

An electron microscopy study of liver and kidney damage in an experimental model of obstructive jaundice



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An electron microscopy study of liver and kidney damage in an experimental model of obstructive jaundice.

With this experimental study we investigated the consequences of ligation of the common bile duct (CBD) on hepatic cells and on the renal ultrastructure by electron microscopy and also determine the effects after liberation of the ductus joint in order to clarify the mechanisms of renal failure commonly observed in cholestatic liver disease.

The study was conducted on 53 Wistar albino rats divided into 4 subgroups. In the comparison group (sham) we proceeded to the simple laparotomy. After preparation of the common bile duct of all the rats of the four groups, and ligation of the duct at the level of the distal third, eight rats in each group were sacrificed on the 3rd, 7th, 10th and 14th day after surgery, taking blood samples to measure the serum levels of ALP and bilirubin, and liver and renal tissue samples for histological evaluation. In four rats of each group the common bile duct was unligated at the same deadlines to obtain free drainage of the bile for a week. At the end of this week, the rats were sacrificed by collecting blood and liver and kidney tissue samples.

RESULTS: *after CBD ligation in both groups, the ALP value, total and direct bilirubin levels were proportionally increased. After duct release, bilirubin levels decreased significantly.*

In group II, while large lipid granules were observed to indicate oxidative damage, mitochondrial swelling and crystals were observed after duct liberation. Areas of glycogen and normal mitochondria were observed in group IV. After duct release in this group, increases in Ito granules, lipid granules and normal mitochondria were observed, which may reflect the evolution of hepatic regeneration.

When renal tissue was examined in group II, fusion processes in the feet, thickening of the basement membrane and mesangium were observed, and mitochondrial crystals were observed in renal tissue as well as in the liver after duct release. Damage in group III and group IV was increased parallel to prolongation of jaundice and after loosening persistent damage with mitochondrial crystals.

CONCLUSION: *Ultrastructural changes in rat liver tissue in conditions of obstructive jaundice may be reversible after restoration of drainage. On the other hand, ultrastructural changes in renal tissue in cases of prolonged jaundice are irreversible even if the internal drainage is restored.*

KEY WORDS: Bile Duct, Liver, Kidney, Obstructive Jaundice

Introduction

Obstructive jaundice, progresses with high mortality and morbidity rates due to many serious complications like

pulmonary, renal disfunction and sepsis. The high mortality rates show correlation with the rise of bilirubin levels related to the duration of the obstruction ¹. Patients are especially under the risk of acute renal failure during major abdominal surgery. The mechanism lying under the development of acute renal failure has not been yet established. But it has been suggested that as a result of bile acids accumulation in plasma being excreted only by the kidneys make it nephrotoxic. The aim of this experimental study is to asses the effects

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of obstructive jaundice in various times and levels on the liver and kidney by electron microscopy and by investigating the changes after bile duct unligation to enlighten the mechanism of renal failure.

Materials and Methods

EXPERIMENT GROUPS AND STUDY PLAN

After the approval of ethical committee, 53 Wistar albino rats with average weight of 266 (250-280) gr divided into 4 subgroups. All of the rats were kept on a standard rat diet of chow and drinking water till the day of the study and were left starved the night before the study.

The rats' anesthetics were given by intraperitoneal injections of 5mg/kg Ketamine HCl (50 mg/ml ketamin hydrochloride E. Werner Lambert). The abdomen was opened by a median laparotomy under sterile conditions. Sham group underwent laparotomy. After exposing the common bile duct of all rats in group I, group II, group III and group IV (n=12), at a level one third distal of the duct ligated with 6/0 PG. Eight rats in each group are consecutively sacrificed at day 3, 7, 10 and 14th days after ligation and blood samples are collected for serum ALP and bilirubine levels.

The groups and the procedures followed are shown below.

GROUPS

Control group (n=5): only median line laparotomy.

Group I, II, III, IV (n=8): After being ligated on 3rd, 7th, 10th and 14th days the common bile duct, the rats were sacrificed and blood and tissue samples were taken from the liver and kidney.

Group Iu, Iiu, IIIu, IVu (n =4): After being ligated the common bile duct was unligated and the rats were sacrificed on the 10th, 14th, 17th and 21st days, tissue and blood samples were taken from the liver and kidney.

BIOCHEMICAL ANALYSIS

Blood samples were obtained by cardiac puncture. 6ml of blood was taken. 3mls of the blood was put in glass tubes for ALP, total bilirubin, direct bilirubin and indirect bilirubin levels. Biochemical analyses were carried out immediately. 3mls of blood was centrifuged at 3000 rpm, 4°C for 10 minutes and serum and plasma was separated. Serum total bilirubin, direct bilirubin and indirect bilirubin were studied by spectrophotometric methods (Hitachi Mod. 912, Boehringer – Mannheim)

ELECTRON MICROSCOPY METHODS

Half thin sections obtained from tissue blocks were stained with toluidine blue and observed by BH2 olympus photomicroscope and captured photos were evaluated. The thin cross section slices taken from the marked samples were stained with lead citrate and uranyl acetate. The samples were studied by Carl-Zeis EM 900 and photos were evaluated.

