Bratisl Lek Listy 2011; 112 (12)
676 – 678

EXPERIMENTAL STUDY

The effect of ankaferd to stop bleeding in experimental partial nephrectomy

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Abstract: *Aims:* In kidney surgery, bleeding is one of the most important issues. In partial nephrectomy, as a "blood stopper", we used surgycell and ankaferd which is used traditionally in Turkish medicine.
Material and methods: 24 Wistar-Albino rats were grouped randomly. Laparotomy was performed in the first group, sham group. In the second group, partial nephrectomy was performed to lower-kidney pole and then, serum physiology was given to the lower part of the kidney. In the third group, partial nephrectomy was performed and surgycell was given over the kidney. In the fourth group, partial nephrectomy was performed and then ABS was applied. In all groups, the gauze was weighted on sensitive lift before and after the operation in order to determine the amount of bleeding. After the subjects were left alive for 5 hours, the levels of blood urea, and creatinine and kidney histopathology were evaluated.
Results: No meaningful difference between the groups was found as for the levels of blood urea, and creatinine and the kidney histopathology. Bleeding amount was diminished significantly in the group 4, to which ankaferd was applied.
Conclusion: Ankaferd is a substance which can be used effectively for controlling acute bleeding in kidney surgery *(Tab. 2, Ref. 19)*. Full Text in free PDF *www.bmj.sk.*
Key words: partial, nephrectomy, hemorrhage, ankaferd, surgery.

Haemostatic agents have important roles in laparoscopic and open renal surgeries. They may be used as a single agent for many conditions. To date many materials have been described to stop hemorrhage during kidney surgery. As renal parenchymal hemaostatic aids, glues and other substances were reviewed by Thompson et al (1), they identified the advances in tissue sealants, and selective renal ischemia and haemostatic cutting modalities. In 1909, Bergel (2) first described the use of dry plasma to facilitate homeostasis. Subsequently fibrin glues, absorbable fibrin adhesive, synthetic hydrogel polymer and liquid albumin-indocyanine green solder have been developed to use with the same purpose as a sealing agent (3–6). The main mechanism in the renal parenchyma during homeostasis is to produce fibrin, which is facilitated by fibrinogen and thrombin. These products generally support this cascade to ensure homeostasis. Special feature of the kidney is that it receives one fifth of the cardiac output each minute, and therefore, any injury to the renal parenchyma may potentially result in hemorrhage (7). That is why it is so important to achieve renal parenchymal homeostasis during nephron sparing surgery and renal trauma. In open and LPN, increased WIT due to uncontrollable bleeding is a restricted factor to preserve sufficient renal function. However, the most difficult aspect of LPN is to achieve sufficient hemostasis and kidney reconstruction within the time constraints of the warm ischemia (7).

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Material and methods

We studied the place of the haemostatic agent ABS in nephron sparing surgery in an animal model. According to our knowledge, we used ABS as a haemostatic agent in a PN model for the first time in urology. ABS is a unique folkloric medicinal plant extract that has historically been used in Turkish traditional medicine. ABS is composed of a mixture of 5 plants, each has some effect on endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cell mediators (8). We evaluated the efficacy and histopathological effect of ABS in a PN model without renal hilar clamping. This study was approved by the local animal review and ethics committee, and the research council at our institution. A total of 24 Wistar-Albino rats weighing 150 to 170 g were divided into 4 groups composed of 6 rats and underwent right lower pole PN. Targeted excised tissue was determined to be approximately 1 cm^2 in each rat.

While the animals lying in backstroke position, peritonium was opened with ventral incision. In the first group (G1), the sham group, only peritoneum was opened, partial nephrectomy was not performed; none of the drugs was given. In the second group (G2), after partial nephrectomy, serum physiology was given. In the third group (G3), after partial nephrectomy, surgical was applied on the kidney cut. In the fourth group (G4),

after partial nephrectomy, ABS was performed. After the operation, abdominal wall was sutured. The gauze that was weighted on the sensitive scale was weight again. The given drug and serum amount were counted and then the total bleeding amounts were determined. After the subjects were left alive for five hours, abdominal wall was opened again and 4 mL blood was taken from vena cava inferior**,** gross specimens and histological sections were evaluated in blinded fashion by blinded pathologist. Sections were stained with hematoxylin and eosin.

Statistical analysis

Statistical analysis was performed using SPSS statistical software package for Windows (version 15). The statistical evaluation of the data was between 95 % and 0.05 meaningfulness; to compare inter-groups Kruskal–Wallis test, to compare intragroups and determine the difference source Mann- Whitney U test and Bonferroni adjustment were used. All variables were stated as medians because non-parametric tests were used in statistical analysis.

Results

The median blood urea nitrogen (BUN) of G1, G2, G3 and G4 were 35.5 (24–49), 51 (28–59), 43 (35–68) and 44 (36–50) mg/dL, respectively and there was no meaningful difference between the groups (p=0.27). Median creatinine levels were 0.695 (0.62–0.83), 0.815 (0.69–0.93), 0.925 (0.76–1.64), 0.85 (0.64–0.91), respectively and there were no statistical differences between the groups (p=0.057) (Tab. 1). The median weights of dry and wet pads of G1, G2 and G3 were 1.712850 (1.5926–1.9648), 1.9306 (1.3739–3.5093), 1.6731 (1.5656–1.9870) g and 3.94405 (2.3486–6.1276), 4.276050 (2.6089–5.3684), 2.32865 (2.1597–2.5726) g, respectively (Tab. 2). Statistical difference was found between the groups according to the dry and wet pad weights (p=0.008). The cause of the difference was evaluated. While there was no statistical difference between G2 and G3 (p= 0.818), between G2 and G4 (p=0.009), and between G3 to G4 (p=0.004) groups, meaningful difference was found in dry and wet par weights. Between the groups, there was no histopathological difference.

