The Effect of Dimethyl Sulphoxide (DMSO) After Reperfusion in Liver Ischemia
(An Electron Microscopical Study)

KARACİĞER İSKEMİ-REPERFÜZYONUNDA DIMETHYL SÜLPHOXIDE'İN (DMSO) ETKİSİ (Elektron Mikroskobik Çalışma)

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Summary

Ischemia is one of the important causes of liver cell injury and it was known that the free oxygen radicals play very effective role in this type of injury. We examined the effect of Dimethyl Sulphoxide (DMSO) after reperfusion in liver ischemia that was produced on rat liver cells in this study.

Fourty rats were divided in two groups as experiment (group A1, A2) and control (group B1, B2) groups. After a laparatomy was performed on , a 1 gr/kg dose of 5% DMSO was administered to groups A1 and A2. In order to cause ischemia portal veins, hepatic artery and bile ducts were clamped for an hour. The animals were sacrificed either on the 2nd (groups A1 and B1) or on the 14th (groups A2 and B2) days and livers were taken out as a whole for pathological examination. Blood samples were drawn to determine the serum levels of SGOT, SGPT, LDH, and AP.

Only SGOT values in group A1 were significantly lower than that of group B1 ($p<0.05$) whereas SGPT,LDH and AP values in group A2 were significantly lower than that of group B2 ($p<0.05$). Among the other blood parameters no significant difference was found.

The tissue samples were examined under electron microscopy. Control group revealed mostly edema in hepatocyte mitochondria; clarification of matrices with homogenization of some; fragmentation and/or loss of cristae; all with reservation of outer membrane. Endoplasmic reticulum (ER) were dilated and ribosomes decreased in number. Experimental group revealed mostly normal hepatocyte mitochondria and other organelles. Ribosomes increased in number.

It was seen that DMSO decreases the effects of ischemia in the liver.

Key Words: Liver; Ischemia, Dimethyl Sulphoxide

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Özet

İskemi karaciğer hücre harabiyeti yapan önemli nedenlerden biri olup, bu tür zedelenmelerde serbest oksijen radikallerinin önemli rol oynadıkları bilinmektedir. Biz bu çalışmada sıçan karaciğer hücresinde oluşturulan iskemi reperfüzyon sonrasında Dimetil Sülfoksid (DMSO)'nun etkisini inceledik.

Kırk sıçan çalışma (grup A1, A2) ve kontrol (grup B1, B2) grubu olarak iki gruba ayrıldı. Laparotomi yapıldıktan sonra A1 ve A2 gruplarına 1gr/kg dozunda %5 DMSO uygulandı. İskemi oluşturmak için portal ven, hepatik arter ve ortak safra kanalı 1 saat süreyle klemplendi.

Hayvanlar 2. (grup A1, B1) ve 14. (grup A2, B2) günlerde öldürüldüler. Patolojik inceleme için karaciğerleri bir bütün olarak çıkarıldı. Serum SGOT, SGPT, LDH, AP seviyelerini tesbit etmek için kan örnekleri alındı.

A1 grubunda sadece SGOT değerleri, B1 grubundaki değerlerden belirgin olarak düşük iken ($P<0.05$), A2 grubunda SGPT, LDH, AP değerleri B2 grubundakilere göre belirgin olarak düşük bulunmuştur ($P<0.05$). Diğer kan parametreleri arasında bir farklılık bulunmamıştır.

Doku örnekleri elektron mikroskobunda incelendi. Kontrol grubunda çoğunlukla hepatosit mitokondrisinde ödem, bazılarında homojenizasyonla birlikte matriks klarifikasyonu, krista fregmantasyonu ve/veya kaybı; hepsinde dış membranın korunduğu tesbit edildi.

Endoplazmik retikulumun (ER) genişlediği ve ribozomların sayısının azaldığı, deney grubunda çoğunlukla hepatosit mitokondrisi ve diğer organellerin normal olduğu görüldü. Ribozomların sayısında artma tesbit edildi.

DMSO'nun karaciğer iskemisine bağlı değişiklikleri azalttığı görülmüştür.

