INTERVENTIONAL RADIOLOGY
Official journal of the Japanese Society of Interventional Radiology and Asia Pacific Society of Cardiovascular and Interventional Radiology

ORIGINAL RESEARCH

Comparison of Renal Damage Following Renal Artery Embolization with Three Different Embolic Mixtures in Swine

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Abstract:
Purpose: Renal artery embolization is a minimally invasive and effective procedure for renal ablation, a complete necrosis of the renal parenchyma. This study aims to compare the extent of renal damage in swine following renal artery embolization with ethanol and N-butyl-2-cyanoacrylate, commonly used as embolic materials in renal ablation.
Material and Methods: Three different embolic mixtures were prepared for renal artery embolization in swine: 33% ethanol-Lipiodol mixture (ethanol:Lipiodol = 1:2; Group A), 67% ethanol-Lipiodol mixture (ethanol:Lipiodol = 2:1; Group B), and 10% N-butyl-2-cyanoacrylate-Lipiodol mixture (N-butyl-2-cyanoacrylate:Lipiodol = 1:9; Group C). Three swine were assigned to each group and underwent embolization of the unilateral renal artery. Renal arteriography was performed before, immediately after, and two days after renal artery embolization. After two days, the kidneys were removed to determine the macroscopic necrosis rate and for histologic examination. Dark tissue regions were considered necrotic.
Results: The macroscopic necrosis rate of the kidneys was 50.3% ± 7.4%, 100% ± 0%, and 100% ± 0% in Groups A, B, and C, respectively. The necrosis rates were higher in Groups B and C than in Group A. Histologically, the renal tubules were damaged in the necrotic areas. In addition, the glomeruli were damaged in Groups A and B but were preserved in Group C.
Conclusions: Sixty-seven percent ethanol-Lipiodol mixture and 10% N-butyl-2-cyanoacrylate-Lipiodol mixture are effective embolic materials in renal artery embolization for renal ablation in swine. Also, ethanol caused partial glomerular necrosis, whereas N-butyl-2-cyanoacrylate preserved the glomeruli. Therefore, ethanol should be used for renal ablation.

Keywords:
ethanol, NBCA, RAE, renal ablation

Interventional Radiology 2023; 8(3): 154-160
https://doi.org/10.22575/interventionalradiology.2021-0031
https://ir-journal.jp/

Introduction

Renal artery embolization (RAE) is a minimally invasive and effective procedure for treating renal tumors (e.g., renal cell carcinoma and angiomyolipoma), renal arteriovenous malformation, polycystic kidneys, and urinary incontinence associated with ureteral ectopia [1-15]. RAE is performed to achieve renal ablation (complete necrosis of the renal parenchyma), leading to tumor size reduction, tumor rupture prevention, and renal function abolition. The embolic materials used in RAE vary among facilities, and examples include metallic coils, gelatin sponges, N-butyl-2-cyanoacrylate (NBCA), and ethanol. RAE using metallic coils is effective for occluding traumatic bleeding and pseudoaneurysms, and gelatin sponges are effective for occluding bleeding from a small distal renal branch. However, metallic coils and gelatin sponges can cause recanalization of the renal artery [11, 16].

Furthermore, metallic coils are expensive and time-consuming to insert. Kauffmann et al. [17] proposed the concept of capillary embolization for tumor ablation. Liquid embolic materials, such as NBCA and ethanol, are useful

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Received: December 22, 2021, Accepted: June 13, 2023, Advance Publication by J-STAGE: September 21, 2023

for capillary embolization and are commonly used for renal ablation. This study aimed to compare the extent of renal damage in swine following RAE with ethanol and NBCA.

Material and Methods

Experimental animals and preparation of the embolic mixtures

Institutional review board approval was obtained for this animal study. Nine healthy, specific-pathogen-free, female swine, weighing 50-52 kg were used in this experiment. The swine were allocated to three groups for unilateral RAE with the following embolic materials: 33% ethanol-Lipiodol mixture (ethanol:Lipiodol = 1:2; Group A), 67% ethanol-Lipiodol mixture (ethanol:Lipiodol = 2:1; Group B), and 10% NBCA-Lipiodol mixture (NBCA:Lipiodol = 1:9; Group C). Three kidneys were assigned to each group.