Discussion

Ankaferd, a medicinal product, has been approved by the Ministry of Health to manage external hemorrhage and dental surgery bleeding in Turkey based on safety and efficacy reports indicating its sterility and non-toxicity (www.ankaferd.com). It comprises of a standardized mixture of plants, including Thymus vulgaris 5 mg, Glycyrrhiza glabra 9 mg, Vitis vinifera 8 mg, Alpinia officinarum 7 mg and Urtica dioica 6 mg, 100 ml Ankaferd solution (Ankaferd ilac Kozmetik A.S, Ankara, Turkey). The basic Ankaferd mechanism of action is the formation of an encapsulated protein network that provides focal points for vital erythrocyte aggregation. The protein network induced by ABS is formed rapidly (less than 1 second). However, blood cells, particularly erythrocytes, participate in protein network formation. It has been shown that the ABS induced protein network is capable of regulating further coagulation and haemostatic reactions. Hence, normal haemostatic elements are spared during formation of the protein network since the blood clotting process is driven by protein agglutination (8). A small number of studies have been performed regarding the efficacy of ABS in visceral organs. In one study, ABS was successfully used in a case of upper gastrointestinal bleeding (9) and also its therapeutic potential was confirmed in managing hemorrhage during open heart surgery (10).This study design was based on the evaluation of the efficacy of ABS for achieving hemostasis in PN models. PN is now an acceptable approach for the management of localized, small renal tumors. Although the complication rate is slightly higher than that of open radical nephrectomy, advantages in terms of renal preservation have become more apparent. As a result, PN is being performed more frequently, even in patients with a normal contra lateral kidney (11). However, expansion of the indications for PN has been shown in publications, especially for tumors up to 7 to 10 cm (12). Recently the development of LPN has gained more popularity in the world, including hilar control, suture repair of the collecting system, suture ligation of blood vessels and capsular closure over Surgicell bolsters (13). Most investigators have limited reconstruction time to 30 minutes, while some believe that it may be extended to as long as an hour (14). Bleeding and ischemic renal damage due to

Tab. 1. Biochemistry results.

	G1	G2	G3	G4	P
BUN* (mg/dL)	35.5 (24–49)	51 (28–59)	43 (35–68)	44 (36–50	N.S
Creatinin (mg/dL)	0.695 (0.62–0.83)	0.815 (0.69–0.93)	0.925 (0.76–1.64)	0.85 (0.64–0.91)	N.S
Blood Urea Nitrogen*					

Tab. 2. Dry and wet weight in the operation pads.

	G2	G3	G4	P
Dry weight (g)	1.712850 (1.5926–1.9648)	1.9306 (1.3739–3.5093)	1.6731 (1.5656–1.9870)	N.S
Wet weight (g)	3.94405 (2.3486–6.1276)	4.276050 (2.6089–5.3684)	2.32865 (2.1597–2.5726	0.08

the warm ischemia period are the most important complications following surgery (15). In order to decrease the WIT and PNT, various tissue sealant and haemostatic agents have been developed to replace the tissue suturing. Several agents have been investigated for their haemostatic potential for managing vascular injury and many have also been evaluated for their efficacy for repairing collecting system injury. In our study, it was found that ABS application decreased bleeding significantly after partial nephrectomy.

Even if there is no histopathological difference between the groups, ABS application in open sore give the impression that it doesn't have toxic effect to the tissues. As a result of the biochemical blood search, levels of urea and creatinine did not differ between the groups, and this showed that Ankaferd application didn't have systemic negative effects on kidneys. In partial nephrectomy model that was used by Huri at al (16), cut parts of the kidneys were sutured, and it was seen that ABS application decreased the bleeding in small veins. (16) In our study, hilar vein occultation was not applied and it was seen that ABS application diminished the bleeding quickly and effectively. Desai et al (17) noted the effectiveness of gelatin matrix in a porcine hand assisted LPN model without hilar occlusion. This avoided the warm ischemia and its associated complications. Gill et al (18) reported that gelatin matrix dramatically decreased the complication rates of renal reconstruction after LPN. Recent study has shown the potential efficacy of these materials for decreasing hemorrhage and urinary leakage in LPN cases (19). Briefly, a decrease in blood loss, WIT and PNT were shown to be a main target in PN cases when using haemostatic agents and glue. However, the molecular and histological effects of these products have not been absolutely determined to date in the literature. Generally these agents have been used as supportive products on sutured kidney during PN. It should be stressed that the ideal topical haemostatic agent for PN should be easy to use, should show its effects within minutes, should be effective for arterial and venous bleeding, should preserve the glomerular structure and renal parenchyma, should provide collecting duct system closure and should not be toxic or anaphylactic. We know that it is too early to declare that Ankaferd is an ideal hemostatic agent for PN in humans. However, preclinical results provide expectations regarding the efficacy of Ankaferd on hemostasis during PN in rats. However, ABS should be tried in a larger animal model with larger caliber vessels before used in humans.

In conclusion, to our knowledge this preclinical experimental series is the first study of ABS as a hemostatic agent in a renal hemorrhage model. ABS facilitated effective hemostasis in PN models. Although the preservation of glomerular histology is not the main effect of ABS, we observed absent glomerular necrosis, which should be evaluated in a controlled study in comparison with a conventional group. Future preclinical and clinical studies are recommended to provide evidence-based medicinal findings regarding the routine application of ABS during renal surgical procedures.

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Received January 30, 2010.
Accepted August 18, 2011.