ultrastructural analysis

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Abstract

Mouse kidney tissues were harvested, fixed in 4% paraformaldehyde (PFA) and embedded in paraffin. All sections (3 micrometers in thickness) were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS, Thermo Scientific) or Masson's trichrome staining (MTS, Sigma) according to the standard procedures or manufacturer's instructions. For transmission electro micrographs (EM), kidneys were fixed in Karnovsky's fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1% cacodylate buffer, pH 7.2 for 2 hrs at room temperature, followed by postfixation with 1% osmunim tetraoxide in cacodylate buffer for 90 min at 4˚C and mordanting with 1% tannic acid in cacodylate buffer.. Ultrathin sections were contrasted with uranyl acetate and lead citrate, and examined with a Hitachi H-7600 transmission electron microscope located in the Imaging Core Facility of the Oklahoma Medical Research Foundation. We followed previously described histological scoring methods (33).

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Protocol

Step 1.

For transmission electro micrographs (EM), kidneys were fixed in Karnovsky's fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1% cacodylate buffer, pH 7.2 for 2 hrs at room temperature,

Step 2.

followed by postfixation with 1% osmunim tetraoxide in cacodylate buffer for 90 min at 4˚C and mordanting with 1% tannic acid in cacodylate buffer.

Step 3.

Ultrathin sections were contrasted with uranyl acetate and lead citrate, and examined with a Hitachi H-7600 transmission electron microscope located in the Imaging Core Facility of the Oklahoma Medical Research Foundation.