Anahtar Kelimeler: Karaciğer, İskemi, DMSO

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Prolonged severe ischemia may be the only reason for tissue death (1). Upon reperfusion of a previously ischemic tissue, a specific injury may result. It is shown that the free radicals which come

out after ischemia-reperfusion have an important role in tissue injury (2,3). This effect of free radicals is shown by peroxidation of lipids and nucleic acids (4,5).Various agents are used to protect cells in case of ischemia as Allopurinol, Deforoxamine, Superoxide Dismutase, Calcium Channel Blockers (as nifedipine, verapamil), active carbon hemoperfusion, etc. (6-12,). The only important reason of injury in liver tissue is ischemia, and some anions (like hydroxyl, hydrogen peroxide, superoxide) appear during the reperfusion (13-15).

In this study; the chemical and histopathological changes, and the effect of DMSO on tissue changes were evaluated after ischemia-reperfusion injury.

Materials and Methods

In this study 40 Wistar albino female rats (200+20gm) were used. The rats were divided into 4 groups as follows:

Group A : Experiment groups A1 : 48 hours A2 : 14 days
Group B : Control groups B1 : 48 hours B2 : 14 days

The rats were left hungry for 12 hours before the surgical procedure was begun. During anesthesia Ketamin (10mg/kg) intramusculary was used for induction and the anestesia was continued by ether.

The animals were laid down on wood stand and abdominal skin was shaved after cleaning with povidone iodine. The abdominal cavity was reached by median incision. The hilus of liver was exposed. The solution of 5% DMSO (1 gr/kg) was injected into the inferior vena cava by using an insulin syringe, 5 minutes before blocking blood flow in the experimental groups (A1,A2). The portal vein, the hepatic artery and the bile ducts going into left, and median lobe of the liver, have been clamped for an hour (16).After an hour, reperfusion was initiated by taking the clamp out.

Animals were sacrificed at the end of the 2nd and 14th day according to their groups. Blood samples were drawn from the heart using 21 gauges syringe. Serum gluthamic pyruvic transaminase (SGPT), serum gluthamic oxaloasetic transaminase (SGOT), alkalen phosphatase (AP) and lactic dehydrogenase (LDH) values were determined in the samples. The livers were taken out and were evaluated histopathologically.

The fresh tissue samples were taken from 12 rats (6 rats from group A1 and 6 rats from group B1). Prefixation and postfixation procedures were obtained by 2.5% phosphate buffered gluteraldehyde and 1% osmic acid, respectively. The specimens were embedded in Epon 812 after dehydration. The cross sections were contrasted by uranylacetate and Reynold's lead stain and were examined under JEM-100B TEM.

The average values and standart deviation of the biochemical results of each group were calculated. Mann-Whitney U test was used for the significance of difference between control and experiment groups. When p is smaller than 0.05 it was agreed to show a statistically significant difference.

Results

Biochemical results: The results of biochemical analysis were shown on Table 1. The difference between the concentration of SGOT in group A1 and group B1 was significant statistically ($p<0.05$). Among the values of SGPT, AP and LDH no significant differences were found between two groups (A1 and B1). In blood samples of the group A2 and B2 which were sacrificed at the end of the 14 th day, SGPT concentrations were found different significantly ($p<0.05$). Especially, the difference between the values of AP and LDH in the groups of A2 and B2 were significant statistically ($p<0.05$). There was no statistically significant dif-

Table 1. The results of biochemical analysis

Days	Group	SGOT (IU/1)	SGPT (IU1)	AP (IU/1)	LDH (IU/1)
2nd	A1	303 ± 60	166 ± 50	233 ± 42	2162 ± 619
day	B1	362 ± 51	194 ± 109	257 ± 70	2652 ± 672
14th	A2	234 ± 80	82 ± 13	71 ± 13	1194 ± 263
day	B2	246 ± 52	98 ± 9	332 ± 67	1606 ± 230

