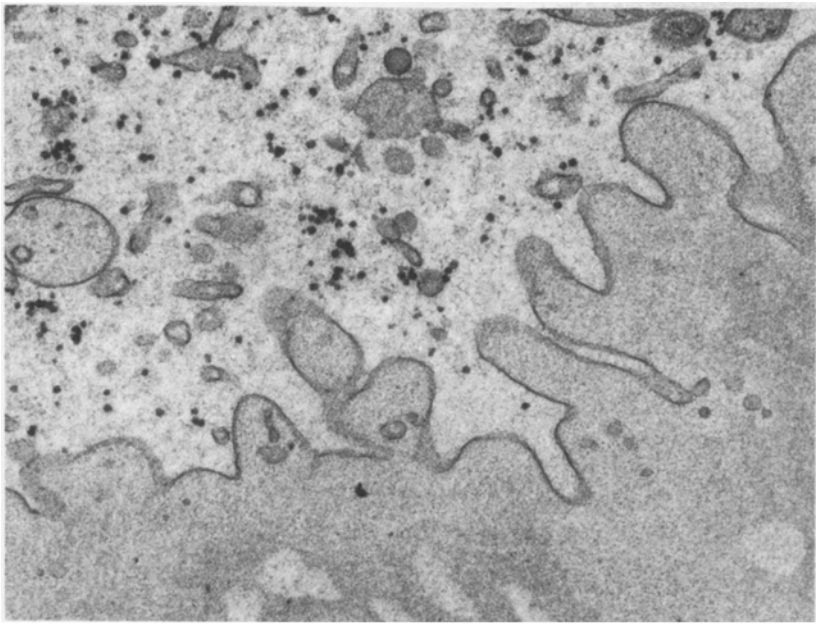

Fig. 5. Partial enlargement of Fig. 4 (x 27, 000)

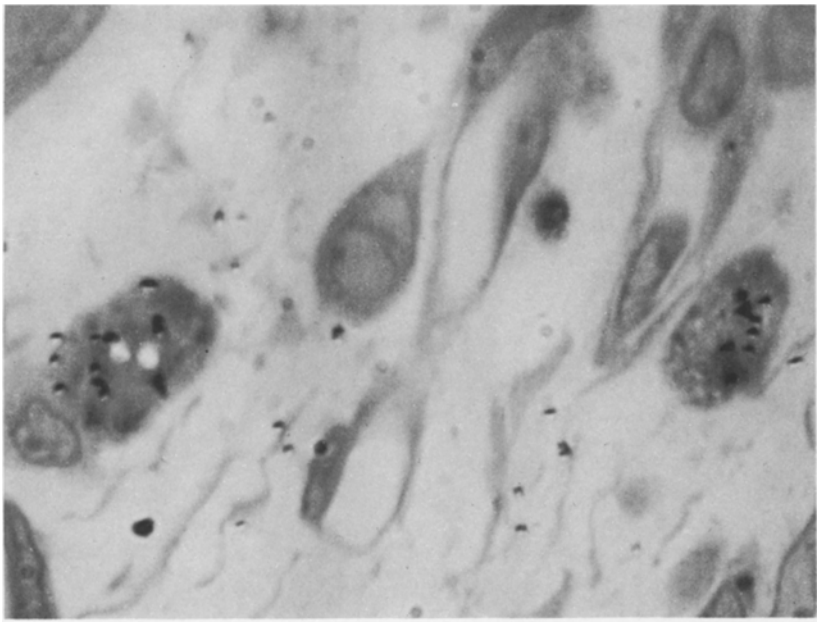

Fig. 6. Macrophages with stored labelled FTAS-degradation products, 14th postoperative day (x 320)

2nd postoperative day (50.5 ± 8.4% of total elimination per 10 days) in a single peak, reflecting the elimination pattern of free ^{125}I Na-Thiosulfate. By contrast, after subcutaneous injection of ^{125}I FTAS (Group B) and also after application of ^{125}I FTAS in a collagen fleece directly to the renal parenchym (Group C) there were two peaks of ^{125}I-excretion, one on the 2nd and one on the 5th postoperative day. The first peak after 2 days corresponded with the maximal excretion of unbound iodine in FTAS which consisted of about 15% of the total applied radioactivity. (The TCA precipitable radioactivity of labelled charges of FTAS amounted to an average of 85%). The operation itself may have delayed the maximum excretion of free, non protein-bound iodine to the second postoperative day.

The second peak of ^{125}I-excretion between the 3rd and 5th postoperative days in animals of Groups B and C coincided with the resorption of the fibrin-clot by macrophages (Group D) (Fig. 5) and may thus be derived from small iodinated fibrin fragments or from iodine freed in the process of clot organisation. These data suggest that the fibrin clot was not dissolved until the third day and could therefore provide haemos-

tasis during this critical time. The protracted secretion of radioactivity after the 5th postoperative day in animals of Group C may be caused by slow release of fibrinolytic fragments from macrophages (Fig. 6). We excluded the possibility that the collagen fleece interfered with the resorption of FTAS by finding that urinary iodine-excretion was identical in Groups B and C.

Wound healing after clot formation is initiated by emigration of granulocytes, macrophages and by capillary sprouting. Bösch (2) claimed that FTAS on a porous carrier accelerated wound healing in bone when compared with controls in which FTAS had not been used. Since proper controls for our kidney resections could not be performed, because the untreated kidney wound would cause recurrent severe haemorrhage (3) and mechanical damage of the kidney tissue may also cause conditions different from surgical treatment, no conclusions concerning the speed of wound healing in our experimental system could be drawn. In addition cyanoacrylat tissue adhesive cannot be used as a control because of its cytotoxic activity. The results of wound healing in rats after partial kidney resection and application of FTAS are similar to studies of wound healing in the rabbit's ear with physiological fibrin concentrations (7). The use of homologous Fibrinogen Cryo-Precipitate excluded any possible influence of foreign protein on haemostasis. Eosinophilic infiltration as a sign of allergic reaction (10) was not observed.

The wedge-shaped ischaemic necroses (3/32) were due to the division at operation of a functional end artery.

Our findings indicate that FTAS may be a useful tool for securing healing in parenchymatous wounds in renal surgery because it is not cytotoxic, can be used on wet surfaces, does not cause immune reactions, and forms a cover for the wound for at least 3 days, and can then be readily organised by granulation tissue.

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