NOTE  Anatomy

The Effects of Microwave-Irradiated Fixation for Postmortem Changes of the Kidney

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ABSTRACT. In the present study, we evaluated the advantages of microwave-irradiated fixation for postmortem autolysis of the kidney. Mouse kidneys, sampled at 0, 1, 3, 5, 10, 15, 20 and 25 hr after death, were fixed with 10% neutral formalin by microwave irradiation (MWI; 20 sec/500 W) and by conventional immersion. They were then examined with light and electron microscopy, morphometrics and immunohistochemicals. Light microscopic and morphometric observations showed that structural preservation effect of MWI was limited to the proximal convoluted tubules at 25 hr. Contrary, mild ultrastructural damage by MWI was found in the glomeruli at 0 and 15 hr. Immunohistochemistry for renin and α-smooth muscle actin showed no apparent differences between MWI and the immersion.

KEY WORDS: microwave irradiation, mouse kidney, postmortem change.

The kidney is a typical organ that shows rapid postmortem autolysis [13, 16, 17], therefore rapid sampling is required to investigate renal morphology. However, in domestic animal clinical cases, many autopsies are obliged to carry out after various postmortem intervals. The use of microwave irradiation (MWI) for tissue fixation is well known as a rapid fixation technique, and its various effects and usefulness have been investigated previously [3, 6–9, 19]. Therefore, we decided to investigate more thoroughly the advantages of MWI when used on kidney tissue with various postmortem intervals.

The study was performed in accordance with the Guidelines for Animal Experimentation of Kagoshima University. Twenty-four female Jcl:ICR mice, 10 weeks old, were used for the experiment. Animals were bred and maintained in a non-barrier conventional room, and received a commercial diet (MF, Oriental Yeast Co., Tokyo, Japan) and tap water ad libitum. All animals were sacrificed by cervical dislocation under anesthesia, using a mixture of ketamine and medetomidine, and were placed at room temperature (22 ± 2°C) until sampling. The kidney sampling was performed at 0 (immediately after death), 1, 3, 5, 10, 15, 20 and 25 hr after death (n=3/each interval). The removed kidneys were immediately sliced about 5 mm thick. The right kidneys were immersed in 10% neutral buffered formalin (NBF) without any treatment. The left kidneys were immediately irradiated at 500 W for 20 sec by a domestic microwave oven, accordance with a common method [11]. After irradiation, the samples were placed in fresh NBF. Two days after sampling, the tissue slices were embedded in paraffin as per routine, and were then cut into 2.0 or 4.0 µm thick sections 1.5 mm below the tissue surface. The 2.0 µm thick sections were stained with hematoxylin-eosin (HE), periodic acid Schiff (PAS) or periodic acid silver-methenamine, and the 4.0 µm thick sections were used in the immunohistochemical detection of renin and α-smooth muscle actin (α-SMA). The detection of renin was performed using the VECTASTAIN Elite ABC Kit (Vector Laboratories, Inc., Burlingame, U.S.A.); rabbit antiserum raised against recombinant renin (supplied by Dr. Kazuo Murakami, University of Tsukuba, Japan) and biotinylated goat anti-rabbit IgG (Vector Laboratories, Inc., Burlingame, U.S.A.); rabbit antiserum raised against recombinant renin (supplied by Dr. Kazuo Murakami, University of Tsukuba, Japan) and biotinylated goat anti-rabbit IgG (Vector Laboratories, Inc., Burlingame, U.S.A.); rabbit antiserum raised against recombinant renin (supplied by Dr. Kazuo Murakami, University of Tsukuba, Japan) and biotinylated goat anti-rabbit IgG (Vector Laboratories, Inc., Burlingame, U.S.A.); rabbit antiserum raised against recombinant renin (supplied by Dr. Kazuo Murakami, University of Tsukuba, Japan) and biotinylated goat anti-rabbit IgG (Vector Laboratories, Inc., Burlingame, U.S.A.). The detection of α-SMA was performed using mouse anti-α-SMA monoclonal antibody (EPOS, peroxidase-conjugated; Dako Cytomation, Inc., Kyoto, Japan). Immunoreactivity was detected by 0.025% 3,3'-diaminobenzidine-0.003% H₂O₂ solution, and counterstaining was performed with hematoxylin. The NBF-fixed tissues that were not used for paraffin embedding were re-fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4) and 1% osmium tetroxide in PB. Then samples were embedded in Epon 812 as per routine for electron microscopic observation. Morphometrical analysis was performed at random using the PAS-stained sections. To determine the structural preservation of tubules, only the tubules that retained structural features of each segment were selected, and their nuclei were counted using a section micrometer (125 × 125 µm). The counting was performed at 40 cortical (625,000 µm²) and 30 outer medullar portions (468,750 µm²). Values were expressed as the mean ± standard error (SE) for each group, and tested using Student’s t-test. Statistical significance was accepted at p<0.01.

