

Application of Transarterial Embolization of Renal Artery in Rabbits with Experimental Hydronephrosis

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ABSTRACT. This study was performed to validate the procedure of transarterial embolization of the renal artery (TAE-RA) using iohexol-ethanol solution in rabbits with unilateral experimental hydronephrosis and to evaluate the embolized kidney and contralateral normal kidney using B-mode ultrasonography and color Doppler ultrasonography. Experimental hydronephrosis was induced at 17 days after ligation of unilateral ureter in 13 rabbits. Renal artery embolization was performed using selective catheterization in the hydronephrotic kidney of eight rabbits and electrocardiography, oxygen saturation, body temperature, pulse, and respiratory rate were within normal ranges during procedures. Iohexol-ethanol solution was used as embolic material. Average ethanol dose for renal artery embolization was 1.4 ± 0.7 ml/kg. There were no rabbits expired after TAE-RA and no side effects associated with regurgitation of iohexol-ethanol solution. In color Doppler ultrasonographic findings, there was no blood flow into the embolized kidneys treated by TAE-RA, however, blood flow signal was found in hydronephrotic kidney not treated by TAE-RA. Ultrasonographically, the mean longitudinal length of the embolized kidney significantly decreased at 2 and 3 months after TAE-RA. No significant difference of resistive index values was found between contralateral normal kidney of rabbits treated by TAE-RA and contralateral normal kidneys of rabbits treated with nephrectomy. We may conclude that TAE-RA with iohexol-ethanol solution is a viable alternative to nephrectomy in rabbits with unilateral hydronephrosis.

KEY WORDS: color Doppler ultrasonography, hydronephrosis, iohexol-ethanol, rabbit, renal artery embolization.

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Nonfunctioning hydronephrosis, even with very thin parenchyma such as a wall of simple renal cysts, expels substantially the urine, and these lesions are progressive and will become symptomatic in the future [12]. Also, in ureteral obstruction of recent onset when tubular function is intact, the renin-angiotensin mechanism may be activated because of increased sodium reabsorption in the proximal tubule, which eventually produces the hypertension [3].

Simple nephrectomy has been routinely performed in patients with nonfunctioning kidneys and recurrent pain, infection or bleeding, however, this procedure is often technically demanding because recurrent infection or inflammation may be encased in dense adherent retroperitoneal tissues, leading to difficult dissection with the potential for serious injury. Patients with a poor general medical condition may also be treated with less invasive therapy, sparing them the additional morbidity associated with an operative procedure. Transarterial embolization of the renal artery (TAE-RA) was initially described in an experimental canine model with the intent of developing a therapeutic technique for treating neoplasia. This technique has been developed into an accepted method of treating advanced or unresectable renal cell carcinoma of human with persistent bleeding, pain or manifestations of the paraneoplastic syndrome [1]. TAE-RA has also been used to infarct end stage kidneys in

human patients with severe hypertension or proteinuria, native kidneys in transplant recipients as well as irreversibly rejected allografts and non functioning hydronephrotic kidneys [13]. For the purpose of renal artery occlusion, several embolic materials have been tried clinically as well as experimentally. These include autologous clot, muscle tissue, gelfoam, cyanoacrylate, and stainless steel coils. The embolic effects of autologous blood clot are ranging from 48 to 72 hr. Isobutyl 2-cyanoacrylate, Gianturco Wallace coil, absolute ethanol and Ivalon showed permanent embolic effects, respectively [2, 4, 5, 7, 10, 11, 18]. Also, iohexol-ethanol solution can be used as a 'visible ethanol' to improve the safety and ease of ethanol embolization [16].

In veterinary medicine, TAE-RA has not been performed to treat the nonfunctioning hydronephrosis nor has been the therapeutic effects of TAE-RA with iohexol-ethanol solution evaluated in laboratory animals with unilateral nonfunctioning hydronephrosis. The purpose of this study is to validate the procedure of TAE-RA using iohexol-ethanol solution in rabbits with unilateral experimental hydronephrosis for further application of TAE-RA to the ablation of nonfunctioning unilateral hydronephrosis in veterinary clinics.

