Özettir

Amaç Çoklu organ yetmezliği riski çoklu yaralanması olanlarda artmıştır. Bu tür olgularda farklı yaralanma tiplerinin birbirine etkisi ve hastaların nasıl etkileyeceği önemsenmesi gerekken ciddi bir sorundur. Bu çalışmada splenektomi ve femur kırığı şeklindeki yaralanmanın bakteriyal translokasyonu ve karaciğer yenilenmesi üzerine etkileri deneySEL hayvan modellinde incelenmesi hedeflenmiştir.

Yöntem Çalışmada elli erkek fare kullanıldı. Gruplara uygulanan cerrahi işlemler: laparotomi + femoral incizyon (Grup I—kontrol), hepatektomi + splenektomi (Grup II), splenektomi + femur kırığı (Grup III), hepatektomi + femur kırığı (Grup IV) ve hepatektomi + splenektomi + femur kırığı (Grup V) şeklindeydi. Cerrahi girişimden üç gün sonra deneklerde bakteriyal translokasyon, karaciğer ağrıları, mitotik aktivite ve kupffer hücre proliferasyonu inceledi.

Bulgular Splenektomili olgularda bakteriyal translokasyonun azaltıldığı ve karaciğer yenilenmesinin arttığı saptanırken (p=0.042, p=0.027), femur kırığı olgularda bakteriyal translokasyonun arttığı ve femoral kırık olgularında ise karaciğer yenilenmesinin azaldığı belirginleşti (p<0.05).

Sonuç Birden fazla yaralanmanın uygulandığı deneySEL bir modelde splenektominin bakteriyal translokasyonu azalttığı ve karaciğer hücre yenilenmesini artırdığı belirlenmiştir.

Anahtar Kelimeler: Splenektomi, Hepatektomi, Femur Kırığı, Bakteriyal Translokasyon, Karaciğer Rejenerasyonu

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INTRODUCTION

Civilian trauma is the leading cause of mortality and morbidity in younger population. As technology advances the spectrum of trauma which is reflected by the catastrophic accidents could be seen every day.

The changing magnitude and impact of trauma have made the clinicians deal with multitrauma patients more frequently. In time, it became evident that this specific group of patients exhibit different characteristics, one of which is the exaggeration of initial impact namely multiorgan failure and this has become a major concern. Multiorgan dysfunction has been tried to be explained by two hit theories and interactions between different subtypes of trauma in order to decrease both the mortality and the morbidity of multitrauma patients.

In accidents with high kinetic energy, laceration of intraabdominal solid organs is usually accompanied by fracture of large bones. These types of injuries, surgical treatment techniques (splenectomy, hepatic resection) and surgical diagnostic techniques (laparotomy) result in changes in inflammatory response which may enhance the original immune reaction. It is well known that bacterial translocation in trauma patients is also an important factor in deterioration of the patients. In trauma patients hemorrhagic shock, parenteral nutrition, burns, intestinal motility disorders and liver resection increase the risk of bacterial...
translocation whereas splenectomy, enteral feeding decrease this risk.

In this study, we tried to evaluate the effects of two-hit model, namely splenectomy and fracture of the femur, on bacterial translocation and liver regeneration in an experimental mouse model.

Material and Methods

This experimental study was conducted in the research laboratory and research animal breeding unit of Suleyman Demirel University Medical School strictly following the guidelines of the local ethical committee. The experiment was carried out in five groups each consisting of ten Wistar-Albino male mice weighing 20-35 grams. The experimental groups were as follows:

Group I: Control group (laparotomy + femoral incision)
Group II: Hepatectomy + Splenectomy
Group III: Splenectomy + Femoral fracture
Group IV: Hepatectomy + Femoral fracture
Group V: Hepatectomy + Splenectomy + Femoral fracture

Sterilization: Sterility was closely monitored in all steps of the experiment and each of the mice were operated using separate sterile surgical equipment.

Anesthesia: All of the mice were anesthetized using 100 mg/kg ketamine hydrochloride (Ketalar, Eczacibasi, Istanbul, Turkey) and 50 mg/kg xylazine hydrochloride (Romphun, Bayer Turk, Istanbul, Turkey) after 12 hours of starvation. Abdominal wall and femoral fracture areas were cleansed using 2.5% povidone iodine and were draped in sterile fashion.