Statistical Analysis

All data collected was evaluated using SPSS 21.0 for windows (SPSS Inc., Chicago, Illinois, USA). All values are shown as mean (SD). The evaluation was carried out using *One-way ANOVA* and *Tukey* posthoc tests. $p < 0.05$ was accepted as statistically significant.

Results

During the experiment no experiment related mortality occurred. The results of biochemical and histopathologic research are given below.

EVALUATION OF BIOCHEMICAL PARAMETERS

The mean serum total bilirubin, direct bilirubin and ALP (Alkaline Phosphatase) values of the groups are given in Table I.

The differences between the total bilirubin values of the control group and group II and group III were found statistically significant ($p < 0.001$, $p < 0.003$). The differences between Group II and group Iu, and the differences between group III and group IIIu were also found significant. After the unligation of the bile duct in both the 10th, 14th day and 17th and 21st day unligation groups, a significant decrease in the bilirubin levels ($p < 0.05$) was observed although the decrease was not to normal levels.

TABLE I - Total bilirubin, direct bilirubin and ALP levels of the groups.

	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	ALP(U/l)
Control	1.66(1.15)	1.0(0.0)	307.5(77.2)
Group I	4.92(1.05)	4.5(0.88)	652.1(33.8)
Group Iu	2.12(2.12)	1.69(1.92)	440.3(34.1)
Group II	8.97(2.03)	8.45(2.18)	647.6(51.3)
Group Iiu	2.64(1.3)	2.09(1.38)	354.0(14.7)
Group III	7.66(1.83)	6.1(1.79)	495.0(166.3)
Group IIIu	2.11(3.02)	1.81(2.7)	364.0(19.46)
Group IV	9.3(7.7)	1.23(2.8)	719.0(28.88)
Group IVu	0.73(0.54)	0.55(0.44)	297.5(10.6)

The difference between the direct bilirubin levels of the control group and the groups of which the bile duct was ligated (Group II, Group III) was also found significant ($p<0.001$, $p<0.014$). The differences between Group II, group IIu, group III and the control group were also considered statistically significant ($p<0.023$, $p<0.014$).

The difference of ALP levels between the control group and group IV, and the difference between group IV and group IVu were also found significant ($p<0.014$, $p<0.023$). The ALP levels increased parallel to the serum bilirubin levels in groups ligated. After unligated it was observed that ALP levels decreased towards normal but no statistically significant difference was observed.

THE ELECTRON MICROSCOPIC ANALYSIS OF THE LIVER

The hepatocytes in group I are totally normal. The nuclear structure, the structure of the granulated endoplasmic reticulum is normal and does not contain dilatations. Normally dark cytoplasm indicates an active cell or apoptosis. But if the cell structure is normal, heterochrome and the cytosol is electron dense this is not a sign of apoptosis (Fig. 1). In Group Iu the findings are close to the control group findings. Its' difference from Group I is that there is a few number of lipid granules in the cytoplasm and this can be accepted as normal (Fig. 2).

In group II the mitochondria are dense due to matrix concentration, the cristae are normal. The number of

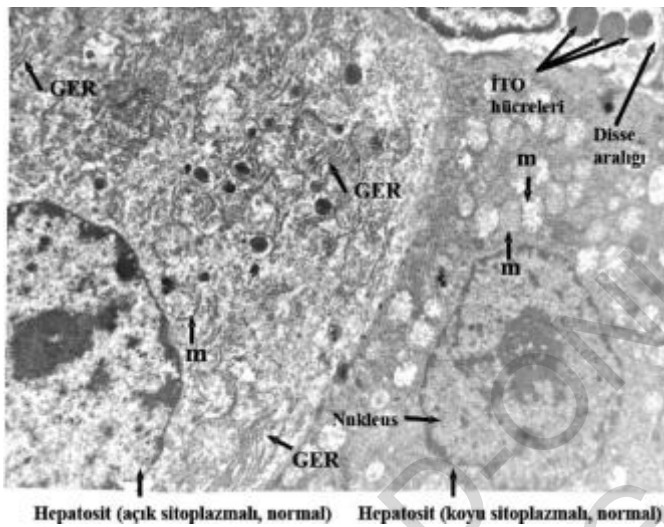


Fig. 1: GrupI: GER=granulated endoplasmic reticulum, m= mitochondria, Uranyl acetate-lead citrate x9000.

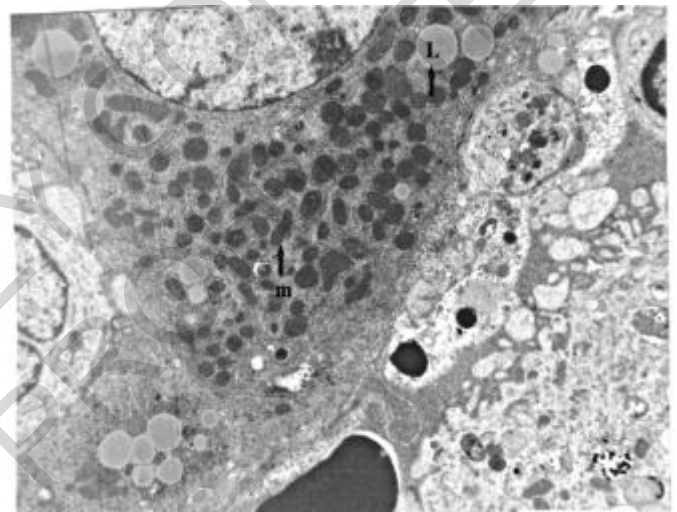


Fig. 3: GrupII: m=mitochondria, L=Lipid granules, Uranyl acetate-Lead citrate x9000.

