Evaluation of renal pelvic glycosaminoglycan (GAG's) layer integrity in a rabbit model after shock wave application

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To evaluate the damaging effects of high energy shock wave application on the integrity of renal pelvic glycosaminoglycan (GAG), an animal model study in rabbits was performed. Following different doses of shock wave application (1000, 2000, 3000 sw) the renal pelvic specimens obtained from treated and untreated kidneys of the animals were examined under light and transmission electron microscopy, after 24 hours and 1 week respectively. Evaluation of our results revealed prominent disruption in renal pelvic GAG layer integrity together with evident increased intracellular protein production and reconstructive process. All our findings may be interpreted as a possible etiologic factor in the long term stone formation following Shock Wave Lithotripsy (SWL), especially in patients with no metabolic abnormality. [Turk J Med Res 1996; 14(2):37-41]

Key Words: Glycosaminoglycan, Renal stones, ESWL

Extracorporeal Shock Wave Lithotripsy (ESWL) has changed stone treatment concepts dramatically in the last 15 years with its successful and effective results. However, despite its safe and practical nature, increasing experience in this field revealed that the procedure has not been found entirely safe. Today it is well known that some acute and chronic side effects may be encountered in any time following shock wave application. Of these chronic adverse effects, new onset of hypertension, patchy fibrosis of renal parenchyma and increased stone recurrence rates are the most debated features to date (1). On the other hand, factors influencing stone recurrence and regrowth rates following ESWL have not been throughly evaluated. In unselected series, a 6% after 1 year and 20% after 4 years recurrence rates have been reported following ESWL (2,3).

Again, both clinical and experimental studies during the last decade indicated the major role of urinary macromolecules in the new crystal formation and crystal regrowth. Glycosaminoglycan (GAG) layer of the urothelium has been found to be important to prevent Ca-oxalate crystals from adhering to the surface and acting as a nidus for stone formation. Thus besides the level of excreted GAG in urine also the integrity of GAG layer in various parts of urinary tract are being considered to be important in the adhesion, formation and growth of urinary crystals (4-6).

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Taking the traumatic effects of high energy shock waves on renal structures into account, in this present prospective study, we aimed to evaluate the traumatizing effects of high energy shock waves on renal pelvic GAG layer integrity in rabbit model, as a possible factor in the long-term stone recurrence rates.

MATERIALS AND METHDOS

18 New Zealand white rabbits, weighing 3-5 kg, were included into the study program. Animals were fed with standard chow and kept in normal room temperature. All animals have been throughly evaluated and no anatomical and biochemical abnormalities or urinary tract infection could be shown. Again all animals were treated with antibiotic and antiparasitic agents, 1 week before the procedure.

Animals were divided in to three main groups and different doses of high energy shock wave application have been applied to the renal pelvis of each animal (1000-2000-3000 SW). Shock wave application has been performed under fluoroscopy after contrast agent injection through ear vein of each animal. All procedures were performed under ketamine hydrochloride (15 mg/kg) anesthesia with Dornier Lithotriptor MPL 9000. Contralateral kidneys of each animal were examined as control kidneys.

Following shock wave application, bilateral flank incision was performed and treated-untreated kidneys of each animal were removed after 24 hours and 1 week.

Evaluation of renal pelvic mucosal GAG layer integrity under light and Transsmission Electron Microscopy (TEM) was performed as follows:

For light microscopic evaluation tissue specimens were first fixed with 10% formaline solution and then were embedded in paraffin blockade. After this process 3-5 micron sections were obtained with microtome for Hematoxilene-Eosin staining. Following all these procedures tissue sections were examined under light microscope (10x40).

On the other hand for TEM evaluation tissues were immediately placed in a mixture of 2% glutaraldehyde solution buffered to pH 7.2 with 0.1 M phosphate and fixed at 4°C for two hours.

After rinsing in several changes of phosphate buffer, the specimens were placed in a veronal acetate buffered (pH 7.2) 2% solution of osmium tetrahydroxide at 4°C for 2 hours. Dehydration was performed in a graded series of ethanol at 4°C; followed by embedding araldite CY212. Sections were cut on an LKB ultramicrotome stained with both uranyl acetate and lead ciltrate, then examined in an EM-10 Zeiss electron microscope.

RESULTS

Evaluation of tissue specimens with respect to renal pelvic GAG layer integrity revealed following results.

I. Light Microscopy Findings

Control Group:

Regular and intact appearance of GAG layer, regular appearance of luminal epithelium, regular orientation of epithelial cells and preservation of connective tissue structures with fibers being in their original order were the main findings observed during examination (Figure 1).

