The histopathological effects of different treatment models on liver tissue of mice pretreated by cyclophosphamide and steroid in E.coli sepsis

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The incidence of gram-negative bacteremia is significantly increasing in recent years by the wide-spread use of cytotoxic and immunosupressive drugs. Although the effective antimicrobial drugs are being used in treatment, the mortality rate is still high. In this study, we searched for the histopathological changes occuring on liver tissue of immunosupressed mice in E.coli sepsis and their severity in different treatment models.

Statistically, there was no difference between the ratios of histopathological changes observed in experimental and treatment subgroups except Kupffer cell hyperplasia and inflammatory infiltration. Consequently, we concluded that the endothelial call damage caused by endoxin shows itself histopathologically on liver tissue whether the effective therapeutic agents are used or not. [Turk J Med Res 1992; 10(6):310-313]

Key Words: E.coli, Sepsis, Immunosupression, Ceftriaxone, lgG

The incidence of gram-negative bacteremia is significantly increasing in recent years (1,2). Many factors contribute to this growing clinical problem such as the wide-spread use of cytotoxic and immunosupressive drugs, and long term survival of patients with chronic diseases characterised by depressed immunity. Also the infections are difficult to eradicate because of microbial drug resistance (1,3-5).

In the effort to control the local disease, administration of antimicrobial agents supresses other drug sensitive organisms, leaving the résistent gram negatives to proliferate and spread into the blood. Although the effective antimicrobial drugs are being used in treatment and proflaxia of gram-negative bacteremia, the mortality rate is still 20-30% and increases to 50-80% if septic shock develops (1,5-8). Among the risk groups, the highest mortality rate is found in immunosupressive drug users (9).

Various therapeutic agents such as monoclonal antibodies against endotoxin, streoid and naloxen have been administrated to the patients, because the antibiotics haven't reduced the mortality rate as might be expected (1,2,6,8,10-19). None of them altered the metabolic and haematologic effects of endotoxin occuring in the body due to the hepatic damage. We planned this study in order to define the histopathological changes in liver of bacteremia and the effects of immunosupression on liver histology.

Geliş Tarihi: 14.8.1992 **Kabul Tarihi:** 10.10.1992

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MATERIALS AND METHODS

In this study, pathogen-free, albino mice weighing 20-25 gr were used. First of all, a total of 120 animals were divided into three groups (Group A,B,C) each having 40 mice (Table 1).

Then the animals in group A received methilprednisolone 16 mg/kg intraperitoneally (IP) and the ones in group B received cyclophosphamide 300 mg/kg IP. No immunosupression had been applied to the animals in group C. Mice of three experimental groups received a bolus of live E.coli (4x10) suspended in 1 ml of 0.09 NaCI with or without additional treatment. Then, the experimental groups of A, B, and C were divided into smaller treatment subgroups each having 10 mice. In each group, mice of first three treatment subgroups received ceftriaxone 200 mg/kg/24 hr, lgG 4 mg, and both ceftriaxone+lgG IP, respectively. The remaining four th group didn't receive any treatment.

In each group, the animals were killed by cervical dislocation after 7 days and the liver tissues were removed immediately. The gross changes were noted. Then the liver samples of 0.5 cm^3 was seperated for frozen section. The remaining tissue was stained with Hematoxylene & Eosin (HE), periodic acid Schiff (PAS), and Gomori's silver impregnation for reticulin.

Table 1. Distribution of mice among experimental groups and treatment subgroups

Treatment	Group A	Group B	Group C
Ceftriaxone	10	10	10
IgG	10	10	10
Ceftriaxone+IgG	10	10	10
No Treatment	10	10	10
Total	40	40	40

RESULTS

The mortality rate of animals in groups A, B and C is shown in Table 2. The lowest mortality rate was determined in mice receiving both ceftriaxone and IgG of all three groups. The difference between the groups A, B and C was not statistically significant (p>0.05).

Macroscopically, multiple cysts were seen in liver of 8 mice. In light microscopic examination, 4 of them were found to be abscess formation and the remaining four were parasitic cysts. Four of the eight liver tissues having multiple cysts were found in mice of group B receiving IgG (2 abscess, 2 parasitic cysts). Two parasitic cysts were found in mice of group B receiving ceftriaxone+IgG and two abscess were found in mice of group C receiving IgG. The parasite seen cysts was Cystiercus fasciolaris. Changes evident in the histology of liver tissue included congestion, proliferated and swollen Kupffer cells, inflammatory infiltration of portal areas, necrosis, degeneration of hepatocytes and fatty change (Table 3,4,5).