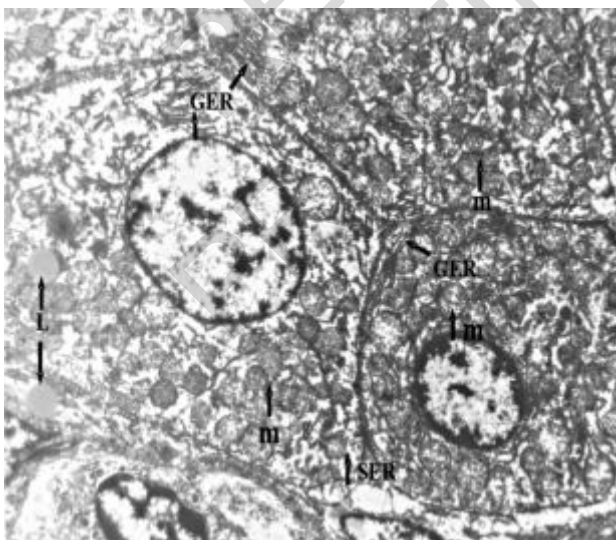


Fig. 2: GrupIu: m=mitochondria, SER=smooth endoplasmic reticulum, L=Lipid granules, Uranyl acetate-lead citrate x9000.

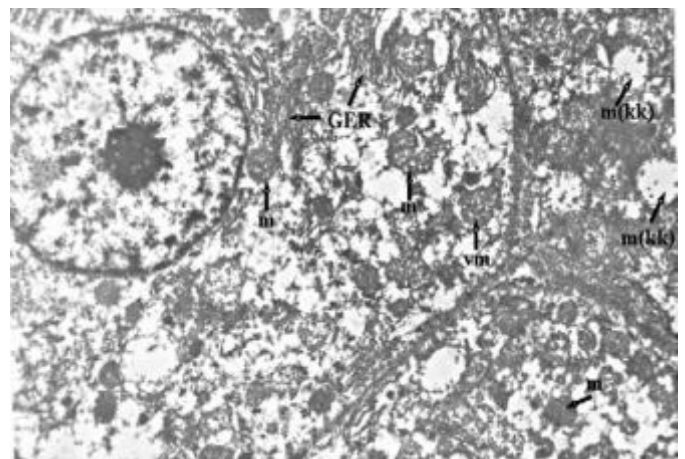


Fig. 4: Grup IIu: m=mitochondria, GER=granulated endoplasmic reticulum, m(kk)= mitochondrial cristae, vm= vesicular mitochondria, Uranyl acetate-lead citrate x9000.

lipid granules is increased and the lipid granules are enlarged (Fig. 3). This indicates to increased steroid synthesis by the endoplasmic reticulum and may be a sign of oxidative injury. The dense mitochondria and the observation of lipid granules may indicate that the cells are overworking.

In group IIu, the hepatocytes are normal, and no crystalizes was observed. Because oxidative phosphorylation cannot be performed, the tubular mitochondria have turned into vesicular mitochondria (Fig. 4). This situation may indicate that antioxidant mechanisms are malfunctioning. In Group IIu, lipids are observed, besides normal mitochondria, mitochondria with cristae loss and swallowed degenerated mitochondria are observed. At the same time the liver sinusoids are totally obstructed and collapsed, even the sinusoid endothelium is nearly unob-

servable. The lumen which is normally totally open and wide is narrowed, only to allow a single erythrocyte pass through.

In group III, all mitochondria are swollen and there is significant mitochondrial cristae loss. The bile canaliculi are dilated and there are significant microvilli in the lumen. Also the endoplasmic reticulum cistern number is reduced, and due to mitochondrial swelling the cytoplasm appears full with mitochondria. The rest of the organelles can not be observed due to the same reason. Also heterogeneous secondary lysosomes that phagocytes the damaged cell elements are present. This indicates to the start of phagocytosis. On the right in the light microscopy cross section many bile ducts are visible and proliferation is seen in these ducts (Fig. 5). In Group IIIu the rough endoplasmic reticulum cisternae are totally

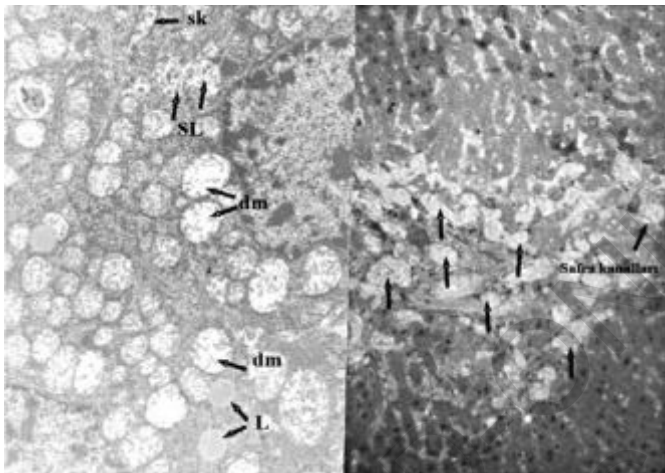


Fig. 5: Grup III: Left side ; SL= secondary lysosome, dm= degenerated mitochondria, L= Lipid, sk= bile canaliculi , Uranyl acetate-lead citrate x13200 Right side light mikroscope , Toluidin blue.