Renal artery embolization

Preanesthesia was induced using a combination of 5 mg/kg ketamine and 0.08 mg/kg atropine sulfate. General anesthesia was maintained with isoflurane gas via intubation. Cardiac and respiratory data were monitored throughout the procedures. Angiography was performed using an X-ray system (Allura Xper FD 20; Royal Philips Electronics, Amsterdam, Netherlands).

Before performing RAE, a 6 Fr sheath (Radifocus Introducer II H; Terumo Clinical Supply, Gifu, Japan) was first inserted via the right femoral artery, followed by a 4 Fr guide catheter (1CJ; Medikit, Tokyo, Japan). The guide catheter was inserted into the unilateral renal artery. We selected the easier side to catheterize based on the renal artery's diameter and/or branch angle.

For RAE using the ethanol-Lipiodol mixture in Groups A and B, a 5.2 Fr balloon catheter with a maximum diameter of 9 mm (Selecon MP Catheter II; Terumo Clinical Supply, Gifu, Japan) was inserted instead of a guide catheter to prevent reflux and dilution of the ethanol. Next, a 1.9 Fr microcatheter (Tellus; Asahi Intecc, Aichi, Japan) was inserted into the renal artery through the balloon catheter, using a 0.016 in microguidewire (Meister, Asahi Intecc, Aichi, Japan). After inflating the balloon, 33% and 67% ethanol-Lipiodol mixtures were used for the RAE of the unilateral renal artery. For RAE using NBCA-Lipiodol mixture in Group C, a 1.9 Fr microcatheter was inserted into the renal artery through the guide catheter using a 0.016 in microguidewire. Next, the RAE of the unilateral renal artery was performed using 10% NBCA-Lipiodol mixture. In all three groups, the RAE's endpoint was complete renal artery occlusion.

Renal arteriography was performed before, immediately after, and two days after RAE (**Fig. 1**). Additionally, fluoroscopic imaging was performed immediately after RAE in Group C.

Evaluation of renal damage

Two days after RAE, the kidneys were removed, fixed with formalin, and cut into 1-cm-thick slices (**Fig. 2**). Dark tissue regions, as visualized in photographs, were assumed necrotic. The necrotic and renal areas were traced using Photoshop CS6 (Adobe Systems Inc., San Jose, CA, USA) for each slice. The necrosis rate was calculated as the total necrotic area (sum of all the slices)/total renal area (sum of all the slices) × 100% (**Fig. 3**). Some tissue samples were stained with hematoxylin/eosin for histologic examination.

Results

Renal arteriography performed immediately after RAE completely occluded the renal artery in all nine swine (i.e., all three swine in each group), without any complications caused by the procedures. Two days after RAE, the renal arteries were partially recanalized in three swine in Group A but remained occluded in three swine in each of Groups B and C (**Fig. 1**).

Macroscopic images of the cut surface of the kidneys removed two days after RAE showed heterogeneous necrosis, with dark and light regions in patches in Group A (**Fig. 4-a**). In Groups B and C, the cut surface was dark, indicating complete necrosis (**Fig. 4-b, c**). Based on the macroscopic images, the necrosis rate at two days after RAE was 50.3% ± 7.4%, 100% ± 0%, and 100% ± 0% in Groups A, B, and C, respectively (**Table 1**). The necrosis rate was numerically greater in Groups B and C than in Group A.

Histological examination confirmed that the dark tissue regions were necrotic. The degree of inflammatory cell infiltration in the renal parenchyma was similar among the three groups. The histological examinations of necrotic regions in Groups A and B revealed the loss or obscurity of nuclei in the tubular epithelium combined with partial necrosis of the glomeruli. In Group C, we observed the loss of nuclei in the tubular epithelium, but all glomeruli were intact (**Fig. 5**). A small number of epithelial cells located immediately beneath the renal capsule were not completely necrotic in all three groups.