Congestion was observed in almost all experimental animals. This morphologic change was seen in 38 (95%), 36(90%) and 40(100%) mice of groups of A, B and C, respectively. The difference between the groups was not statistically significant (p>0.05).

Proliferated and swollen Kupffer cells were more prominent in group C than the groups A and B. They were seen in 70% of mice in group A and 87.5% of group B while 100% in group C.

Histopathological sections of liver tissues of groups A, B and C revealed inflammatory infiltraiton of portal areas in 70%, 57.5% and 82.5% of mice, respectively. This infiltration was mainly composed of lymphocytes and scattered plasma cells and polymorphonuclear leukocytes (PMNLs). It was found that the ratio of inflammatory infiltration in group B was statistically decreased when compared with group C (P<0,05).

Three types of necrosis were observed in animals. Those were single cell necrosis, focal and confluent necrosis. The percentage of necrosis, whatever the type, was found as 62.5%, 72.5% and 67.5% •n groups A, B and C, respectively. Most of them were focal hepatocellular necrosis, which all were located in midzonal area. In 18 of mice, two types of necrosis were observed at the same time. Twelve of them were focal and confluent necrosis, five of them were focal and single cell necrosis, and one was single cell and confluent necrosis. Only confluent necrosis was seen in 9 mice, and only single cell necrosis was seen in 6. The difference between the experimental groups and treatment subgroups were not statistically significant (p>0.05).

Table 2. The mortality rate of animals

Treatment	Group A	Group B	Group C
Ceftriaxone	6/10	6/10	5/10
IgG	7/10	10/10	8/10
Ceftriaxone+IgG	3/10	3/10	3/10
No Treatment	9/10	10/10	9/10
Total	25/40	29/40	25/40

Table 3. The ratio of histopathological changes in liver of mice pretreated by steroid

Group A (Steroid)	Cef.	IgG	Cef+IgG	NoTr	Total
Congestion	7	9	10	10	36
Pro. Kupffer Cells	6	6	7	9	28
Inflammation	6	5	3	9	23
Fatty change	1	4	0	• (2-:.;	• 7-!ä
Degeneration	4	7	2	5	18
Necrosis	8	7	4	10	29

Table 4. The ratio of histopathological changes in liver of mice pretreated by cyclophosphamide

Group B (Cyclop.)	Cef.	IgG	Cef+IgG	NoTr	Total
Congestion	10	9	10	9	38
Pro.Kupffer Cells	8	10	10	7	35
Inflammation	6	7	10	5	28
Fatty change	1	2	0	2	5
Degeneration	3	4	2	4	13
Necrosis	6	4	8	7	25

Table 5. The ratio of histopathological changes in liver of mice with no immunosupression

Group C	Cef.	IgG	Cef+IgG	NoTr	Total
Congestion	10	10	10	10	40
Pro.Kupffer Cells	10	10	10	10	40
Inflammation	-fi	9	9	8	33
Fatty change	0	0	0	0	0
Degeneration	4	5	2	1	12
Necrosis	8	7	8	4	27

The difference between the ratios of degenerative changes seen in hepatocytes, such as cellular swelling and vacuolar degeneration in experimental groups and treatment subgroups were not statistically significant ($p>0.05$).

Fatty change of hepatocytes was observed in 5 (12.5%) of group A, and in 7 (17.5%) of group B. There was no fatty change in group C. The difference between the immunosupressive groups and group C was statistically significant ($p<0.05$).

DISCUSSION

The hight mortality rate in gram-negative bacteremia directed the interest of researchers towards the physiopathological mechanisms and the histopathological changes in different organs. The most studied organ is liver, because the vascular reactions caused by endotoxin couldn't be observed on lung, spleen or renal parenchyma. From this point of view, it is concluded that hepatic vascular changes are the main responsibles of metabolic and heamathologic alterations observed in gram negative bacteremia (8,20-23).

In this study, we searched for the histopathological changes which are the indicators of hepatocellular dysfunction as well as the changes indicating mononuclear phagocyte system (MPS) deficiency.

The decrease in the ratio of Kupffer cell proliferation and inflammatory infiltration in groups A and B comparing group C is an expected results of immunosupression. In addition, we also know that the phagocytic activity of MPS is impaired by a reduction in hepatic blood flow (24). So, the process of disseminated intravascular coagulaiton in gram-negative sepsis leading to liver cell necrosis also inflences the phagocytic activity of mononuclear cells.