MATERIALS AND METHODS

Experimental animals: Thirteen New-Zealand white rabbits with body weight ranging from 3 to 4.2 kg were used. The rabbits were housed in indoor cages and diet (Jerony[®],

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Table 1. Individual characteristics of rabbits

Group	Animal No.	Body weight (kg)	Sex	Hydronephrosis	TAE-RA*	Resection of hydronephrotic kidney
Group I	1	3.6	Male	O	O	
	2	4.2	Male	O	O	
	3	3.6	Female	O	O	
	4	3.4	Female	O	O	
	5	3.4	Male	O	O	
	6	3.8	Male	O	O	
	7	3.3	Female	O	O	
	8	4.2	Male	O	O	
Group II	9	4.2	Female	O		O
	10	3.1	Male	O		O
Control	11	3.3	Male	O		
	12	3.0	Female	O		
	13	3.6	Male	O		
Mean						
± SD		3.59 ± 0.41				

*: Transarterial embolization of renal artery. O: The procedure is performed in the rabbit. S.D.: Standard deviation.

Che-il Jedang, Korea) and water were supplied *ad libitum*. Experimental animals were used without distinction of sex (Table 1). The Committee for the Care and Use of Laboratory Animals in Seoul National University reviewed the procedures of this experiment according to the Guidelines and Regulations for Use and Care of Animals in Seoul National University.

Experimental unilateral hydronephrosis: Thirteen rabbits were anesthetized with 10 mg/kg of ketamine HCl (Ketalar®, Yu-han Yanghang Co. Ltd., Korea) by intramuscular injection and maintained with isoflurane (Aerane(r), Choongwae medical Co. Ltd., Korea).

During surgery, oxygen saturation (SpO₂) probe was applied to rabbit's tail and SpO₂ was monitored and lead II of electrocardiography and rectal temperature were monitored with anesthesia monitoring system (Vet-Ox® plus 4700, U.S.A.). The mid-abdomen was shaved and the animal was fastened to an operating table. The surgical area was scrubbed with povidone-iodine solution then draped in a sterile fashion. Under sterile conditions, a 7 cm midline incision was made around the umbilicus. A segment of the unilateral ureter was located and freed by blunt dissection so as not to damage any of the associated vascular structures. Then, the proximal part of the unilateral ureter was ligated with 2-0 black-silk in two places adjacent to renal pelvis. Carprofen (Rimadyl®, Pfizer, U.S.A.) was administered before extubation to all rabbits and was repeated at 4 to 6-hr intervals during the next 12 hr. On the 18th day after ligation of the unilateral ureter, hydronephrosis was confirmed by evaluation of resistive index (RI) and kidney size using ultrasound.

Selective angiography and renal artery embolization: Eight rabbits with unilateral hydronephrosis were anesthetized with same protocol on the 18th day after ligation of the unilateral ureter. Under aseptic conditions, a stab incision

was made on the inguinal region where pulsation was detected and a femoral artery was bluntly isolated. The distal portion of the artery was ligated while tension was applied to the proximal artery with 4-0 silk. The artery between the silk placement sites was punctured and the catheter (Fas-tracker® 18, length: 150 cm, outer diameter: 2.5F, Target Therapeutics Inc., Fremont, U.S.A.) and the 'J' shaped guide wire (Seeker®-16 Flexible guide wire, length: 175 cm, diameter: 0.016 inch, Target Therapeutics Inc., Fremont, U.S.A.) were introduced into the femoral artery. Under fluoroscopy (Dong-a X-ray R/F TV System, Korea), the catheter with guide wire was selectively introduced into ipsilateral renal artery of hydronephrosis. Iohexol (Omnipaque® 300 mgI/ml, Nycomed Ireland Ltd., Ireland) was used as the contrast agent. One thousand mgI/kg of contrast agent was used as a maximum dosage for selection of renal artery. During procedures, 0.3 ml of saline was administered to flush the remnant contrast agent in the catheter after every injection of contrast agent. The arteriogram of the renal artery was recorded with videotape.

Arteriogram being finished, ethanol (ethyl alcohol, Sigma, U.S.A.) mixture (iohexol: pure ethanol = 1:1) was injected through the catheter in 8 rabbits with unilateral hydronephrosis and saline was injected in 3 rabbits with unilateral hydronephrosis. During renal artery embolization, to avoid the regurgitation of embolic material into abdominal aorta, slow infusion (approximate 1 ml/min) was made under fluoroscopy. Then, angiogram was acquired immediately after TAE-RA through selected catheter by 1 ml bolus injection of iohexol. After selective angiography and renal artery embolization were performed, the catheter was retrieved and the femoral artery was ligated with 4-0 silk.

Nephrectomy of hydronephrotic kidney: Two rabbits with unilateral hydronephrosis were anesthetized and prepared with same protocol on the 18th day after unilateral ligation.