Study Group:

24 Hours: Irregularity of luminal epithelium and deterioration of renal pelvic GAG layer were the leading features. On the other hand, evident of granule formation, vacuole formation with empty vacuoles together with wideniog of interstitial part of connective tissue due to edema formation were also demonstrated. Indented nucleus were seen in the majority of evaluated cells. All of these findings which became more prominent with increasing number of ESWL indicated a deteriorated cell structure following ESWL application (Figure 2).

7 **Days:** Despite gradual decrease in vacuolization and granule formation together with disorientation of connective tissue fibers and edema, irregular appearance of luminal part of the epithelium persisted to some extend especially in animals treated with higher number of shock waves (Figure 3).

II. TEM Findings

Control Group: Regularly oriented epithelial cells and well preserved GAG layer can easily be distinguished. Luminal epithelium is regular and a thin layer of GAG has been observed (Figure 4).

Study Group: Disruption of renal pelvic GAG layer together with indistinguishable luminal part of the epithelium from the other structures can easily be see following ESWL application. Again apparently enlarged and vacuolated granulated endoplasmic reticulum (GER) together with evident increase in the number of ribosomes located on GER wall were other pathologic features. Prominent increase in mitochondrial activity with enlarged mitochondria and cellular debris in the apical part of the membrane could also be noted. All of these pathologic findings again indicated an increased intracellular activity which in turn may be interpreted as repair process (Figure 5).

DISCUSSION

Experimental studies have indicated that the surface epithelium of the urinary tract should be resistant to binding of a large number of substances (i.e. crystal etc.) in urine will be eliminated from the body. Although the mechanism of inhibitor activity produced by GAG's is uncertain, it appears to be related to the binding of inhibitor on the crystals surface where it inhibits inducation of new crystal growth and aggregation. Thus, it seems logical that the structure of the urothelial surface may play a vi-

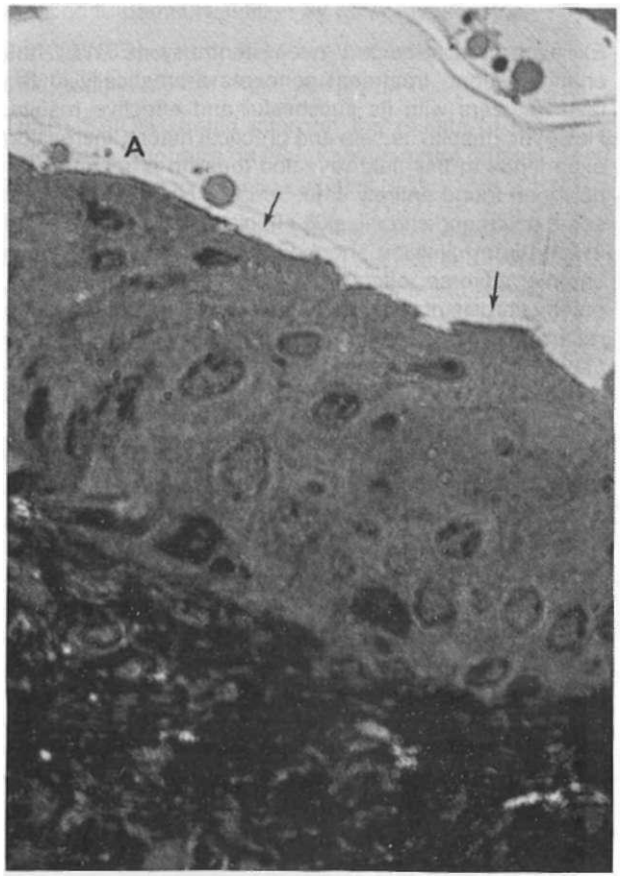

Figure 1. Control group: Regular appearance of GAG layer (see arrows), regular luminal epithelium (A), regular orientation of epithelial cells and connective tissue (CT) can be seen (GAG layer Toluidine blue, H.E.xIOO).

Figure 2. Study group (24 hours, 1500 SW)
Irregular luminal epithelium, loss of GAG layer integrity (see arrows), can be seen on the apical part (A), evident granule formation (G), widening of interstitial part of connective tissue (CT) due to edema formation are being demonstrade (H.E.xlOO).

