DENEYSEL EXTRAHEPATİK KOLESTAZDA SOLUBLE İNTERCELLULAR ADHESION MOLECULE-1 VE BU MOLEKÜL ÜZERİNE DEKSAMETAZONUN ETKİSİ

SOLUBLE INTERCELLULAR ADHESION MOLECULE-1 IN EXPERIMENTAL EXTRAHEPATIC CHOLESTASIS AND THE EFFECT OF DEXAMETHASONE ON THIS MOLECULE

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Özet

Amaç: Extrahepatik kolestazda solüble ICAM-1 (sICAM-1) aktivitesini ve bu parametre üzerine deksametazonun etkisini inceledik.

Metod: Otuz tavşan 10'u 3 gruba ayrıldı. Sham kontrol grubuna laparotomi yapıldıken, diğer 2 grupta ana safra kanalı bağlandı. Çalışma grubunda 10 gün boyunca deksametazon, kontrol grubunda ise izotonik salin solüsyonu im verildi. Tüm deneklere 10 günün sonunda relaparotomi uygulandı. Laparotomiden önce ve relaparotomi sırasında serum sICAM-1, alkalen fosfataz (ALP), gamma glutamil transferaz (GGT), aspartat transferaz (AST), alanin transferaz (ALT), total bilirubin (T. Bil.) ve direkt bilirubin (D. Bil.) değerleri incelendi.

Bulgular: Sham kontrol, kontrol ve çalışma grubunda preoperatif dönemdeki sICAM-1 değerleri 12.60±2.22 ng/mL, 11.80±2.39 ng/mL, 11.00±2.40 ng/mL; postoperatif dönemdeki değerler ise 16.05±10.51 ng/mL, 38.05±10.16 ng/mL, 29.10±7.46 ng/mL idi. Preoperatif dönemdeki 3 değeri birbirinden farklı değildi. Kontrol ve çalışma grubunun postoperatif değerleri, tüm grupların preoperatif değerlerinden ve sham kontrol grubunun postoperatif değerinden yüksekti. Çalışma grubunun postoperatif sICAM-1 ve T. Bil. değerleri kontrol grubundan önemli derecede düştü (p<0.05 ve p<0.01, sırasıyla).

Sonuç: sICAM-1 değerleri extrahepatik kolestazda yüksekmektedir ve deksametazon bu artışın değerleri düşürmektedir.

Anahtar kelimeler: Soluble intercellular adhesion molecule-1, Extrahepatik kolestaz, Deksametazon

Summary

Objective: We investigated soluble intercellular adhesion molecule-1 (sICAM-1) concentration in extrahepatic cholestasis and the effect of dexamethasone on this parameter.

Methods: Thirty rabbits were divided into 3 groups of 10. In the sham control group, the rabbits underwent laparotomy. In the other 2 groups, the rabbits had common biliary duct ligation. In the study group, dexamethasone was injected, and in the control group, isotonic saline solution was injected for 10 days. All rabbits had relaparotomy at the end of the 10th day. Serum sICAM-1, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), aspartate transferase (AST), alanine transferase (ALT), total bilirubin (T. Bil.) and direct bilirubin (D. Bil.) values were investigated before the laparotomy and during the relaparotomy.

Results: In the sham control, control and study groups, sICAM-1 values obtained preoperatively were 12.60±2.22 ng/mL, 11.80±2.39 ng/mL and 11.00±2.40 ng/mL, and those obtained postoperatively were 16.05±10.51 ng/mL, 38.05±10.16 ng/mL, 29.10±7.46 ng/mL, respectively. Preoperative values of the groups were not different from each other. Postoperative values of control and study groups were higher than preoperative values of all groups and postoperative values of sham control group. Postoperative sICAM-1 and T. Bil. values of study group were significantly lower than those of control group (p<0.05 and p<0.01, respectively).

Conclusion: sICAM-1 values are increased in extrahepatic cholestasis and dexamethasone decreases the increased levels.

Key words: Soluble intercellular adhesion molecule-1, Extrahepatic cholestasis, Dexamethasone
Introduction
Long standing obstructive jaundice can result in parenchymal injury, renal failure, sepsis and bleeding [1]. The pathophysiological events preceding liver injury in obstructive jaundice are not well understood. Neutrophils infiltrate the liver after mechanical obstruction of the biliary tract and may be an important source of tissue damage. Neutrophils first adhere to sinusoidal endothelial cells, and this involves the expression of adhesion molecules [2].

Adhesion molecules are important for neutrophil localization at the site of inflammation [3-5]. The immunologically active adhesion molecules are subdivided into the immunoglobulin supergene family, the integrin family and the selectin family [5-7].