Under aseptic conditions, an incision was made on the mid abdomen and the hydronephrotic kidney was freed from its sublumbar attachments, using a combination of blunt and sharp dissection. The kidney was elevated and retracted medially to locate the renal artery and vein on the dorsal surface of the renal hilus. The renal artery and the renal vein were double ligated with non-absorbable suture and the kidney and the ureter were removed.

Ultrasonography and color Doppler ultrasonography: Ultrasonography and color Doppler ultrasonography were performed using a Toshiba SSA-260A machine (Japan) with a 7 MHz electronic sector probe at 0, 4, 9 and 17 days after surgery and 0, 1, 3, 5, 10, 30, 60, and 90 days after renal artery embolization or nephrectomy. Both kidneys of experimental group I (hydronephrosis + TAE-RA), experimental group II (hydronephrosis ± kidney resection) and control group (hydronephrosis) were examined. The same Doppler settings (pulse repetition frequency of 6,000 Hz, medium gain setting, wall filtering of 100 Hz, and low to medium color Doppler flow setting) were utilized to minimize technical errors. After shaving the right and left flank area, coupling gel was applied to the sites and the longitudinal and transverse scans were taken. In dorsal plane of kidney, length of longitudinal and transverse axis of kidney and length of renal pelvis and renal medulla were measured by built in caliper (Fig. 1). Then, color Doppler ultrasonography was performed initially to identify the interlobar or the arcuate artery for subsequent spectral Doppler analysis. Once a particular vessel was localized, interrogation using the Doppler gate to obtain a spectral waveform and quantitative information was performed. RI was determined by: $RI = (\text{peak systolic velocity} - \text{end diastolic velocity}) / \text{peak systolic velocity}$. The RI was calculated for each vessel as an average value obtained from three similar-appearing Doppler waveforms to reduce the effects of physiologic variation.

Serum profile: Blood was collected on 0, 4, 9 and 17 days after experimental hydronephrosis and 0, 1, 3, 5, 7, 10, 30, 60 and 90 days after renal artery embolization. Serum blood urea nitrogen, creatinine, calcium, phosphorus, and alanine aminotransferase levels were measured using enzymatic methods by automatic blood chemical analyzer (Selectra2®, Merck, Netherlands).

Statistical analysis: Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) statistical computer program. According to property of sample, one-way analysis of variance (ANOVA) and paired *t*-test were applied to data analysis.

RESULTS

Renal artery embolization: A single ipsilateral renal artery of obstructed kidney was selectively catheterized in 8 rabbits and immediate interruption of arterial flow was obtained in all cases.

Iohexol-ethanol solution was infused slowly at an approximate rate of 1.0 ml/min into the renal artery in 8 rab-

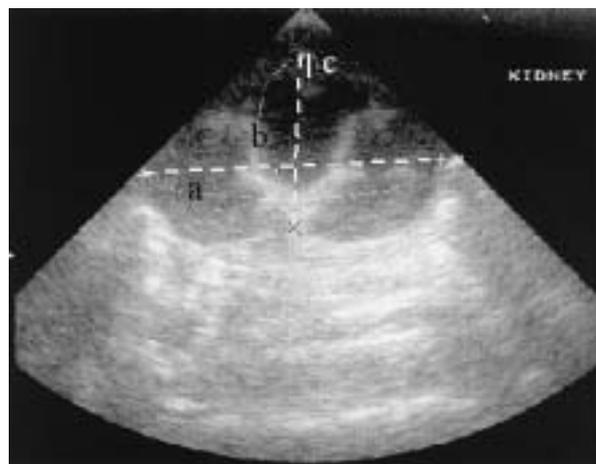


Fig. 1. The length of longitudinal axis (a), transverse axis (b) was measured in dorsal plane of kidney using built in caliper. The length of renal medulla and renal pelvis was calculated by subtraction of the length of renal cortex (c) from the length of transverse axis.

