THE EFFECTS OF ENDOGENOUS EPIDERMAL GROWTH FACTOR DEFICIENCY AND EXOGENOUS EPIDERMAL GROWTH FACTOR ADMINISTRATION ON RAT KIDNEY

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ABSTRACT
The objective present study was to determine sialoadenectomy and exogenous EGF administration effects on light microscopic changes in a rat kidney. Adult female Wistar albino rats used. The animals divided into three groups randomly. Group-I: The animals served as control (n:10). Group-II: Sialoadenectomy (n:10), Group-III: Sialoadenectomy+EGF administration (n:10). After 27 days sialoadenectomy, Control and experimental groups rats were killed by pentobarbital and their kidney removed. The tissue samples were examined under light microscope. Light microscopic evaluation indicated that the kidney tubules epithelium and glomerules in control group showed a normal appearance while changes were observed in all experimental groups. However, SX+ exogenous EGF administration group was performing a nearer light microscopic view of to the control group. In this study we indicated that short term exogenous EGF supplementation in sialoadenectomized rats can relatively increase kidney light microscopic improvoment.

Introduction
Epidermal Growth Factor (EGF) is a mitogenic polypeptide hormone which in vivo stimulates ectodermal (epithelia) and endodermal cell growth and in vitro growth of epithelial cell and fibroblasts. EGF is formed in salivary glands, kidney tubules and intestinal tract and occurs in nanogram quantities in plasma. EGF is a small, 53 amino acid, single chain polypeptide that is found at highest concentrations in salivary glands. (1). Having previously documented increased expression of the proproliferative and antiapoptotic growth factor, epidermal growth factor (EGF).

In 1991, Montesano et al. (2) demonstrated that hepatocyte growth factor (HGF) could induce Madine-Darby canine kidney (MDCK) cell-derived cysts to form tubular structures when cultured suspended in a collagen matrix. Since then, several other growth factors have been found to modulate tubulogenesis in specific epithelial cell types. These include positive stimulators such as the EGF receptor ligands, epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) and negative regulators such as TGF-β. EGF and EGFR are expressed in embryonic kidney and some genetic backgrounds EGFR knockout mice reveal a defective collecting duct system (3, 4). In contrast, mice lacking HGF or its receptor c-met do not show any abnormal kidney development. However, since those mice die by embryonic day 14, one cannot exclude the possibility that HGF may be involved in the later stages of kidney tubule development. Among the most prominent negative regulators is TGF-β. TGF-β was shown to primarily inhibit
HGF induced tubule branching but not the elongation of the tubule (5).

The present study was designed to investigate the possible role of sialoadenectomy in kidney damage and administration of improvement agent exogenous EGF.

Materials and Methods

Thirty adult female Wistar albino rats, 180-200 days old and 220-250 g in weight were obtained from the Department of Medical Science Application and Research Centre of Dicle University (DÜSAM)- Diyarbakir. They were housed in individual cages in temperature-controlled environment (22°C) with a 12:12 h light-dark cycle. All rats were fed standard pellet food and ad libitum tap water, which were performed according to the Declaration of Helsinki with the permission of the Governmental Animal protection committee.

Thirty adult female Wistar albino rats were divided into three groups, a control and two experimental groups.

Group-I: The animals served as control. The first group of rats were not subjected to sialoadenectomy (n: 10).

Group-II: The second group of rats were anesthetized with an intramuscular injection of Ketamine HCl (10 mg / 100 g) and xylazine (0.8 mg / 100 g). To remove the salivary glans, a 15 mm incision was made below mandible, bilaterally submandibular glands removed (n: 10).

Group-III: Sialoadenectomy + EGF administration (n:10).

After 22 days of sialoadenectomy operation, each rat was given drinking water added 75 microgram / 5 days exogen EGF (Epidermal Growth Factor- Human Recombinant- Sigma E9644)

After 27 days, Control and experimental groups rats were killed by pentobarbital and their kidney removed. The tissues were fixed for either 6-8 hours in 10% formalin solution at 4 °C. They were dehydrated though increasing concentrations of the ethanol series and the tissues were embedded in paraffin and cut into 4-5μm transversally. The sections were cut and stained with Hematoxyline-Eosin and Hematoxylen-Van Gieson. The specimens were examined and photographed with light microscope (Nikon Eclipse-400).

