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Histological evaluation of kidney development in young rats after a surgery and management of wound with fibrin glue

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ABSTRACT

Aim. The subject of this study is evaluation of histological hallmarks of kidney growth in rats after a surgery and subsequent management of the wound with fibrin glue.

Material and Methods. Sixty-five Wistar rats underwent a surgical procedure resulting in parenchymal injury by incision followed by an application of fibrin glue. Kidneys have been examined and evaluated histologically after 4 weeks (group I) and 26 weeks (group II) after the surgery

Results. When fibrin glue was used, in both groups, regular anastomoses of the wound edges of the kidney after surgery were noted. Histological features of kidney growth (renal glomeruli, renal tubules and blood vessels) were observed in the parenchyma and in the postoperative scar. In the second group kidneys grew on average by 3.5mm in the longitudinal section and by 1.89 in the cross section. In this group, the number of renal glomeruli nearly doubled. Glomeruli and tubules were also found in the postoperative scar.

Keywords: kidney, histology, surgery.

Introduction

Because good adhesive and haemostatic properties [1–4], fibrin based glues are used in surgical management of parenchymal organs in elective surgeries and post-traumatic wounds. Tissue adhesion occurs via activation of physiological haemostatic pathways. As a result, the inflammatory reaction around the wound is minimal, which shortens the healing period. In the end deposited fibrin is completely resorbed [5–7]. In this study Beriplast glue by Behring Company was used. It is a two-component adhesive containing fibrin-

ogen, thrombin, Ca⁺⁺ ions, factor XIII and fibrinolysis inhibitor. It causes fibrinogen to convert into fibrin with clot formation. Thrombin activates factor XIII, which catalyses the process of bonding fibrin polymers and clot stabilisation [8, 9].

We chose to study wound healing properties of fibrin glue in kidneys surgeries because they are technically difficult and carry a high risk of complications [10–12].

Additionally, interesting question was how histological structure of kidney is affected by

surgery and application of fibrin glue in developing organs. We have found that fibrin glue not only shortens the healing time of the wound, but also enables a regular development of the organ.

Aim

The aim of this work was to evaluate histological changes in developing rat kidney in rats that underwent a surgery and had the wound treated with fibrin based glue.

Material and Methods

A total of 65 Wistar rats, both male and female, aged 5–7 weeks, weighing 43–57g were chosen for the study. The research was approved by Bioethics Committee.

Surgery

All animals underwent a surgery of bipolar incision of the renal parenchyma. The resulted wound was treated with Beriplast FS fibrin glue (CSL Behring, King of Prussia, PA). Rats were anaesthetised with inhalatory ether. The limbs were secured with tape. Anaesthesia was maintained by administering drops of ether onto a mask of cotton wool. The shaved skin of the abdomen was disinfected with water solution of Hibitan. The abdominal cavity was opened with a left flank cross incision below the coastal margin. In order to secure skin flaps, two stitches were placed on the edges of the skin. This provided free access to the kidney. Left kidney has been visualized by moving away the intestinal loop. Subsequently, kidney was rolled up to the level of the lining of the stomach and put on a wet gauze pad. A two millimetre deep longitudinal incision of parenchyma along the side edge from upper towards lower pole was performed with a surgical knife.

After brief separation of the incised kidney, fibrin glue was applied to the bleeding surface of the parenchyma. The edges of the cut were brought together and lightly pressed with a gauze pad for about 30 seconds.

The animals were sacrificed and kidneys were harvested for histological examination 4 weeks after the surgery (group I) and 26 weeks (group II).

Histological examination

Examination of rat kidneys fixed in 10% buffered neutral formalin was carried out according to the routine histological technique. The specimens were embedded in paraffin, cut at 3–5 μm thickness using a Leica microtome and mounted on microscope slides. The sections were stained with haematoxylin and eosin.