ICAM-1 is a cytokine-inducible member of the immunoglobulin supergene family which is expressed on the surface membrane of cells of multiple lineages at sites of inflammation and immune reactivity [2,8]. ICAM-1 mediates the recruitment of neutrophils through the endothelium to the site of inflammation by the ICAM-1 / Mac-1 and ICAM-1 / IFA-1 adhesion pathways [3,9,10]. In extrahepatic cholestasis, recruitment of neutrophils is a main feature of the inflammatory infiltrate in areas of parenchymal damage. ICAM-1 cell surface expression has been reported in a variety of inflammatory disorders, including human liver allograft rejection, viral hepatitis B, autoimmune liver disease, extrahepatic cholestasis and inflammatory liver diseases [3-5, 11, 12]. sICAM-1 levels have been decreased with many immunosupressive agents in many disorders like liver allograft rejection and Graves ophthalmopathy [8]. But studies of preventing liver tissue in extrahepatic cholestasis with many agents which decrease ICAM-1 activity are insufficient. So, we intended to investigate sICAM-1 activity in extrahepatic cholestasis and the effect of dexamethasone on this activity.

Materials and Methods
Thirty male Albino rabbits weighing 2000-2200 g were used throughout the study. Animals were quarantined for 1 week in standard laboratory conditions so that they could adapt to the environment. After fasting rabbits were anesthetized with ketamine HCl (85 mg/kg/im) and xylazine (6 mg/kg/im), they were divided into 3 groups of 10 as sham control, control and study group.

Sham control group: The rabbits had only laparotomy via a midline incision.

Control group: The rabbits had laparotomy via a midline incision and the common biliary duct was ligated. They were given isotonic saline solution (0.3 mg/kg/day) im in the morning far 10 days beginning the first postoperative day.

Study group: The rabbits had laparotomy via a midline incision and the common biliary duct was ligated. They were given dexamethasone (0.3 mg/kg/day) im in the morning for 10 days beginning the first postoperative day.

No animal died during the study. After anesthetized, all rabbits had relaparotomy at the end of the 10th day.

Fasting blood samples were taken from all animals before the first operation from ear vessels and during the second operation from inferior vena cava. Serum was obtained at 3000 rpm for 10 min, and was stored at -80°C until analyzed. Then serum sICAM-1, ALP, GGT, AST, ALT, T.Bil. and D.Bil values were determined.

These parameters were measured with commercially available kits (Boehringer Mannheim, Germany) in an autoanalyzer (Hitachi 717).

Table 1. The Parameters Obtained Preoperatively in the Sham Control, Control and Study Groups (means±SD)

<table>
<thead>
<tr>
<th>parameters</th>
<th>sham control group (n=10)</th>
<th>control group (n=10)</th>
<th>study group (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (IU/L)</td>
<td>100.00±34.80 *</td>
<td>67.30±14.20</td>
<td>115.00±30.70 **</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>8.80±7.10</td>
<td>11.90±5.90</td>
<td>9.60±3.90</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>109.40±10.45</td>
<td>160.40±53.00</td>
<td>155.20±52.00</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>47.90±28.10</td>
<td>61.20±31.60</td>
<td>74.30±27.00</td>
</tr>
<tr>
<td>T.Bil. (mg/dL)</td>
<td>0.31±0.07</td>
<td>0.28±0.07</td>
<td>0.33±0.15</td>
</tr>
<tr>
<td>D.Bil. (mg/dL)</td>
<td>0.14±0.05</td>
<td>0.14±0.05</td>
<td>0.16±0.11</td>
</tr>
<tr>
<td>sICAM-1 (ng/mL)</td>
<td>12.60±2.22</td>
<td>11.80±2.39</td>
<td>11.00±2.40</td>
</tr>
</tbody>
</table>

* p<0.05 sham control vs control group  ** p<0.001 sham control vs study group
Table 2. The Parameters Obtained Postoperatively in the Sham Control, Control and Study Groups (means±SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham control group (n=10)</th>
<th>Control group (n=10)</th>
<th>Study group (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (IU/L)</td>
<td>238.30±57.60 a,c</td>
<td>838.80±487.60</td>
<td>918.70±894.30</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>8.40±3.70 a,c</td>
<td>538.50±337.20</td>
<td>614.40±324.80</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>64.50±24.10 a,c</td>
<td>759.30±493.10</td>
<td>537.80±202.90</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>75.00±16.90 a,c</td>
<td>352.70±146.20</td>
<td>316.90±128.70</td>
</tr>
<tr>
<td>T.Bil.(mg/dL)</td>
<td>0.34±0.13 a,c</td>
<td>15.39±2.94</td>
<td>6.94±5.36 e</td>
</tr>
<tr>
<td>D.Bil.(mg/dL)</td>
<td>0.16±0.05 a,c</td>
<td>9.77±2.96</td>
<td>4.01±3.19</td>
</tr>
<tr>
<td>sICAM-1 (ng/mL)</td>
<td>16.05±10.51 b,d</td>
<td>38.05±10.16</td>
<td>29.10±7.46 f</td>
</tr>
</tbody>
</table>

a: p<0.001, b: p<0.01 sham control vs control group, c: p<0.01, d: p<0.01 sham control vs study group, e: p<0.01, f: p<0.05 control vs study group

Measurement of sICAM-1:

Test principle: The assay is based on the quantitative “enzyme-immuno-assay” principle, using two monoclonal antibodies, directed against different epitopes of sICAM-1. Both antibodies recognize sICAM-1. During the first incubation step, SICAM-1 in standards/samples is simultaneously bound by the biotin-labeled antibody and the peroxidase-conjugated detection antibody, forming a complex which binds via the biotin-labeled antibody to the streptavidin-coated surface of the microliter plate. Subsequent to the washing step, the peroxidase bound in the complex is developed by tetramethylbenzidine as a substrate and determined photometrically. The developed colour is proportional to the concentration of sICAM-1.