Laparotomy: 4 cm midline laparotomy was performed, the ligaments of liver and spleen were disattached but no resection was carried out. The abdomen was closed with full thickness 3/0 interrupted polypropylene sutures.

Dissection of the femur: A 1 - 1.5 cm incision was made over the lateral thigh of the right leg and subcutaneous tissue, superficial fascia and muscle were cut open in order to expose the femur. The wound was closed with 3/0 polypropylene interrupted sutures without creating a femoral fracture.

Hepatectomy: Standard 2/3 partial hepatectomy described by Higgins and Anderson was performed in Group II, IV and V. After laparotomy the liver was exposed and the pedicules of median and left lateral lobes of the liver were tied using 3/0 silk sutures, leaving right lateral lobe and caudate lob intact.

Splenectomy: The pedicle of the spleen was tied using 3/0 silk sutures and splenectomy was completed preserving the pancreas in Group II, III, and V.

Femoral Fracture: A surgical femoral fracture was created in Group III, IV, V. After homeostasis the wound was closed with 3/0 polypropylene interrupted sutures. In order not to increase the rate of translocation and infection, the fracture was stabilized with medical band-aid.

All of the mice had free access to standard mouse chow ad libitum after the operation. On the third postoperative day all of the mice were sacrificed by cervical dislocation. Antisepsis was assessed by cleaning the abdominal incision site using 2.5% povidone iodine and sterile drapes were applied to the surgical site in order to avoid contamination of the abdominal cavity in order not to render the results of bacterial translocation.

Microbiological Method: Abdominal incisions were opened using separate sterile equipment for each mouse. All of the regenerating liver tissue was resected and put into sterile petri dishes sterilized and weighed beforehand. The wet tissue weight was measured using electronic scale. A small piece was preserved in formalin for histopathologic examination.

The mesentery was excised, put into sterile petri dishes sterilized and weighed beforehand. Wet tissue weight of the mesentery was measured using electronic scale. The specimens were minced and were homogenized with 1 ml of sterile saline. Samples taken from this homogenate using 1/100 sterile loop were inoculated to blood agar and Mac Conkey agar. Colonies which grew were identified using conventional methods. The cultures were evaluated after they were incubated for 24 hours under 37°C. The possible translocated bacteria in the cultures were chosen as Escherischia coli, Enterobacter sp. and Enterococcus sp.

Histopathologic Examination: The weight of the regenerating liver remnant, mitotic index and Kupffer cell activity were taken as criteria to evaluate liver regeneration. The weight of the regenerating liver was calculated as follows: Wet tissue weight of the liver of sacrificed mouse (K)/Total weight of the mouse x 100 = Weight of the regenerating liver.

5 micron thick samples of 10% formaline fixed, paraffin embedded liver tissue were stained using hematoxyline-eosin and examined in the light microscope under x10, x40 and x100 magnification. The mean mitotic index of each tissue sample was calculated by dividing the total number of cells showing mitosis in ten different microscopic fields under x40 magnification. The arithmetical mean of the mitotic index mice in the same group was taken as the mitotic index of the group. Microscopic findings like hyperemia of the liver, degeneration, intraparenchymal and subcapsular bleeding, microvesicular fatty degeneration, reactive hepatitis were not taken into account for evaluation of the liver specimens. The arithmetical mean of the number Kupffer cell count detected in ten different microscopic fields under x40 magnification was taken as the Kupffer cell activity of the specimen. This activity was graded as minimal activity (+), moderate activity and (++) and maximal activity (+++).

Statistical analysis: The data were analyzed using SPSS 8.0. Mann Whitney-U, Chi square test and Student t test were used and p<0.05 was accepted as statistically significant.

Results

Mortality: In the control group, 2 mice died whereas only one mouse died in group II. Three mice died in Group IV and in Group V. No mortality was encountered in Group III.
statistically significant difference could be found between the control group and the other groups.

**Bacterial Translocation:** The translocated bacteria were E.coli, Enterococcus sp, enterobacter sp. Bacterial translocation was maximal in Group IV, followed by Group V and II whereas it was minimal in Group III. Bacterial translocation was found to be statistically significant when control group and study groups were compared (p=0.028). When Group II and III were compared for bacterial translocation, there was no statistically significant difference (p=0.294). Bacterial translocation was less in Group III in comparison to Group IV and V. The difference was found to be statistically significant (p=0.042). Bacterial translocation data are given in Table I and Figure I.