The fatty change noted in our animals may be attributable to one or more causes: mobilization of fatty acids from body stores as a result of adrenal cortex stimulation by endotoxin, or decreased synthesis of apolipoproteins in hepatocytes (23,25). One of the interesting findings in our study is the absence of fatty change in nonimmunosupressive groups. However it is concluded that the metabolic derangements leading to fatty change in gram negative sepsis do not always affect hepatocytes, because the highest ratio of fatty change, which is observed in group B (%12.5), can be accepted as low in reality.

Although it is generally accepted that endotoxin inhibits oxidative metabolism, the pattern of damage present in the liver of animals is not typical of hypoxic hepatic injury as it is seen in man, ie, centrilobular (23). Instead, hepatocytes most severely affected were in the mid-zonal region.

In our study, the percentage of necrosis, whatever the type, was found to be as %62.5, %72. 5 and %67.5 in groups A, B and C, respectively. Almost all of them were focal hepatocellular necrosis located in mid-zonal area. Additionally, the degenerative changes in hepatocytes were most prominent in mid-zonal areas.

In order to explain the mid-zonal localization of hepatocellular changes, Balis et al suggest that the phagocytosis of endotoxin by leukocytes in hepatic sinusoids leads to extensive microtrombosis. This microcirculatory failure progresses to hepatocellular necrosis primarily affecting the mid-zonal regions of liver (20,21). This microcirculatory failure is also associated with activation of both coagulative and fibrinolytic pathways leaeding to progression of DIC (21). Moreover, some investigators have showed that endogenous mediators are the responsibles of tissue injury in gram negative sepsis, not the endotoxin itself (26,27). It is appearent that, tumor necrosis factor (TNF) is the main mediator which triggers the inflammatory events (28). TNF is synthesized by macrophages, lymphocytes, astrocytes, microglial cells and Kupffer cells. These cells are activated by various inflammatory stimulae triggering TNF biosynthesis. One of these inflammatory stimulae is bacterial endotoxin. In response to endotoxin, large amounts of TNF are released within minutes. After intravenous (IV) administration of endotoxin in man, TNF levels peak within two hours (28). A similiar observation by Hesse et al. indicates that, after IV administration of live E.coli in baboons TNF levels peak within 90 minutes to 20500 pgr/ml (27). Larger quantities of endotoxin stimulate much higher TNF concentrations that trigger tissue injury (28).

Tracey at al. also showed that septic shock can be prevented by administration of monoclonal anti-TNF antibody two hours before infusion of a lethal intraeenous dose of live E.coli (28).

Administration of TNF produces lethal tissue injury that is almost identical to fatal endotoxic or septic shock syndrome (26,29). TNF-induced tissue injury is partly mediated by enhanced endothelial procoagulant activity that promote intravascular coagulation and capillary trombosis (28).

In our study, we couldn't observe significant difference between the ratios of hepatocellular changes such as necrosis and degeneration both in experimental groups and treatment subgroups. In other words, neither immunosupression nor different treatment models affected the severity of tissue injury on liver tissue. This conclusion confirms that the effect of an endogenous mediator, probable TNF, on leukocytes and endothelial cells can not be reduced after the cells have been exposed to endotoxin and the endothelial cell damage caused by endotoxin in acute-stage of gram negative bacteremia shows itself histopathologically on liver tissue whether affective therapeutic agents are used or not.

Siklofosfamid ve steroid verilen farelerde oluşturulan deneysel E.Coli sepsisinde uygulanan farklı tedavilerin karaciçpr histopatolojisi üzerine etkileri

Son yıllarda sitotoksik ve immunosüpresif ilaçların yaygın olarak kullanılmasıyla birlikte gram-negatif bakteriyemi insidansında anlamlı bir artış gözlenmektedir. Tedavide etkili antimikrobiyal Haçlar kullanılmasına karşın mortalité oranı hala yüksektir. Bu çalışmada, immunsüpresyon uygulanmış farelerdeE.coli sepsisinde karaciğerde ortaya çıkan histopatolojik değişiklikler ve uygulanan farklı tedavi modellerinin bunlara etkisini araştırdık.

Histopatolojik değişikliklerin görülme oranları yönünden deney ve tedavi alt grupları arasında istatistiksel bir karşılaştırma yapıldığında Kupffer hücre hiperplazisi ve iltihabı infiltrasyon dışında anlamlı bir farklılık bulunmadı. Sonuç olarak, etkili terapötik ajanlar kullanılsın ya da kullanılmasın endotoksinin neden olduğu endotel harabiyeti karaciğer dokusunda histopatolojik olarak kendisini gösterir. [Türk Tıp Araştırma 1992; 10(6):310-313]

Anahtar Kelimeler:E.coli, Sepsis, immunosüpresyon, Seftriakson, lgG

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