Discussion

We selected three mixtures to use as embolic materials: 33% ethanol-Lipiodol mixture (ethanol:Lipiodol = 1:2), 67% ethanol-Lipiodol mixture (ethanol:Lipiodol = 2:1), and 10% NBCA-Lipiodol mixture (NBCA:Lipiodol = 1:9). In earlier embolization procedures, the concentration of ethanol ranged from 50% to 99% [1, 2, 4, 5]. Park et al. reported that the infusion of ethanol-Lipiodol mixtures containing 50% or 75% ethanol resulted in embolization equivalent to that achieved using absolute ethanol [18]. Tanaka et al. reported that a concentrated mixture of 2:1 ethanol-Lipiodol achieved remarkable shrinkage of enlarged kidneys [12]. Based on those findings, we prepared the ethanol-Lipiodol mixture at a ratio of 2:1 in this study. Because the ratio of ethanol used

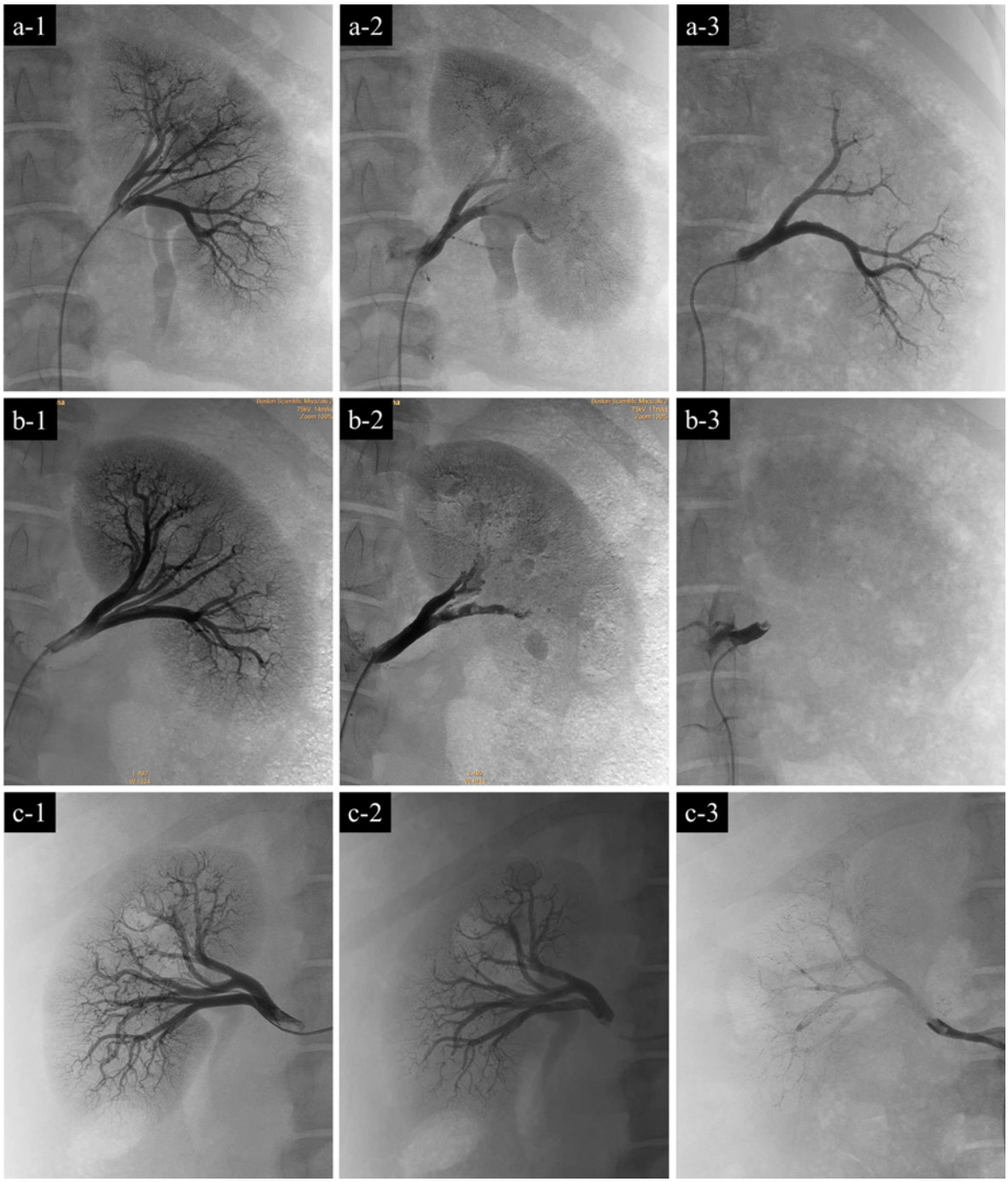

Figure 1. Images obtained before, during, and two days after renal artery embolization (RAE) using 33% ethanol (a; Group A), 67% ethanol (b; Group B), or 10% NBCA (c; Group C) in swine. (a-1) Left renal arteriography (RA) before RAE. (a-2) Left RA immediately after RAE showing complete occlusion of the left renal artery. (a-3) Left RA two days after RAE showing partial recanalization of the left renal artery. (b-1) Left RA before RAE. (b-2) Left RA immediately after