**Table I. Bacterial Translocation Data**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>MLN CFU/mg</th>
<th>L CFU/mg</th>
<th>SUM</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>3.1</td>
<td>0</td>
<td>1/16</td>
<td>6.2</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>108.3</td>
<td>5</td>
<td>17,5</td>
<td>38.9</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>91.5</td>
<td>3</td>
<td>166.7</td>
<td>25.0</td>
</tr>
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<td>4</td>
<td>7</td>
<td>1020.4</td>
<td>5</td>
<td>1020.4</td>
<td>71.4</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>28.6</td>
<td>4</td>
<td>28.6</td>
<td>50.0</td>
</tr>
</tbody>
</table>

MLN: Mesenteric lymph node, L: Liver, CFU/mg: Colony Forming Unit per milligram, *p<0.05 statistically significant

**Figure I: Bacterial Translocation in Groups**

**Liver Regeneration:** When liver weights of the sacrificed mice were compared, the least increase in liver weight was found to be in Group IV. The liver weight in Group II and Group III had increased significantly when compared with the control group (p=0.002, p=0.045 respectively). When the liver weight increase of Group II and III were compared, there was a statistically significant increase in Group III (p=0.018). The comparison of Group III and V showed a significant result in favor of group III (p=0.027). Liver regeneration data are given in Table II.

**Mitotic Index:** The arithmetical mean of mitotic indices of each group was calculated and compared with the mitotic index of each group. There was a statistically significant difference between the control group and Groups II, III, and IV and V (p=0.001) (Table III).

The highest mean mitotic index value was found in Group II. When the mitotic index values of Groups II, III, IV and V were compared, the only statistically significant difference was found between Group II and IV (p=0.045). The mitotic index values of each group calculated according to mean mitosis values is presented in Figure II.

**Table II. Increase in Liver Weight**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Mean Weight Increase (mg±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>6.2 ± 0.8</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>5.0 ± 0.4*</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>5.8 ± 0.8</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>4.7 ± 1.1*</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>5.1 ± 0.8</td>
</tr>
</tbody>
</table>

*p<0.05 statistically significant

**Table III. Mitotic Index**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Mean Mitotic Index (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>3.9 ± 1.5*</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>2.8 ± 1.2</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>2.7 ± 1.1*</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>3.4 ± 1.0</td>
</tr>
</tbody>
</table>

*p<0.05 statistically significant

**Figure II: Distribution of Mitotic Index**

**Kupffer Cell Activity:** Minimal Kupffer cell activity was present in most of the mice in the control group whereas there was no significant difference when an intergroup analysis was done among the other groups for the number of mice with minimal Kupffer cell activity.

Moderate Kupffer cell activity was most prominent in Group IV (p=0.023). There was no difference for the number of mice exhibiting moderate Kupffer cell activity when other groups were compared.

Maximal Kupffer cell activity was significantly prominent in groups II, III, IV and V when compared with the control group (p=0.031). When groups II, III, IV and V were compared for the number of mice showing maximal Kupffer cell activity, no statistically significant difference could be found. The highest mean was in group II and the least mean was in group IV. The values in group III and V were close and there was no significant difference (Table IV). The distribution of Kupffer cell activity which is the mean of the number of Kupffer cells in each group is presented in Figure III.
Bacterial translocation can be defined as the movement of live intestinal intraluminal bacteria from the mucosa of the epithelium towards the lamina propria, followed by mesenteric lymph nodes and remote organs. Bacterial translocation can be the result of a wide spectrum of insults to the organism. E. coli is the most frequently encountered microorganism for bacterial translocation. The prominent microorganism in our study was E. coli.

Spleen has a major role in body defense against infections. Clinically, it is well known that asplenics patients are significantly prone for development of lethal sepsis. Spaeth et al. investigated the effect of bacterial translocation simulation created by endotoxin application in splenectomy. It was shown that splenectomy did not increase bacterial translocation and splenectomy indeed decreased bacterial translocation after splenectomy. Our findings suggest that this could be a possible mechanism. Filtration function is lost but as the clearance of leukocytes is diminished; the cytokine release triggered by these leukocytes in presence of bacterial translocation and their impact on Kupffer cell proliferation might be increased. Although Alexander et al showed that bacteria are translocated to enterocytes by direct penetration; the route of bacterial translocation to blood and remote organs after major liver resections is not clear yet.