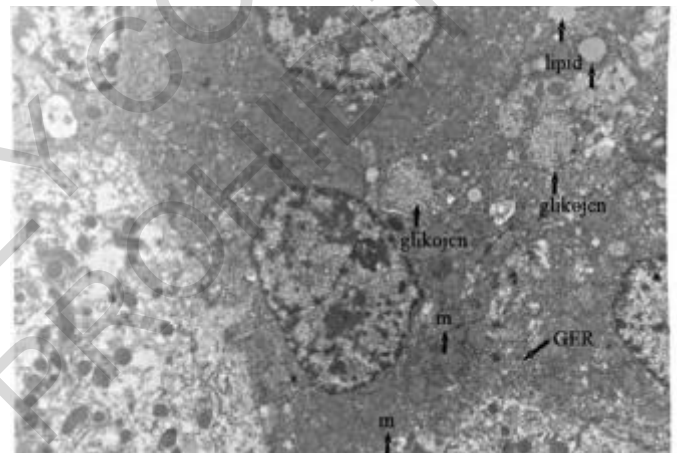


Fig. 7: Grup IV: m=mitochondria, GER= Granulated Endoplasmic Reticulum, Uranyl acetate-lead citrate x9000.

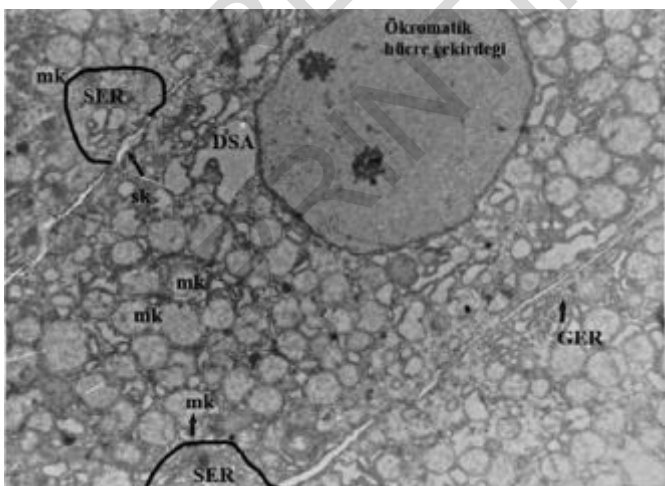


Fig. 6: Grup IIIu: SER=smooth endoplasmic reticulum, GER= Granulated Endoplasmic Retikuculum, mk= mitochondrial crystallization , sk= bile canaliculi, DSA= degenerative cytoplasmic area Uranyl acetate-lead citrate x9000.

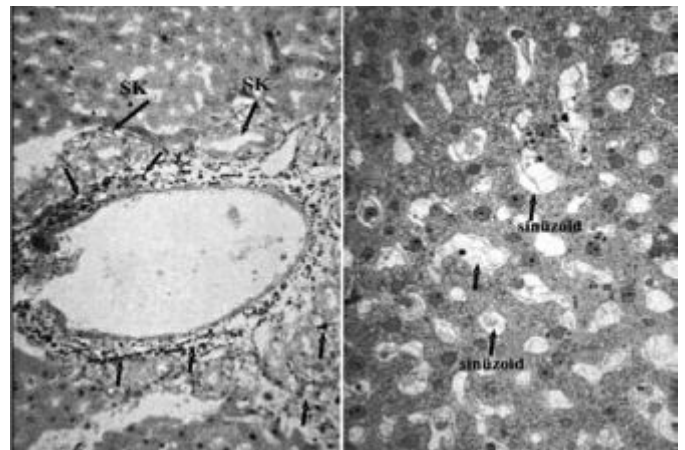


Fig. 8: Grup IVu: left side light microscope, Toluidin blue, SK= bile canaliculi, right side EM, Uranyl acetate-lead citrate x9000.

dilated and all mitochondria have lost their cristae. Smooth endoplasmic reticulum is also dilated and damage has increased. At the same time there are degenerative cytoplasmic areas, the nucleus is totally euchromatic, healthy nuclei are not seen, and the nucleus of the cell going to necrosis and the nucleus inner and outer membrane can be totally distinguished (Fig. 6). In this group despite unligation the damage is irreversible.

In group IV, the sinusoids are totally collapsed and an acinar appearance can be observed. The mitochondria are dense; the matrix is dense although the cristae are healthy (Fig. 7). The rough endoplasmic reticulum is slightly dilated in comparison to the control group and group I and there are significant areas of glycogen accumulation. This indicates that regeneration has started (NO effect?). In group IVu, under light microscopy neutrophil infiltration, connective tissue increase, fibroblast increase, collagen increase (toluidine blue), bile ducts and the portal area can be seen on the left side. The sinusoids with acinar appearance that function as secretory glands can be observed. In Ito cells lipid granules are increa-

sed by number and there are large lipid granules (Fig. 8). This shows that active cell synthesis and glycogen synthesis has begun. The cell nucleus in normal heterochromia, smooth endoplasmic reticulum is partially dilated but it is near to normal. The lipid granules are increased by number, the inner nucleus structure is normal and the sinusoids are collapsed.