RAE showing complete occlusion of the left renal artery. (b-3) Left RA two days after RAE showing complete occlusion of the left renal artery. (c-1) Right RA before RAE. (c-2) Fluoroscopic image immediately after RAE showing retention of 10% NBCA in the right kidney. (c-3) Right RA two days after RAE showing complete occlusion of the right renal artery.

was generally 50% or higher, we decided that ethanol with a ratio of <50% should also be evaluated. Therefore, we prepared the ethanol-Lipiodol mixture at a ratio of 1:2. Takasawa et al. reported that NBCA-Lipiodol mixture with a low concentration of NBCA can embolize more peripheral and smaller diameter arteries in RAE and achieve embolization of a larger vascular bed [19]. Morishita et al. reported successful RAE using 10% NBCA in three patients with polycystic kidney disease [14]. Based on these earlier findings, we prepared the NBCA-Lipiodol mixture at a ratio of 1:9. We did not infuse saline-Lipiodol mixture into the renal artery as a control group because Park et al. reported that pure Lipiodol did not achieve renal embolism [18]. Konya et al. also reported that the congestion caused by transient capil-

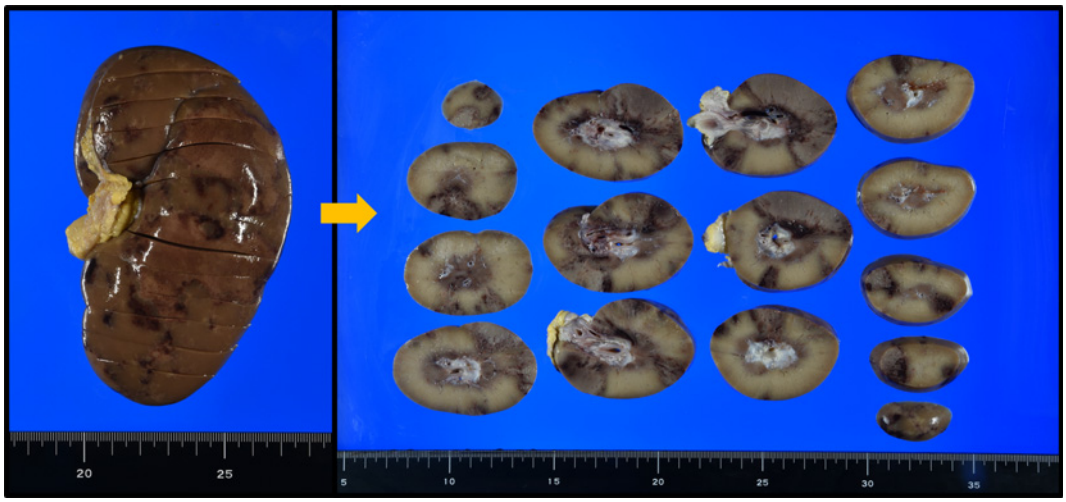

Figure 2. Representative images of the kidney slices (1 cm thick).