Kupffer cell counts are decreased in primary biliary cirrhosis and other types of cirrhosis. The decrease in phagocytic activity of Kupffer cells is a bad prognostic sign in liver diseases. Although 80-90% of reticuloendothelial system function is covered by the liver. It is shown those six hours after 70% hepatectomy, the remnant 30% liver tissue is sufficient enough to clear translocated bacteria from systemic circulation. When a 70% hepatectomy is performed, there is an immediate increase in reticuloendothelial system activity in spleen. Cytokines and prostaglandins secreted from the spleen induce local or splanchnic hyperperfusion, permeability increase in the intestinal wall, intestinal edema and intestinal necrosis. Although bacterial translocation is reduced after splenectomy, the impairment in bacterial activity and phagosytic activity of alveolar macrophages supports this hypothesis.

In our study, bacterial translocation was increased both in mesenteric lymph nodes and liver after major liver resection. This finding is consistent with the literature. The least bacterial translocation was encountered in Group III. Although 80-90% of reticuloendothelial system function is covered by the liver. It is shown those six hours after 70% hepatectomy, the remnant 30% liver tissue is sufficient enough to clear translocated bacteria from systemic circulation. When a 70% hepatectomy is performed, there is an immediate increase in reticuloendothelial system activity in spleen. Cytokines and prostaglandins secreted from the spleen induce local or splanchnic hyperperfusion, permeability increase in the intestinal wall, intestinal edema and intestinal necrosis. Although bacterial translocation is reduced after splenectomy, the impairment in bacterial activity and phagosytic activity of alveolar macrophages supports this hypothesis.

Liver regeneration is the most accelerated tissue regeneration encountered in mammals. The magnitude of regeneration process is parallel to the volume of the remnant liver. Regeneration stops when the remnant liver reaches the volume of the original liver. Weight increase and increased mitotic activity are useful tools to evaluate liver regeneration. Mitotic activity of the hepatocytes are

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**Table IV. Mean Kupffer Cell Activity**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>+ (%)</th>
<th>++ (%)</th>
<th>+++ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>62.5*</td>
<td>25.0</td>
<td>12.5</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>11.1</td>
<td>33.3</td>
<td>55.6</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>20.0</td>
<td>30.0</td>
<td>50.0</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>28.6</td>
<td>42.8*</td>
<td>28.6*</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>28.6</td>
<td>28.6</td>
<td>42.8*</td>
</tr>
</tbody>
</table>

*p<0.05 statistically significant

**Figure III. Mean Kupffer Cell Activity in Groups**

Maximal kupffer cell activity was significantly prominent in groups II, III, IV and V when compared with the control group (p=0.031).

Discussion

Bacterial translocation can be defined as the movement of live intestinal intraluminal bacteria from the mucosa of the epithelium towards the lamina propria, followed by mesenteric lymph nodes and remote organs. Bacterial translocation can be the result of a wide spectrum of insults to the organism. E. coli is the most frequently encountered microorganism for bacterial translocation. The prominent microorganism in our study was E. coli.

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In our study, bacterial translocation was increased both in mesenteric lymph nodes and liver after major liver resection. This finding is consistent with the literature. The least bacterial translocation was encountered in Group III. This could be explained by the decrease of bacterial translocation and the increase in Kupffer cell count due to splenectomy. The maximal bacterial translocation was encountered in Group IV. The bacterial translocation rate was higher than the rate in group II and group V. This difference was statistically significant (p=0.042). The absence of splenectomy, which reduces bacterial translocation, and trauma and immunosupression caused by femoral fracture, can be held responsible for this result. Yet further studies are needed to clearly identify the mechanism(s) responsible for the increase in bacterial translocation.