THE ELECTRON MICROSCOPIC EVALUATION OF THE KIDNEY

In group I the podocytes, the filtration splits foot processes (pedicles), the basement membrane, the mesangial cells and mesangium are totally normal (Fig. 9). The criteria for the kidneys are irregularity in the basement membrane (thickness, thinness), fibrillary appearance in the basement membrane and fusion of the foot processes. The obstruction of filtration splits causes derangement in filtration. The endothelium, basement membrane, the blood-urine barrier, open filtration splits and foot

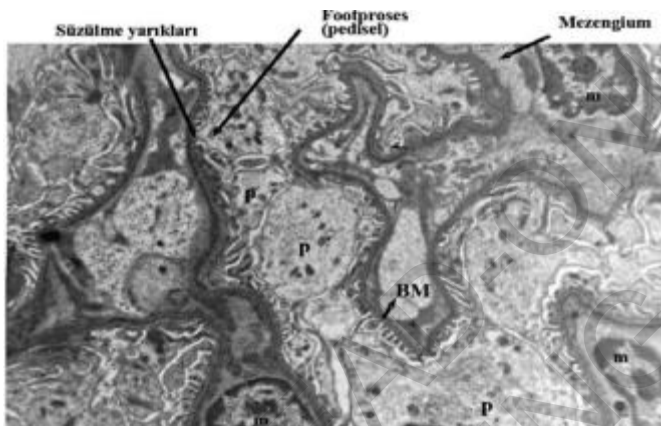


Fig. 9: Grup I: p= podoisit, BM= Basement membrane, m= mezenjial cell, Uranyl acetate-lead citrate x9000.

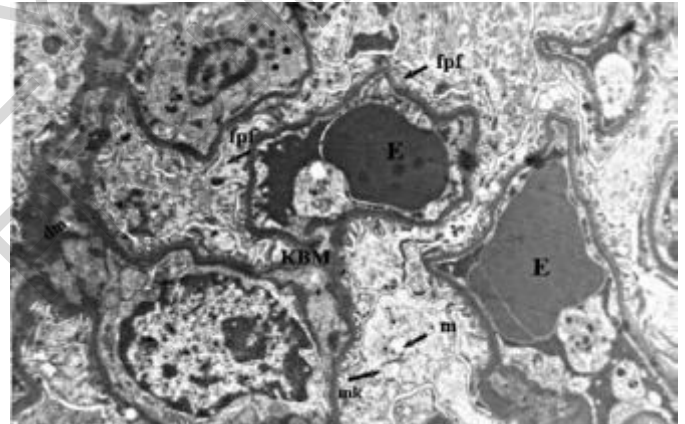


Fig. 11: Grup II: KBM= dens basement membrane, fpf= foot process fusion, E= Eritocyte, mk= mitochondrial crystallosis, m=mitochondria, dm= dens mesengium.

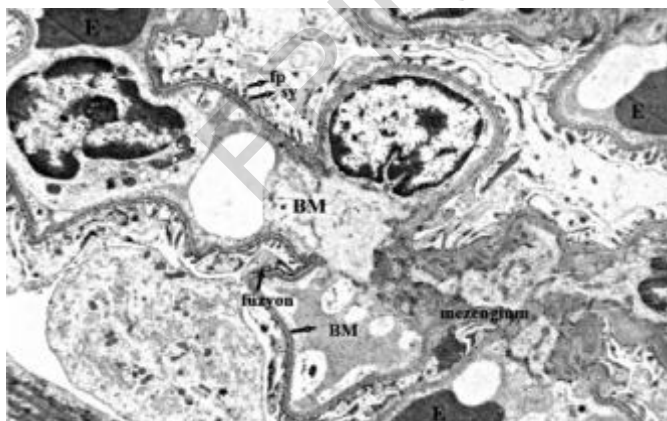


Fig. 10: Grup Iu: E= Eritocyte, BM= basement membrane, fp= foot process, sy= filtration splits, Uranyl acetate-lead citrate x9000.

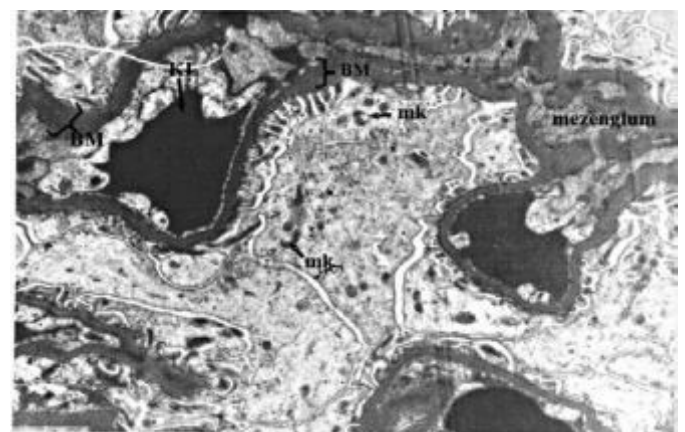


Fig. 12: Grup IIu: BM= Basement Membrane, mk= Mitochondrial crystallosis, KL= capillary lumen, Uranyl acetate-lead citrate x13200.

processes without fusion are easily visible. The relationship between the endothelia cells and the tubular epithelia composes the basement membrane.