Results and Discussion

Control Groups

We observed normal histological appearance of kidney in control groups. The glomerules and tubules were seen normal appearance (Fig. 1).

Experimental Groups

Light microscopic findings, the most significant findings were noted after sialoadenectomized (SX) rat kidney. When the kidney light microscopic data analysis detected significant difference between control and experimental groups.

Sialoadenectomy (SX) result in histological changes in kidney, as well as a glomerular structure and tubules cells in rats. In the light micrograph of SX group significant changes were observed. Especially the tubulus epithelium of degeneration and necrosis. We saw remarkable ruptured proximal tubules (Fig. 2a).

Desquamation of proximal tubule epithelium was apparent Fig. 2a. In these changes
the greatly increasing in these groups. We seen normal collogen fibrils in kidney cortex. We observed basal membrane of glomeruluse and proximal tubule (Fig. 2b). In the light micrograph of SX+EGF group significant changes were observed. Especially the proximal tubules and glomerulus was apparent slight degeneration. SX+ exogenous EGF group was performing a nearer view of to the control group, but we observed partly degeneration of proximal tubulles, but in these cells the greatly improving of proximal tubule epithelium was also observed (Fig. 3a).

We clearly observed normal collogen fibrils, basal membrane of glomeruluse and proximal tubule in kidney cortex of SX+exogenous EGF group (Fig. 3b).

Our findings demonstrate the importance of Epidermal Growth Factor (EGF) in the proliferation of kidney tubule cells, and suggest that submandibular glands activation is one mechanism by which kidney disorder susceptibility may be increased. This study showed that salivary glands play an important role in kidney epithelium.
integrity and proliferation tubule cells. The removal of salivary glands greatly increased the susceptibility of kidney tubule to the formation of lesions and this accompanied by marked reduction of exogenous EGF administration occurred light microscopic changes.

Epidermal growth factor (EGF) is a peptide that stimulates proliferation, and acts as a survival factor in the developing rat kidney. EGF is normally synthesized by distal tubular cells, with increasing expression during maturation (6). Chronic unilateral urethral obstruction (UUO) suppresses renal EGF production in neonatal rats or in infants and children (6, 7). Exogenous EGF reduces tubular apoptosis by 80% in the neonatal rat with chronic UUO, and enhances recovery after relief of obstruction (8, 9). Exogenous EGF also inhibits tubular apoptosis in the adult rat subjected to UUO (10). The present study demonstrates several findings in relation to the role of the EGF in the renal histology in EGF deficiency on rat model. After 27 days of sialoadenectomy on rat, cell proliferation was no longer different between control, experimental groups. These findings were accordance with Kennedy et al. (10).

Results of the present study clearly reveal that exposure of sialoadenectomy produces histopathological changes of kidney.

In previous studies, Chevalier et al (1998) found exogenous EGF reduces tubular apoptosis of kidney. We have reported recently that in vitro stretching of rat tubular cells (a simulation of stretched cells lining dilated tubules in the degeneration. On histological evaluation of kidney from sialoadenectomized, it was observed that there was severe degeneration of glomeruli showing shrinkage (Fig. 2a) along with disrupted proximal tubules.

Epidermal growth factor (EGF) is a peptide that stimulates proliferation, and acts as a survival factor in the developing rat kidney (11). We didn’t encounter any light microscopic study related to the effect of sialoadenectomy on rat kidney.

Exogenous EGF may be clinically therapeutic in promoting the repair of tissues damaged by bowel inflammation (12).

These data showed that sialoadenectomy leads to an increase kidney damage, decrease and light microscopic changes in sialoadenectomized rats. The dramatic differences in the renal cellular response to exogenous EGF administered to rats compared to sialoadenectomized rat kidney.

REFERENCES