Liver regeneration is the most accelerated tissue regeneration encountered in mammals. The magnitude of regeneration process is parallel to the volume of the remnant liver. Regeneration stops when the remnant liver reaches the volume of the original liver. Weight increase and increased mitotic activity are useful tools to evaluate liver regeneration. Mitotic activity of the hepatocytes
increases only to stop when original volume of the liver is restored and no abnormal increase in liver volume and abnormality in liver function is ever encountered. DNA synthesis in hepatocytes starts at postoperative 15-16 hours and peaks at postoperative 24 hours after 70% hepatectomy.\(^{(1)}\) Higgins and Anderson showed that the most prominent increase in liver mitotic activity and paranchymal weight takes place on the third postoperative day.\(^{(1)}\) The regenerative rate of a normal adult rat hepatocyte is one mitosis per year under normal circumstances. This rate increases under maximal stimuli. After a 70% hepatectomy, the remnant liver weight doubles in the first 48 hours and reaches the original weight before resection in 7-15 days.\(^{(1,10,11)}\) Splenectomy is known to affect liver regeneration. In splenectomized rats, there is an increase in the rate of liver weight gain in the early postoperative period and this effect fades in the late postoperative period. The promoting affect of splenectomy in liver regeneration has been supported by many studies. Perez et al. postulated that the positive effect of splenectomy in liver regeneration is of humoral origin and could be attributed to the absence of an inhibitor factor secreted by the spleen after splenectomy.\(^{(22)}\) Using primary hepatocyte cultures of rats, Ohira et al. studied the effects of splenic extracts on liver regeneration and discovered that certain extracts contained factors which inhibited hepatic regeneration.\(^{(23)}\)

When we analyzed remnant liver weight increase in our study, the least weight increase was found in Group IV. When Group II and Group IV and Group I were compared, the differences were statistically significant (p=0.002, p=0.045 respectively). Least weight gain in the remnant liver encountered in Group IV can be explained by the absence of splenectomy and immunological effects and trauma caused by femoral fracture. There was no significant difference in remnant liver weight gain in groups where hepatectomy was accompanied by splenectomy (Group II and Group V) which pointed to the positive effect of splenectomy on liver regeneration, consistent with the literature. Although femur fracture was present in Group III and Group V, the positive effect of splenectomy came over the negative effect of femur fracture. In group III where hepatectomy and femur fracture were not accompanied by splenectomy, the remnant liver weight gain was minimal.

In our study, the mitotic index of the groups was consistent with the literature. The mitotic index of Group II, III, IV and V were found to be greater than the control group. When the mitotic index of Group II and IV were compared, mitotic index of Group II was significantly higher than that of Group IV (p=0.045). The difference could be explained by the positive effect of splenectomy in Group II and negative effect of femur fracture on regeneration and mitotic activity in Group IV.

Liver regeneration is a basic and planned response against loss of liver tissue. In 2/3 partial hepatectomy of rats, the residual liver lobes regenerate until the original liver volume is reached and this process takes 7-10 days.\(^{(11)}\) This regenerative process is under complex humoral and cellular interactions. Kupffer cells are known to secrete most of the major growth factors and cytokines responsible for hepatic regeneration like hepatocyte growth factor (HGF), transforming factor-β (TGF-β), interleukine-6 (IL-6), tumor necrosis factor-α (TNF-α) and therefore they are crucial for liver regeneration.\(^{(23,24)}\) In a study by Boulton et al, early postoperative phase of 70% liver resection after selective Kupffer cell depletion was investigated. It was show that Kupffer cells have both an inhibitory and stimulatory effect on hepatocyte DNA synthesis and that the early phase of regeneration is prolonged and regeneration is belated.\(^{(23)}\) Takashi et al. investigated the role of Kupffer cells in liver regeneration. In 2/3 hepatectomy model, on the fifth posthepatectomy day, they selectively depleted kupffer cells of mice by injection of liposome encapsulated dicloro-methylene diphosphonate (lipo MDP). As a result, liver regeneration was decelerated after Kupffer cell depletion.\(^{(24)}\)

In our study, the highest Kupffer cell activity was encountered in Group II. This finding suggests that both splenectomy and increased Kupffer cell count has a stimulatory effect on regeneration. The lowest Kupffer activity was encountered in Group IV which can be explained by the negative effect created by the absence of splenectomy and the negative effect of stress caused by femur fracture. There was no statistical difference among Group II, III and V which is an evidence for the positive effect of splenectomy on liver regeneration.

In this non lethal trauma model, we investigated the effect of splenectomy and femur fracture on bacterial translocation and liver regeneration. Splenectomy decreased the rate of bacterial translocation while promoting liver regeneration after 2/3 partial hepatectomy. Femur fracture increased bacterial translocation and rendered liver regeneration after 2/3 partial hepatectomy.

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REFERENCES