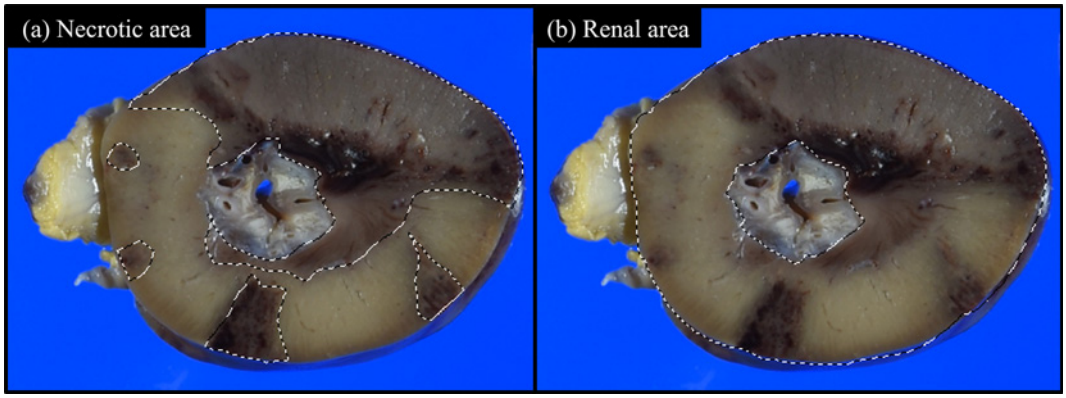

Figure 3. Calculation of the renal necrosis rate.
The dark regions are necrotic. The necrotic area (outlined in a) and the renal area (outlined in b) were traced on each slice. The necrosis rate was calculated as the total necrotic area (sum of all the slices) /total renal area (sum of all the slices) × 100%.

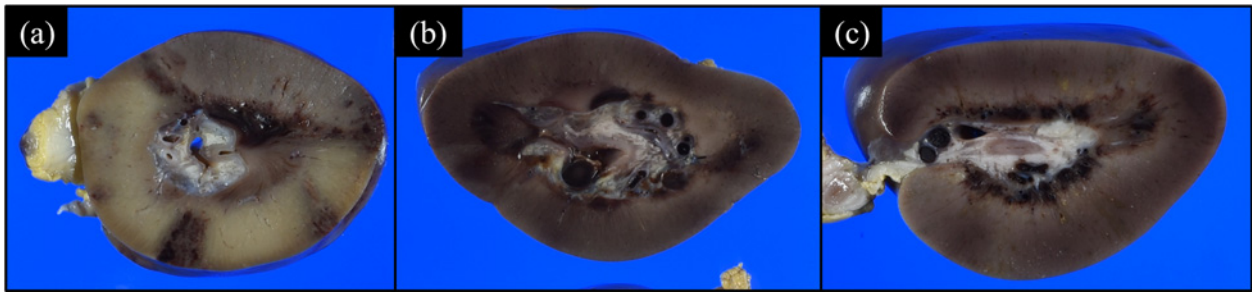

Figure 4. Representative macroscopic images of the kidneys in the three groups.
(a) Group A (33% ethanol): the cut surface of the kidney showing dark and light regions in patches. (b) Group B (67% ethanol): the entire cut surface is dark. (c) Group C (10% NBCA): the entire cut surface is dark.

lary occlusion following infusion of pure Lipiodol resulted in inflammation but not embolization of the renal parenchyma [20].