bits. The iohexol-ethanol solution was visualized faintly and distributed at thinned renal cortex under fluoroscopy when it was infused into the renal artery. The mean dose of iohexol-ethanol solution was 10.2 ± 5.2 ml ranging from 4.4 to 19 ml. The mean dose of absolute ethanol solution per body weight was 1.4 ± 0.7 ranging from 0.6 to 2.5 ml/kg (Table 2). The SpO₂, body temperature, pulse, and respiratory rate remained within the normal range during TAE-RA. The fasting serum levels for blood urea nitrogen, creatinine, calcium, and phosphorus level remained within the normal range for 90 days after TAE-RA. The longitudinal length of embolized kidney was significantly decreased at 60 days (37.5 ± 2.5 mm, $p < 0.01$) and at 90 days (27.8 ± 1.9 mm, $p < 0.001$) compared to that of before TAE-RA (51.0 ± 3.9 mm) (one-way ANOVA, Fig. 2). Also, the transverse length of embolized kidney was significantly decreased at 30 days (25.0 ± 2.9 , $p < 0.01$), 60 days (21.1 ± 0.7 mm, $p < 0.001$) and at 90 days (32.2 ± 2.4 mm, $p < 0.001$) compared to that of before TAE-RA (51.0 ± 3.9 mm, one-way ANOVA, Fig. 3).

Selective angiography: All 8 rabbits underwent successful complete obstruction of the renal artery, as demonstrated by the absence of blood flow to the embolized kidneys on immediate postoperative angiograms (Fig. 4).

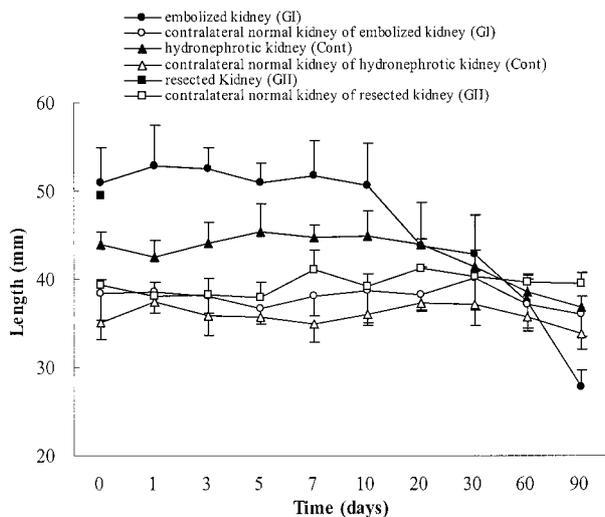
RI of contralateral normal kidney after TAE-RA: All 8 rabbits underwent successful complete obstruction of the renal artery, as demonstrated by the absence of blood flow to the embolized kidneys on color Doppler ultrasonography throughout the observation period. However, blood flow signals were detected at the thinned renal cortex of the hydronephrotic kidney in 3 rabbits not treated by TAE-RA throughout the observation period.

The mean RI values of interlobar artery or arcuate artery of kidney are summarized in Fig. 5. The mean RI values of contralateral normal kidneys in experimental group I

Table 2. Characteristics of the rabbits with unilateral hydronephrosis treated by transarterial embolization of renal artery

Animal No.	Body weight (kg)	Related to renal artery embolization	
		Dose of iohexol-ethanol solution (ml)	Dose of pure ethanol (ml/kg)
1	3.6	8.4	1.2
2	4.2	8.1	1.0
3	3.6	5.4	0.8
4	3.4	9.8	1.4
5	3.4	4.4	0.6
6	3.8	19	2.5
7	3.3	14.8	2.2
8	4.2	11.6	1.4
Mean \pm SD	3.7 \pm 0.3	10.2 \pm 5.2	1.4 \pm 0.7

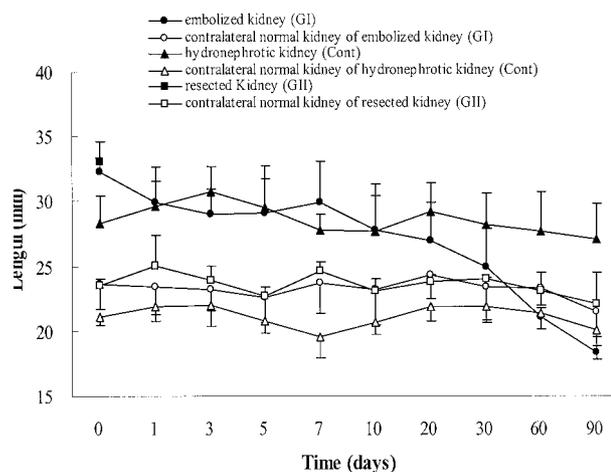
S.D.: Standard deviation.

Fig. 2. Longitudinal length of kidney treated by transarterial embolization of renal artery. The longitudinal length of embolized kidney is significantly decreased after 60 days ($p < 0.01$) and 90 days ($p < 0.001$) after transarterial embolization of renal artery (one-way analysis of variance). GI: Group I, GII: Group II, Cont: Control group.