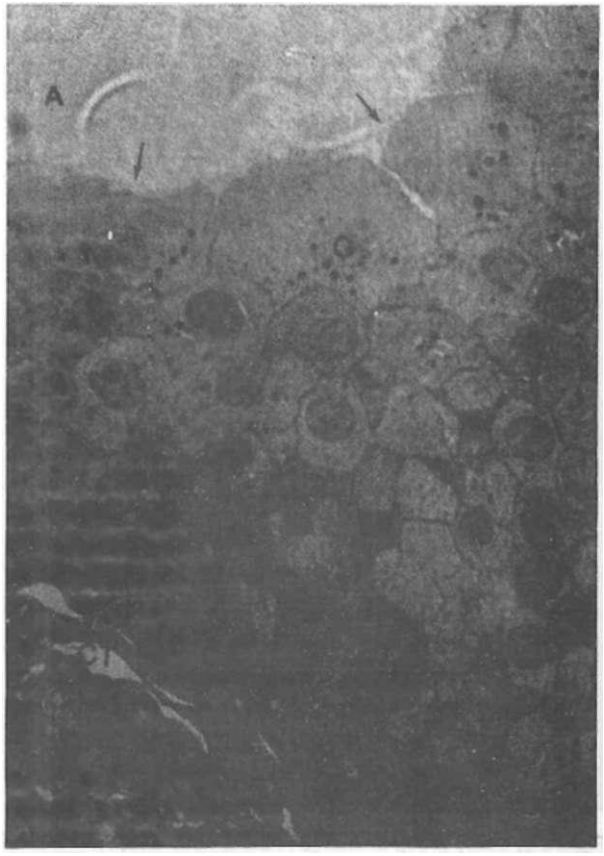

Figure 3. Study group animal (7 days, 3000 SW)
Gradual decrease in vacuolization and granule formation (G), irregularity of luminal epithelium and GAG layer (see arrows), disorientation of connective tissue (CT) fibers are being shown (H.E.xlOO).

tal role in determining nucleation and whether Ca-oxalat stone crystals will adhere to the urothelium and thus remain in urinary tract where further fixed particle growth in to a macroscopic stone could occur. As a result urothelium GAG layer integrity is an important barrier for the adhesion and aggregation of stone crystals (4,5,7,8).

Taking all these features of urothelium in to account, one may propose that any traumatizing agent, which may deteriorate the integrity of GAG layer covering urothelium will predispose crystal adhesion and growth thorough disrupted parts of GAG layer (4,5).

On the other hand, studies dealing with the true stone recurrence rates following SWL, could not show definite factors which may directly affect this phenomenon. Although the size, localization and residual parts of the treated stones have been regarded as important factors in the formation of new crystals, stone recurrence in patients with no residual fragment or no metabolic active state led the physicians evaluate factors other than the stone itself (2,3).

In this present experimental study we aimed to show the deteriorating and traumatizing effects of high energy shock waves on normal renal pelvic mucosal GAG layer in rabbits. Evaluation of our light microscopy and TEM examination results after 24 hours post procedure revealed a marked disruption of GAG layer, together with increased intracellular activity in the treated cells. All of these findings again led us to consider" a prominently increased intracellular activity with increased protein production. Taking the damaged and disrupted appearance of GAG layer integrity in to account, these findings have been interpreted as reconstructive intracellular activity in the affect kidney cells.

Again, TEM evaluation of renal pelvis with respect to GAG layer integrity demonstrated same deterioration on this level due to traumatizing effect of HESW. Evaluation of specimens on the 7 day follow-up revealed the continuation of these pathologic findings despite a gradual improvement in intracellular activity. Thus our results indicated a prominent damage of renal pelvic mucosal GAG layer following SW application.

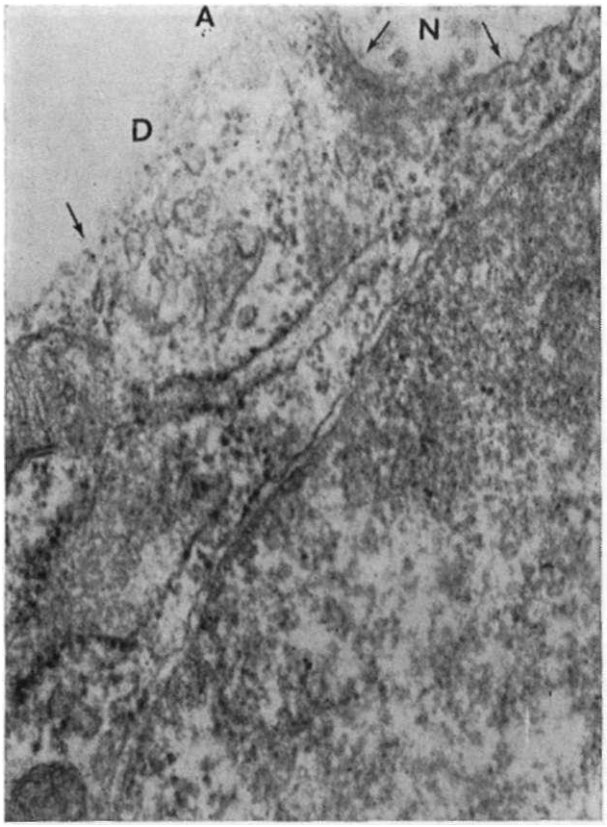

Figure 4. Study group animal (24 hours, 1500 SW)
Disruption of pelvic GAG layer to some extend can be seen (D). (See normal GAG layer on upper right part of the figure (N). (TEMx36000).