The method used to evaluate the extent of renal necrosis was appropriate because the dark tissue region on a photograph of the kidney slice was consistent with the necrotic tissue region detected by histological examination. However, some epithelial cells located immediately beneath the renal capsule were not completely necrotic in all three groups. This may be due to the inflow of arterial blood through the renal capsular artery. Therefore, the macroscopic renal necrosis rate may be overestimated.

Wright et al. reported that Lipiodol enhances the embolic effect of ethanol and suggested that this is because the vis-

cous Lipiodol stagnates the blood flow and the renal arteries in the target area filled with a homogenous mixture, prolonging the effects of ethanol on the vessels [21]. However, in our study, the embolic effect of the 33% ethanol-Lipiodol mixture in Group A was weaker than that of the 67% mixture in Group B. This is probably due to partial recanalization of the renal arteries, as confirmed by angiography two days after RAE in Group A (**Fig. 1**). We assume that embolization was incomplete because of the reduced embolic effect due to the lower ethanol concentration and the release of blood stasis over time due to the higher Lipiodol content. Our results agree with the suggestion by Park et al. [18] that a 50% ethanol-Lipiodol solution is radiopaque and an effective agent for renal arterial ablation. Therefore, to achieve strong embolization, Lipiodol's amount should be limited to the minimum required to ensure it is visible during the procedure.

Table 1. Renal Necrosis Rates Determined by Imaging.

Swine	Group A (33% ethanol)	Group B (67% ethanol)	Group C (10% NBCA)
1	50.9	100	100
2	40.9	100	100
3	59	100	100
Mean ± SD (%)	50.3 ± 7.4	100 ± 0	100 ± 0

NBCA: *N*-butyl 2-cyanoacrylate; SD: standard deviation

The macroscopic necrosis rate was 100% following RAE with 67% ethanol and 10% NBCA. In an earlier study, Morishita et al. [14] performed renal ablation using the 10% NBCA mixture. Since the mixture caused total renal necrosis in our study, it may be an alternative to ethanol for renal ablation in ethanol-intolerant patients.

RAE using ethanol has been shown to cause complete cellular death with total vascular occlusion due to a combination of vascular thrombosis, cohesion of erythrocytes, vascular endothelial damage, and necrosis of perivascular areas, thereby preventing late revascularization by collateral vessels [22]. Following intravascular injection, NBCA polymerizes upon contact with plasma, forming a mold-like sclerotic material or thrombus, which adheres to the vessel wall, causes endothelial damage, and achieves vessel embolization [23]. The histological examinations in our study revealed necrosis of the tubular epithelia in all three groups, and partial necrosis of the glomeruli in Groups A and B (ethanol) but not in Group C (NBCA). Two possible reasons were hypothesized for this. First, the glomeruli may be more resistant than the tubular epithelia to ischemia. Second, NBCA is less toxic than ethanol. Based on our results, we hypothesize that the effect of RAE using NBCA may be weaker than that of RAE with ethanol. Therefore, we believe that ethanol should