Photographic documentation of the whole kidney, 40-fold magnified, was made using an Olympus BX 43 microscope and a XC 30 digital camera. Using computer software cellSens (Olympus), calibrated with the microscope, renal glomeruli were counted, and the postoperative scar was measured at the base in the cortex of the kidney.

Statistical analysis

Following parameters were calculated, for each of the testing group: average values, standard variance, minimal and maximal values.

All experimental results were analysed with Shapiro-Wilk method for assessing normal distribution. When the variant did not show normal distribution, a nonparametric test of Mann-Whitney test was used.

Significance of the statistical analysis results was set as $p < 0.05$.

The above analyses were performed using *Statistica 12* (Statsoft, Inc.) software.

Results

Group I

In all animals the wound healed. Average width of the scar, measured at the base of the cortex of the kidney was 1167.155 μm . In the bonding area lymphocytic infiltration was observed, with hemosiderin deposits. In 8 animals apart with lymphocytic infiltration, foci of parenchymal necrosis were visible. Presence of blood vessels in the scar was observed (**Table 1**).

Group II

In all animals the wound healed. Average width of the scar, measured at the base of the cortex of the kidney was 764.77 μm . In the area of the scar glomeruli and tubules were found. Proliferation of blood vessels was also visible in this area. Small foci of lymphocytes were observed in the scar or its proximity (**Table 2**).

Table 1. Descriptive statistics of results in Group I

	Group I				
	N	Average value	Minimum	Maximum	Std variance
Number of glomeruli	33	118.545	98.000	151.000	11.832
Longitudinal section (cm)	33	6.485	6.000	7.000	0.507
Cross section (cm)	33	4.455	4.000	5.000	0.506
Scar width (μm)	33	1167.155	688.580	1454.870	217.348

Table 2. Descriptive statistics of results in Group II

	Group II				
	N	Average value	Minimum	Maximum	Std variance
Number of glomeruli	32	212.344	159.000	247.000	22.075
Longitudinal section (cm)	32	10.000	8.000	11.000	0.622
Cross section (cm)	32	6.344	6.000	7.000	0.4823
Scar width (μm)	32	764.779	539.510	1100.640	150.265

Discussion

Fibrin glue is commonly used in laparoscopic procedures and in partial nephrectomy surgeries [13, 14].

Results of our research showed that when fibrin glue was applied to surgical wounds in the experimental model of parenchymal injury the organ development continued normally and histological structure has been maintained.

Based on the calculations of macroscopic length of longitudinal and cross sections of the kidney, the difference in both groups studied is statistically significant.

In animals examined 26 weeks after the incision of renal parenchyma, the scar was significantly thinner than in the group 4 weeks after the surgery. The kidney in those animals was also bigger. A larger number of glomeruli and tubules was also observed. The difference in the number of glomeruli between both groups was statistically significant ($p < 0.05$). The surgery did not result in disruption of kidney development.

It should also be noted that in both groups the markers of inflammation in the scar were visible. However, in the group examined 26 weeks after the surgery the histological findings were indicating less intensity of the inflammatory process.

In group II in the area of the scar, regular glomeruli, tubules and blood vessels appeared. No inflammation of the scar or renal interstitial fibrosis were observed. Similar results regarding the presence of renal tubules were presented by Wang PF and co-authors. The authors noted the presence of additional tubules on the 21st day after the surgery in rats [15].

The results obtained indicate that the surgery did not have an adverse effect on the development of the kidney in general and its gross microscopic structure. Despite of concern for haemorrhage in a well-vascularised organ as kidney no macroscopic or microscopic events were observed. This may be secondary to beneficial haemostatic properties of fibrinogen glue.

Conclusions

1. In all subject animals, when fibrin glue was used for bonding of experimental surgical parenchymal wound, the observed scar edges were regular.
2. The development of the kidney was not disturbed, which was confirmed by macroscopic and microscopic studies.

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Conflict of interest statement

The authors declare no conflict of interest.

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