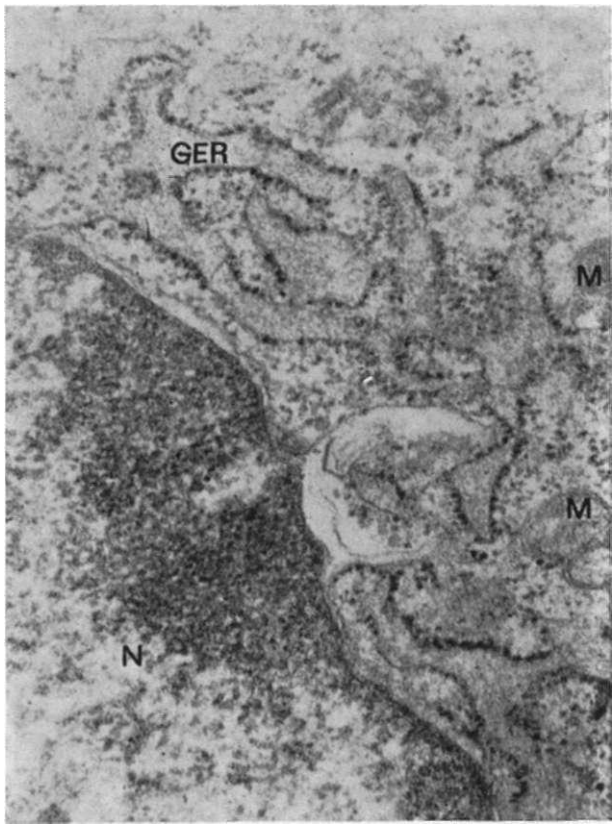

Figure 5. Study group animal (24 hours, 3000 W)
Apparently enlarged and vacuolated granulated endoplasmic reticulum (GER) together with evident increase in the number of ribosomes located on GER wall together with evident increase in the number of ribosomes located on GER wall were other pathologic features. Prominent increase in mitochondrial activity with enlarged Mitochondria (M), evident nucleus (N) and cellular debris in the apical part of the membrane (A) could be seen on the figure (TEMx29000).

In other words, besides the deterioration of renal pelvic GAG layer during SW application, at the same time adhesion of crystals may easily occur in these traumatized parts of renal pelvis and this may lead to stone recurrence in long term follow-up evaluation.

Taking the inhibitory effect of GAG layer against stone formation into account, all these pathologic findings may be responsible for long-term stone recurrence rates by producing open gate for crystal adhesion and aggregation. If we add, the shock wave induced trauma to these GAG defects occurred, during teratment of the stones, also some stone crystals could be embedded into these defected parts of GAG layer. To our knowledge, such a subject has not been evaluated in the literature to date and we believe that larger group of patients with other parameters are certainly needed in order to give more reliable data on the major inhibitory role of GAG layer covering all parts of urothelium.

Şok dalga uygulamasını takiben tavşan modelinde renal pelvik glukozaminoglikan (GAG) tabakasının bütünlüğünün değerlendirimesi

Yüksek enerjili şok dalga uygulamasının renal pelvik Glukozaminoglikan (GAG) tabakası üzerine olan zedeleyici etkilerini araştırmak amacıyla tavşanlarda bir hayvan çalışması gerçekleştirildi. Değişik sayıda şok dalga (1000-2000-3000) uygulamasını takiben, hayvanların tedavi edilen ve edilmeyen böbreklerinde alınan renal pelvis örnekleri 24 saat ve 1 hafta sonra olmak üzere ışık mikroskopisi ve transmisyon elektron mikroskopisi (TEM) ile incelendi. Elde edilen sonuçların incelenmesi bize işlem sonrasında belirgin renal pevlik GAG tabaka defektinin oluştuğunu ve hücrelerin içerisinde belirgin protein birikimi ile karakterize yenileyici işlemlerin valığını göstermiştir. Bütün bulgularımız ESWL uygulamasını takiben uzun dönemde ortaya çıkan taş oluşumunda özellikle metabolik anormalliği olmayan hastalarda olmak üzere muhtemel bir faktör olarak bu tabaka defektinin göz önüne alınması gerektiğine işaret etmektedir.
[Türk J Med res 1996; 14(2):37-41]

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