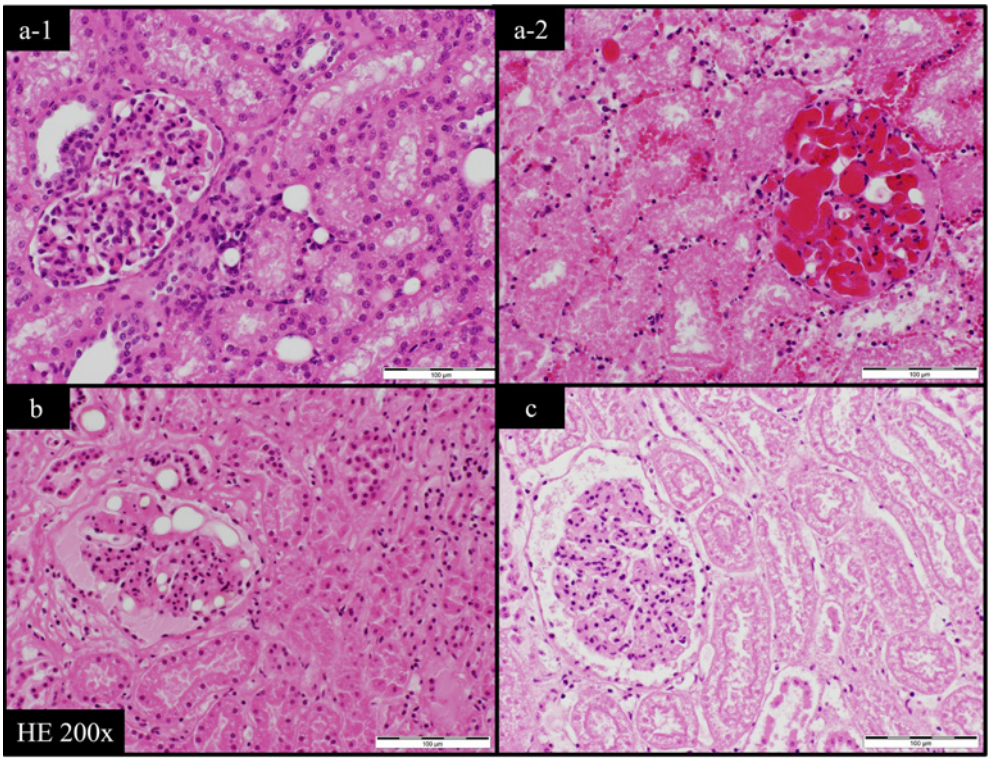

Figure 5. Representative histological findings.
(a) Histological images of the non-necrotic (a-1) and necrotic (a-2) regions from Group A (33% ethanol) showing the loss or obscurity of nuclei in the tubular epithelium combined with partial necrosis of the glomeruli. (b) Histological image from Group B (67% ethanol) showing the loss or obscurity of nuclei in the tubular epithelium, combined with partial necrosis of the glomeruli. (c) Histological image from Group C (10% NBCA) showing the loss of nuclei in the tubular epithelium. All glomeruli were intact. HE = hematoxylin and eosin

be used for renal ablation.

Since the reflux of the embolic material during RAE can cause serious complications, such as colonic infarction [24] and spinal cord infarction [25], we used a balloon catheter to prevent reflux. The balloon catheter also stopped the inflow of blood into the renal artery and prevented ethanol dilution, which could theoretically enhance the effects of ethanol. However, Taniguchi et al. [26] reported that balloon occlusion might not affect tumor shrinkage when embolizing renal angiomyolipomas with a mixture of ethanol and Lipiodol.

This study has four limitations. First, the number of animals used was small, and long-term evaluation was impossible considering animal welfare. Second, the macroscopic renal necrosis rate may be overestimated because histological evaluation revealed that some epithelial cells located immediately beneath the renal capsule were not completely necrotic. Third, in the histological examination, we could not evaluate the distribution of the embolic materials. Finally, in this study, RAE was performed in normal kidneys, not in abnormal kidneys affected by renal tumors.

Conclusion

We found that the 67% ethanol-Lipiodol mixture and the 10% NBCA-Lipiodol mixture are effective embolic materials in RAE for renal ablation in swine. We also found that ethanol caused partial glomerular necrosis, whereas NBCA preserved the glomeruli. Therefore, ethanol should be used for renal ablation.

Conflict of Interest: None
Funding: This study was not supported by any funding.
Author Contribution: Ryota Tanaka, Tetsuo Sonomura, Hirotatsu Sato, Kodai Fukuda and Nobuyuki Higashino contributed to the implementation of the experiment.
Ryota Tanaka, Tetsuo Sonomura, Ryuki Shimono, Akihiko Kumamoto, Akira Ikoma and Hiroki Minamiguchi contributed to the preparation of the paper.
Masataka Koike and Shin-ichi Murata contributed to the pathology.
IRB: The animal experiment was approved by the institutional ethics review committee and was performed following the "Act for the Protection and Management of Animals" and the "Standards for the Care and Storage of Laboratory Animals and Alleviation of Pain" of Japan.
Informed Consent: Not applicable.
Consent for Publication: Not applicable.

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