Standards of defined concentrations are run in each assay allowing the construction of a calibration curve by plotting absorbance versus concentration. The sICAM-1 concentration of unknown samples is then calculated from this calibration curve. Sensitivity of this kit is 3.4 ng/mL.

All results were presented as mean value±standard deviation (SD). Differences between groups were carried out by Mann-Whitney-U test. Values obtained before and after surgery were compared with Wilcoxon-signed rank test. For correlation analysis, Spearman’s rank correlation was used. A p value lower than 0.05 was accepted as significant.

Results

In the evaluation of the intraabdominal organs at relaparotomy, the intraabdominal organs were normal (the mean diameter of the gall bladder was 1x0.5 cm and the mean diameter of the common biliary duct was 0.1cm) in the sham control group. In the other 2 groups, the mean diameters of the gall bladders had increased to 5x3 cm from 1x0.5 cm, and the mean diameters of the common biliary ducts had increased to 0.8 cm from 0.1 cm. All intraabdominal organs were icteric and the livers were congestioned.

As shown in Table-1, preoperative parameters of all groups were not different from each other except ALP (p >0.05). There were significant differences between preoperative ALP values of sham control and control (p<0.05) and control and study group (p<0.001). In the comparison of postoperative ALP, GGT, AST, ALT, T.Bil. and D.Bil. values, there were significant differences between sham control and control (p<0.001) and sham control and study group (p<0.001). Postoperative sICAM-1 values of control and study groups were higher than those of sham control group (p<0.01). While the other postoperative parameters did not show a significant difference between the control and study groups, T.Bil. and sICAM-1 values of study group were significantly lower than those of control group (p<0.01 and p<0.05, respectively) (Table-2).

In the control and study groups, postoperative values were significantly higher than preoperative values (p<0.01 for all parameters). On the other hand, in the sham control group only postoperative ALP values were significantly higher than the preoperative values (p<0.01). According to Spearman’s rank correlation, a significant correlation was found only between postoperative sICAM-1 and AST values in sham control group (r=0.73, p<0.05) and between preoperative sICAM-1 and ALP values in control group (r=0.77, p<0.05).

Discussion

The pathogenesis of hepatocellular death in extrahepatic cholestasis is not fully understood. However, there is evidence implicating a cellular
immune mechanism. The infiltration of inflammatory cells such as neutrophils, monocytes, and lymphocytes plays an important role in the induction and maintenance of liver damage. Leukocyte cytotoxicity requires cell adhesion, both to target cells and to other immune cells [13]. ICAM-1 is an adhesion factor from the immunoglobulin supergene family and is involved in mediating the migration of immune cells into inflamed tissues and inducing cell-cell contacts during antigen recognition [2, 8]. SICAM-1 appears as a serum protein most likely after proteolytic cleavage of the complete extracellular part of the molecule close to the cell membrane [5, 14]. Increased sICAM-1 levels have been previously described in cholestatic autoimmune liver diseases as primary biliary cirrhosis or primary sclerosing cholangitis as well as in patients with acute or chronic hepatitis of different etiology [3-5, 9, 12].

The study was performed to investigate sICAM-1 activity in extrahepatic cholestasis in an animal model. Serum sICAM-1 values were investigated in the rabbits that had only laparotomy (sham control group) and the rabbits that underwent the common biliary duct ligation (control group and study group) before the first operation and after the second operation. Preoperative sICAM-1 values of all 3 groups were not different from each other. On the contrary, postoperative sICAM-1 values of the control and study groups were higher than the preoperative values of sham control group.

sICAM-1 levels may be decreased with many immunosuppressive agents. In a current study, increased sICAM-1 levels in liver allograft rejection and Graves opthalmopathy had been decreased with steroid therapy [8]. So, we aimed to investigate the effect of dexamethasone on the increased sICAM-1 levels in extrahepatic cholestasis. The rabbits that underwent common biliary duct ligation were given dexamethasone 0.3 mg/kg/day for 10 days beginning the first postoperative days. That dose is the maximal anti-inflammatory dose of dexamethasone. Postoperative sICAM-1 values of this group were statistically significantly lower than those of the rabbits that underwent common biliary duct ligation and were given placebo. Some investigators have used anti-ICAM-1, or anti-LFA-1 monoclonal antibodies to inhibit the enhancement of hepatocyte-leukocyte adhesion [13, 15, 16].

Postoperative ALP values of sham control group were higher than preoperative values of all groups. This may be due to the effects of laparotomy and to manipulation of the intestines. Postoperative sICAM-1 and bilirubin values of study group were statistically significantly lower than those of control group. This indicates that dexamethasone may decrease serum sICAM-1 and bilirubin values.

We concluded that sICAM-1 values were increased in extrahepatic cholestasis and dexamethasone decreased the increased sICAM-1 and bilirubin values.

References

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