In group Iu, partial increase of mesengium, healthy foot processes, healthy filtration splits in most areas and besides these fusion between filtration splits and erythrocytes are observed (Fig. 10). They are very similiar to the control group.

In group II, in some areas of membrane thickening is observed and in most areas there is fusion in the foot processes. When fusion occurs the filtration splits close and the ultra-filtrate cannot be filtrated. The mesengium is dense. This can be accepted as the initiation of fibrosis. 2 erythrocytes can be seen in the field (Fig. 11). Crystallysis is observed in the podocyte's mitochondria. In group IIu, basement membrane irregularity and disintegration of the blood – urine barrier can be seen. Partial mitochondrial crystallysis in the podocyte cytoplasm and

degenerative cell particles in the capillary lumen are seen (Fig. 12). The damage is significant.

The basement membrane in group III is irregular just like as in thin basement membrane nephropathy. The membrane is thin in some areas and thick in other areas. The mesangium appears dense and increased (Fig. 13). The areas where the endothelium lumen and capillaries should be are totally full with cell waste. Significant fusion is present in the foot processes. Also the filtration splits are obstructed. In group IIIu, crystallysis is significant in the mitochondria and the cell nucleus is euchromatic. Mesangial accumulation and protein accumulation are present just as in nephritis. The protein accumulation is due to the disfunction of the blood-urine barrier. Also fusion is present in the foot processes. And there is significant crystallysis in the podocyte mitochondria (Fig. 14).

In group IV, the foot process fusion is significant and



Fig. 13: Grup III: BM= Basement membrane, fpf= foot process fusion, Uranyl acetate-lead citrate x9000.

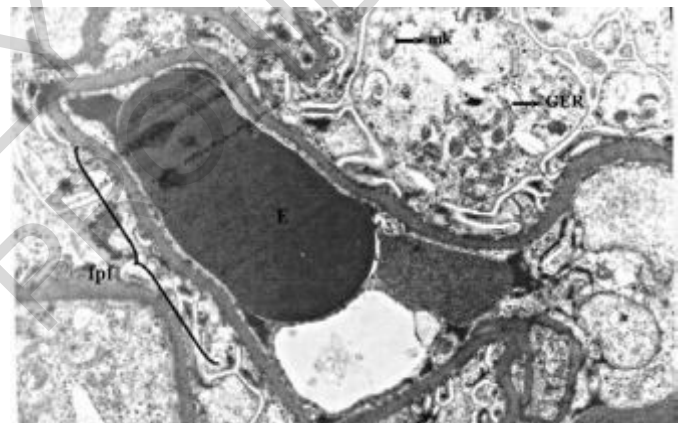


Fig. 15: Grup IV: fpf=foot process fusion, E= Erythrocyte, GER= Granulated Endoplasmic reticulum, mk= mitochondrial crystallization, Uranyl acetate-lead citrate x21000.

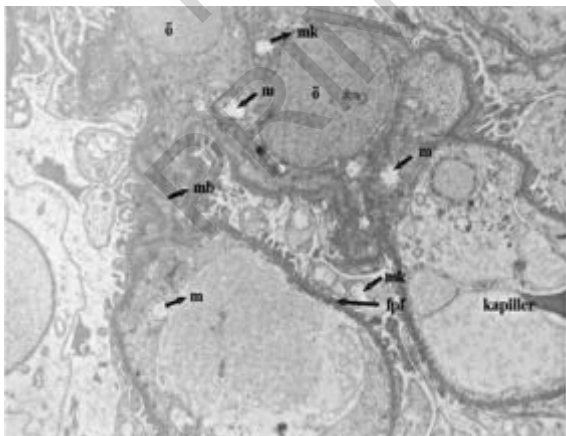


Fig. 14: Grup IIIu: mb= mesangial storage, fpf= foot process fusion, mk= mitochondrial crystallization, m=mitochondria, ö= , euchromatic cell nucleus Uranyl acetate-lead citrate x9000.

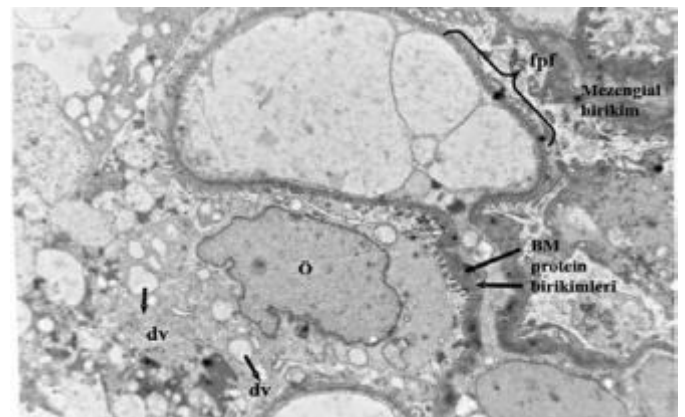


Fig. 16: Grup IVu: dv= degenerative vacuole, fpf= foot process fusion, ö= euchromatic cell nucleus, BM= Basement membrane, Uranyl acetate-lead citrate x9000.

the rough endoplasmic reticulum cisternae are dilated, also significant mitochondrial cristae loss is present. The filtration splits are almost unobservable. The foot processes are lost and there is no barrier present. Mitochondrial crystalalysis is seen (Fig. 15). In group IVu, many degenerative vacuoles and significant nucleus invasion is present. The fusion of the foot processes is marked. Significant protein accumulation is present in the basement membrane and the epithelium cells appear degenerated (Fig. 16). In comparison to group IV the filtration splits are more regular but in some areas no filtration splits are present.