In light microscopy, no preservation effects of MWI were seen from 0 to 20 hr after death, including brush border structure. However, a remarkable difference between MWI and non-MWI preserved kidneys was observed at 25 hr after death, and it showed that the collapse of tubules with MWI was milder than with non-MWI preservation (Fig. 1). Figure 2 shows the number of cortical tubule nuclei that retained structural features of each segment. A statistically significant difference was detected at 25 hr in the proximal convoluted tubules (PCT), with the value for MWI kidneys.
being significantly higher than the value for non-MWI kidneys. In outer medullary tubules, mild drawback of MWI was detected in the distal straight tubules in samples taken 0 hr after death in the present study (data not shown).

On electron microscopy, although ultrastructural preservation following MWI was recognized in the PCT at 25 hr, mild damage by MWI was found in the glomeruli (Fig. 3). At 0 hr after death, the contrast of the three-lamina structure in the glomerular basement membrane (GBM) was better appreciated in the non-MWI specimens than that in the MWI kidneys. At 15 hr, collapse of the podocyte was more severe in the MWI kidneys than in the non-MWI kidneys.

In immunohistochemistry, there were no differences between the MWI and non-MWI methods. In both MWI and non-MWI preserved kidneys, reactions for renin and \( \alpha \)-SMA did not become weak or diffuse in any of the samples taken from 0 to 25 hr after death (Fig. 4).

In the present histological and morphometric observations, the MWI method was markedly better for delineating renal morphology in the PCT in samples taken 25 hr after death. The collapse of the PCT in the MWI kidney samples that was visible with light microscopy was obviously milder than that observed in non-MWI kidney samples. This observed preservative effect was confirmed quantitatively, since it was statistically significant. From these findings, it would appear that collapse of the PCT during late postmortem periods progresses very rapidly, though these rapid autolysis changes were kept to a minimum by MWI.

In domestic animal clinical cases, electron microscopic diagnosis from autopsy samples is frequently obliged to perform using NBF fixed stock tissues. Therefore present electron microscopic examination was performed using NBF fixed kidneys. As the results, MWI induced some artifacts seen in the GBM and podocyte. Since collapse of GBM or podocyte is very important cytopathologic finding to diagnose the glomerulonephritis or lipoid nephrosis [2], we considered that apply of MWI in NBF fixation is not suitable for electron microscopic diagnosis of the renal disease.

The inappropriate enhancement of renin-angiotensin system (RAS) activity and over-expression of \( \alpha \)-SMA are important risk factors for various types of renal failure [1, 4 12, 18]. In domestic animal clinical cases, the abnormal enhancement of RAS activity and \( \alpha \)-SMA expression was demonstrated in cats with chronic renal failure [5, 10, 14, 15]. In the present study, we felt that MWI method was safely used but not effective for investigating the expression of these two proteins that are important in renal pathology.

From the present findings, we concluded that the use of the MWI method in formalin fixation might be effective when it is necessary to take samples of the kidneys a long time after an animal’s death (25 hr in the present study), especially in histopathologic diagnosis of the domestic animal clinical cases.

REFERENCES


Fig. 3. Electron micrographs of the glomeruli. a: MWI preserved kidney at 0 hr after death. b: non-MWI preserved kidney at 0 hr after death. c: MWI kidney sample at 15 hr after death. d: non-MWI kidney sample at 15 hr after death. At 0 hr, three-lamina structure of the basement membrane of the non-MWI sample (b) is better preserved than the MWI sample (a). At 15 hr after death, the collapse of the podocyte pedicles with MWI (c) is more severe than in the non-MWI specimens (d)×10,000 (a and b) and 12,500 (c and d).

Fig. 4. Immunohistochemical detection of renin and α-SMA 25 hr after death. a: renin detection in MWI kidney sample. b: renin detection in non-MWI kidney sample. c: α-SMA detection in MWI kidney sample. d: α-SMA detection in non-MWI kidney sample. No differences were found between the MWI and the non-MWI methods. ×125 (a and b) and 62.5 (c and d).