(hydronephrosis + TAE-RA) were not significantly different from that of contralateral normal kidneys in experimental group II (hydronephrosis + nephrectomy) and that of contralateral normal kidneys in control group (hydronephrosis) throughout the observation period.

DISCUSSION

TAERA is accepted in the ablation of diseased kidney in human patients. Embolic materials include the Gianturco steel coil, gelfoam, Ivalon particles, polyvinyl alcohol, autologous clot and detachable balloon catheters. Also, there have been many experimental studies done about the embolization of kidneys with various embolic materials [2, 4, 5, 7, 10, 11, 18]. One of the techniques available for per-

Fig. 3. Transverse length of kidney treated by transarterial embolization of renal artery. The transverse length of embolized kidney is significantly decreased after 30 days ($p < 0.01$), 60 days ($p < 0.001$) and 90 days ($p < 0.001$) after transarterial embolization of renal artery TAE-RA (one-way analysis of variance). GI: Group I, GII: Group II, Cont: Control group.

manent vascular occlusion seems to be the use of the Gianturco stainless steel coil [4, 18, 19]. Ekelund *et al.* used steel coils for therapeutic embolic occlusion of the renal artery in nine human patients with inoperable renal carcinoma, and no complications were seen without any evidence of recanalization at follow-up arteriograms [8]. However, they reported the risk of peripheral embolization, pseudoaneurysm formation and drawback of Gianturco coils and therefore, obliteration of the renal artery by ethanol injection was very safe and convenient in experimental model. Miyazono *et al.* reported that infusion of pure ethanol into the adrenal artery was difficult because of its radiolucency through their initial experience with TAE of aldosteronomas, therefore, iohexol-ethanol solution could be an alternative to pure ethanol in experimental study [16]. In this study, technique of renal artery ablation by iohexol-ethanol solution was very

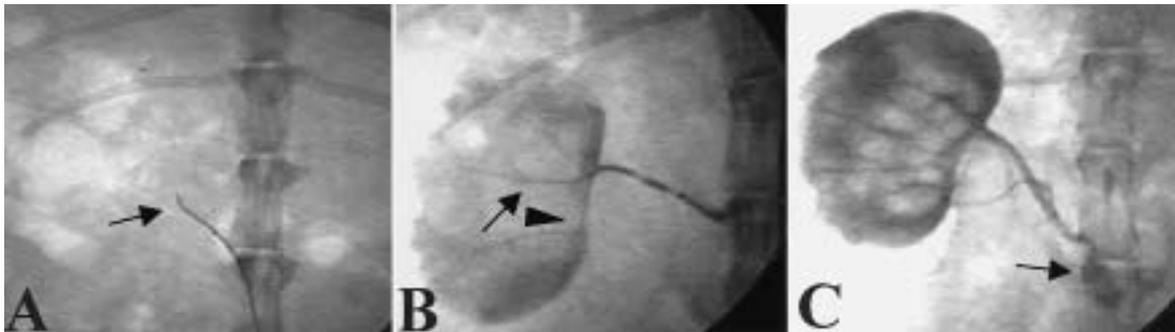


Fig. 4. Transarterial embolization of renal artery (TAE-RA) and angiographic findings immediately after TAE-RA. A. The catheter (arrow) with guide wire is selectively introduced into the renal artery of hydronephrotic kidney. B. Iohexol-ethanol solution is injected into the renal artery via selected catheter. Iohexol-ethanol solution is visible at the interlobar artery (arrow) and thinned renal cortex (black arrow-head). C. After TAE-RA, contrast agent injected into the renal artery is regurgitated into the abdominal aorta (arrow) due to the obstruction of the renal artery. The enhancement of the renal artery and the renal parenchyma is due to the accumulation of injected iohexol-ethanol mixture during TAE-RA.