Discussion

Obstructive jaundice is characterized by the accumulation of hepatotoxic agents, mitochondrial dysfunction and the disintegration on the livers' antioxidant defense system^{1,2}. In cholestatic liver lesions, bile salts and bilirubin accumulated in the intracellular compartment act like mediators causing systemic complications and lead to hepatocyte damage³. It has been proven that tissue injury and endotoxemia cause an increase in the formation of free oxygen radicals and reactive oxygen metabolites which leads to increased lipid peroxidation^{4,5}. The accumulation of hydrophobic bile acids and the insufficiency of energy production of the mitochondria are shown as the main reason of hepatotoxicity⁶. Both the accumulation of bile acids and the disintegration of the mitochondrial metabolism lead to increased production of free oxygen radicals which causes oxydative injury^{7,8}.

Free radicals cause a series of events leading to cellular injury either directly themselves or by causing cellular antioxidant systems to malfunction⁹⁻¹².

Experimental studies have shown that obstructive jaundice increases lipid peroxidation, causes mitochondrial disfunction and decrease of glutatyon levels^{13,14}.

Many studies drawing attention to the role of free radicals in cholestatic liver injury have shown that the antioxidant capacity of the liver is highly affected by the duration of cholestasis. It is for this reason that the timing of surgical intervention to prevent irreversible liver injury is critical.

In this study on an experimental model of obstructive jaundice the ultrastructural changes in the liver and kidneys have been observed under electron microscopy in short and long term (3rd, 7th, 10th and 14th days) and after unligation (or internal drainage).

In this study the increse of bilirubin and alkaline phosphatase levels after ligation and the decrease after unligation shows that an obsrtuctive jaundice model has been created.

In studies it has been mentioned that the cause of hepatic injury may be the reactive oxygen radicals formed as a result of oxydative stress. Because the mitochondria is the organelle where reactive oxygen molecules are for-

med most the mitochondria is the organel where changes at the cellular level occur the most. The mechanisms that favor linking to oxidative phosphorylation in electron transport dysfunction causing autoxidation forming superoxides. 2-5% of the oxygen used in hepatocyte mitochondria is turned into reactive oxygen metabolites and the rest is turned into H₂O. TNF- α , bile acids, ischemic-reperfusion injury, ceramides increase the formation of reactive oxygen metabolites and this increase blocks the electron transport system¹⁵⁻¹⁸.

Although alkaline phosphatase (ALP) levels rise under many other pathologies it is actually an ectoenzyme of the plasma membrane and the rise of serum ALP levels is related to the injury of liver cell membrane injury. The reasonable rise of ALP levels may also be an indication of cholestasis and cellular injury.

In early ligation groups I and Iu, histologic findings were all close to those of the control group, only in group Iu a low number of lipid granules were observed.

In group II which was ligated for 7 days oxidative injury initiates. In group II, vesicular mitochondria are seen which may be due to cessation of oxidative phosphorylation. In group II mitochondrial swelling and cristae loss is also present. In previous studies, the liver tissue damage in rats with ligated bile ducts is shown to be necrosis, mitochondrial swelling, crystalalysis and apoptosis^{15,19}.

The most marked liver tissue damage occurred in group III (ligated on the 10th day). In this group due to degenerated mitochondria other organelles cannot be distinguished. Also in group III lysosomes that phagocyte other cellular elements are present. In group IIIu the injury can be accepted as irreversible despite unligation. In this group the mitochondria have lost their cristae.

In the final group IV mitochondria with intact cristae and glycogen accumulation areas are seen. In this group after unligation an increase of Ito granules occurs and lipid granules and normal mitochondria are observed. This may indicate to initiation of regeneration. Hepatic endothelial cells and Ito cells can synthesize nitric oxide by iNOS expression²⁰.

Any hemodynamic stimulus that can cause tension stress in the liver leads to nitric oxide release and thus initiates the liver regeneration cascade. Obstructive jaundice causes functional disorder in hepatocytes. In our study due to the induced regeneration on the 14th day injury has been reduced.

Patients with intra or extrahepatic biliary obstruction are under the risk of acute renal failure during major abdominal surgery. The mechanism lying under the renal injury during obstructive jaundice has not been enlightened yet. But it has been suggested that because they can only be excreted by kidneys, the bile acids accumulated in the plasma act as nephrotoxins²¹. But there is not enough evidence to support this hypothesis. In a study of Kaler et al. it has been shown that 3-4 days after the bile ducts were ligated non-specific changes

occured in the proximal tubules, and that these changes were most significant when the the plasma levels of bilirubin and bile acids were highest ²¹.

The importance of this study is that concurrently formed ultrastructural changes of the kidney and liver have been investigated both in the presence of elongated jaundice and after unligation (or internal drainage). When the kidney tissue of 3 day ligation rats were evaluated the findings were similiar to the control group and even though fusion is present in filtration splits in some areas after unligation, the kidney histology is close to the control groups. These findings show that kidney damage does not occur in the early stage of obstructive jaundice and this is parallel with the study of Kaler et Al.

In the 2nd group that is after 7 day ligation basement membrane thickening in some places, fusion of the foot processes and dense mesengium is observed. The formation of fusion means the ultra-filtrate is not being excreted and the clinical reflection can be oliguria. After unligation in this group disintegration of the blood-urine barrier and crystalalysis of the podocyte mitochondria has been observed. The decreased excretion of acumulated bile acids in plasma due to oliguria and their effects as nephrotoxins in a longer time span despite unligation may explain this situation ²²⁻²⁴. Also because of the concurrent liver injury the decrease of antioxidan enzyme levels will potentially increase the injury ²⁵.

In the rats of group III the basement membranes are fairly irregular, and the findings are similiar to those of the thin basement membrane nephropathy. This shows that injury significantly increases as jaundice continues. Even after unligation injury has continued to increase. Mitochondrial crystalalysis and euchromatic cell nucleus are present. In this group it has been observed that the rough endoplasmic reticuli are dilated and secondary lysosomes phagocytating intracellular waste has appeared. This shows that oxydative stress has increased and waste formed after tissue damage is trying to be digested.

In group IV, the 14 day ligation group, foot process fusion is marked, the rough endoplasmic reticulum cisternae are dilated and mitochondrial crystalalysis is very significant. In this group even after unligation many degenerative vacuoles have been observed and endothelium injury and mesangial accumulation is significant. Interestingly in this group despite the regenerative capacity of the liver the kidney injury can be said to be irreversible. In one out of ten patients developing obstructive jaundice acute renal failure develops after major surgical intervention ²⁶. Although there are many factors in the etiology of renal failure, there are no studies in literature exploring the long term effects and electron microscobic changes.

Conclusion

Finally, we can say that changes observed in rat models

with obstructive jaundice can be reversible after drainage related to the duration of ligation. The ultrastructural changes observed in the kidney are especially irreversible in elongated jaundice despite internal drainage and this is associated with higher morbidity and mortality is the most defining factor.

In patients with elongated obstructive jaundice precautions like hydration and hemodialysis accelerating bile acid excretion could prevent the damage to reach irreversible levels. Because all these results and interpretations depend on an experimental study, it has to be supported by clinical trials to explain the changes in humans.

Riassunto

Con questo studio sperimentale abbiamo indagato le conseguenze della legatura del dotto biliare comune (CBD) sulle cellule epatiche e sull'ultrastruttura renale mediante microscopia elettronica e determinare anche gli effetti dopo liberazione del dotto per slegatura, al fine di chiarire i meccanismi di insufficienza renale comunemente osservati nella malattia epatica colestatica.

Lo studio è stato condotto su 53 ratti albin Wistar divisi in 4 sottogruppi. Nel gruppo di confronto (sham) si è proceduto alla semplice laparotomia. Dopo preparazione del dotto biliare comune di tutti i ratti dei quattro gruppi, e legatura del dotto a livello del terzo distale, otto ratti in ciascun gruppo sono stati sacrificati in 3^a, 7^a, 10^a e 14^a giornata dall'intervento, prelevando campioni di sangue per dosare i livelli sierici di ALP e bilirubina, e campioni di tessuto epatico e renale per la valutazione istologica.

In quattro ratti di ciascun gruppo il dotto biliare comune è stato slacciato alle stesse scadenze per ottenere il libero drenaggio della bile per una settimana. Alla fine di questa settimana i ratti sono stati sacrificati raccogliendo campioni di sangue e di tessuti epatici e renali. Risultati: dopo la legatura del CBD in entrambi i gruppi, il valore di ALP, i livelli di bilirubina totale e diretta sono risultati proporzionalmente aumentati. Dopo la liberazione del dotto i livelli della bilirubina sono diminuiti in modo significativo.

Nel II gruppo, mentre si erano osservati grossi granuli lipidici ad indicare il danno ossidativo, dopo liberazione del dotto si è osservato rigonfiamento mitocondriale e presenza di cristalli. Nel gruppo IV sono state osservate aree di glicogeno e mitocondri normali. Dopo la liberazione del dotto in questo gruppo sono stati osservati aumento dei granuli di Ito, granuli lipidici e mitocondri normali, che possono riflettere l'evoluzione di una rigenerazione epatica.

Quando il tessuto renale è stato esaminato nel gruppo II, sono stati osservati processi di fusione nei piedi, ispessimento della membrana basale e del mesengio, e dopo la liberazione del dotto sono stati osservati cristalli mitocondriali nel tessuto renale come nel fegato.

Il danno nel gruppo III e nel gruppo IV è risultato aumentato parallelamente al prolungamento dell'ittero e dopo la slegatura un danno persistente con cristalli mitocondriali.

In conclusione i cambiamenti ultrastrutturali del tessuto epatico del ratto in condizione di ittero ostruttivo possono essere reversibili dopo ripristino del drenaggiobiliare. D'altra parte i cambiamenti ultrastrutturali del tessuto renale nei casi di ittero prolungato sono irreversibili anche se il drenaggio interno è restaurato.

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