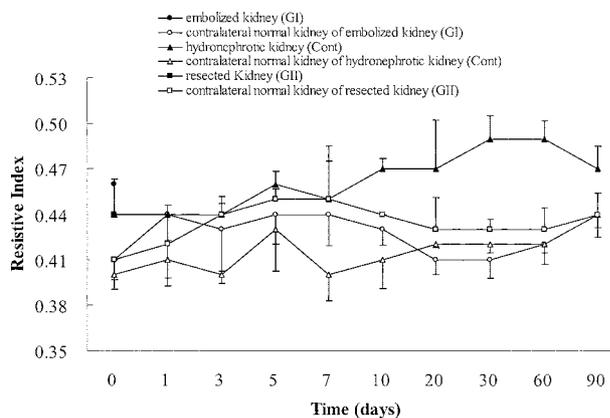


Fig. 5. Resistive index of kidney treated by transarterial embolization of renal artery. The mean resistive index values of contralateral normal kidney in embolized group was not significantly different from that of contralateral normal kidney in rabbits with nephrectomy and that of contralateral normal kidney in control group throughout the observation period (paired *t*-test). GI: Group I, GII: Group II, Cont: Control group.

easy to perform in the experimental hydronephrosis of rabbits. Reflux into the aorta might be prevented by a slow injection of the solution, and even if small amount of solutions occurred, this would not seem to be harmful and there were no change of serum profile in 8 rabbits after TAE-RA.

The number of reports of interventional treatment for nonfunctioning hydronephrosis is relatively small in human patients. A case of posttraumatic renal hypertension secondary to unilateral hydronephrosis who underwent ablative TAE-RA with Gianturco-Wallace coils presented successful control of renal hypertension over 24 months; however, the fate of the hydronephrosis was not mentioned [17]. Another case of nonfunctioning hydroureteronephrosis who received TAE-RA with 90% ethanol underwent percutaneous nephrectomy resulting in the removal of a total 16 g of renal parenchyma. The renal volume estimated by ultrasound

was reduced from 129.7 ml at the time of operation to 17.1 ml at 90 days after the procedures [15]. Total ablative treatment for nonfunctioning hydronephrosis combined with TAE-RA and percutaneous sclerotherapy of the renal pelvis and ureter with absolute ethanol have been reported to be safe and less invasive alternative to surgical nephrectomy in human patients [12].

However, the procedure of treatment for hydronephrosis with TAE-RA with Iohexol-ethanol solution has not been reported yet. In this experiment, the rabbits had small diameter of the renal artery and the femoral artery which could not enable us to use the balloon catheter and Gianturco-Wallace coil widely used for human patient. However, a small catheter such as the Fas-tracker 18 with outer diameter of 2.5F which is designed for interventional procedures of human cerebral diseases could be introducible into the renal artery of rabbits. Iohexol-ethanol solution was employed for the treatment of hydronephrosis because it is easy to deliver through small catheters. Also, it is inexpensive, easy to handle, and it provides complete infarction with both cellular death and secondary vascular thrombus without evidence of collateralization, and shows a low risk of complications such as reflux to other organs [8, 9].

For nonsurgical interventional treatments, a precise follow-up evaluation is needed. Follow-up studies of nonfunctioning hydronephrosis in human patients treated with TAE-RA using absolute ethanol were performed periodically by enhanced computed tomography and found that marked shrinkage of hydronephrosis without any enhancement of the parenchyma [12]. In case of traumatic renal hypertension secondary to unilateral hydronephrosis in human patients treated TAE-RA using Gianturco-Wallace coil, revascularization was evaluated with abdominal aortogram and selective left renal arteriogram and they demonstrated the total occlusion of the left renal artery distal to the coils [17]. In our study, the embolized kidneys were evaluated using color Doppler ultrasonography and no blood flow signals in the embolized kidneys in 8 rabbits treated by

TAE-RA were detected without general anesthesia and the use of contrast medium and this results was quite correspond with the selective angiogram findings. Also, the longitudinal length of kidney estimated by ultrasound was significantly reduced at the time of 60 and 90 days after TAE-RA and it is very similar with previous clinical study performed in human patients [12].

Chen *et al.* observed no difference in the RI values in kidneys without obstruction among the human patients with obstructive uropathy and normal controls *et al.* [6]. In this study, the mean RI values of contralateral normal kidneys in experimental group I (hydronephrosis + TAE-RA) were not significantly different from that of contralateral normal kidneys in control group (hydronephrosis) and that of contralateral normal kidneys in experimental group II (hydronephrosis + nephrectomy) throughout the observation period. The fact that the normal contralateral kidneys in control group and in experimental group had no elevated RI suggested that the hemodynamic changes in obstructed kidneys are local intrarenal rather than systemic events as suggested by Ichikawa and Brenner [14] in an animal study and Chen *et al.* [6] in human clinical study. Also, it is implied that TAE-RA after hydronephrosis does not alter the hemodynamics of contralateral normal kidney. Thus, it is considered that TAE-RA with iohexol-ethanol solution is a viable alternative to nephrectomy in rabbits with unilateral hydronephrosis.

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