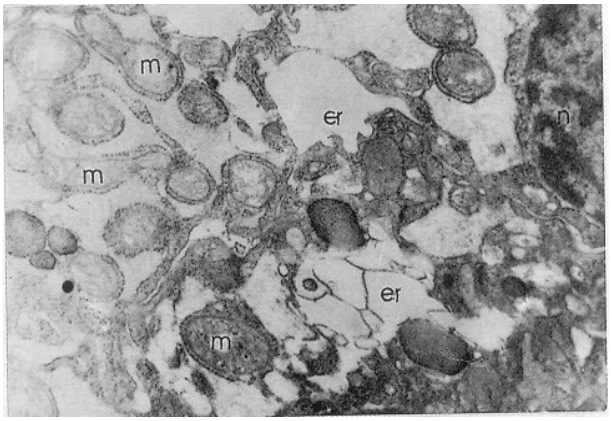

Figure 1. Control group (Ischemia) in hepatocytoplasma:
(m) Edema in mitochondria, widened, outer membrane spaces, clarification of some matrices, homogenization of some, destruction of cristae.
(er) Prominent dilation of the Endoplasmic reticulum, destruction of the canals (distrophic changes), loss of ribosomes.
(n) Nuclear roughness is seen
X 12000

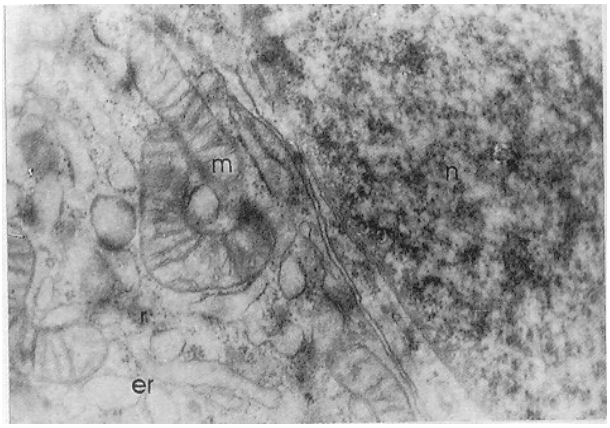

Figure 2. Experimental group (Ischemia + DMSO) in hepatocytoplasma
(m) Normal mitochondria
(er) Wide, non destructed endoplasmic reticulum
(r) Free ribosomes
(n) Increase of chromatin in nucleus is seen
X 18000

ference between the SGOT values in these groups, on the other hand.

Histopathological Changes

Electron microscopical evaluation of the slides of the control group revealed: mostly edema in hepatocyte mitochondria; clarification of matrices with homogenization of some; fragmentation and/or loss of cristae; all with reservation of outer membrane. Endoplasmic reticulum (ER) were dilated and ribosomes decreased in number (Figure-1). Besides these reversible changes some irreversible changes such as further destruction of the organelles were seen.

Electron microscopical evaluation of the slides of the experimental group revealed mostly normal hepatocyte mitochondria, ER and other organelles. Ribosomes revealed increase in number (Figure-2). İrreversible changes were not seen.

Discussion

The liver ischemia may occur during organ injuries, transplantations and cancers. It's a well known phenomen that insufficiency of liver happens after hemodynamics or cardiogenic shock. The increase in the number of liver transplantation cases brings that liver should be protected for longer time within invitro conditions (8,12).

Liver cells resist to ischemia at least for 30 to 60 minutes. Longer ischemia results in cell death (17). After 60 minutes of ischemia, in the 5 th minute the free oxygen radicals.begin to increase and in the 15 th minute they reach maximum level (2). For this reason radical scavengers should be given before ischemia or before the beginning of reperfusion. The importance of free oxygen radicals in cell injury is shown in small intestine, heart, kidney, brain and skeleton muscles (10). The decrease in the effect of free oxygen radicals is shown with xantine oxidase inhibiton in vital organs such as the heart and the kidneys in great amounts. Studies for protecting the liver was found less effective (9,11). Oxygen radical scavengers may have an important role in protecting liver cells (10,14,15). For this reason in many studies the effect of chemicals which prevent forming of the free oxygen radicals or clean free oxygen radicals to come out are studied for protecting liver cell. The DMSO protecting effect for acute lung injury is bound to its radical cleaning effect after thermal trauma on skin (16). DMSO has a cell protecting effect and on the other hand many pharmacological and therepeutic properties and antiinflamatory effects shown in animals and human beings. It's used in cerebral edema and increased intracranial pressure experimentally and clinically (16,19-21). When DMSO is used for cell protective effect (cry-

opreservative) with a 10 % or more concentration in invitro condition the rat hepatocytes survive longer (22).

Prevention of hepatotoxicity in rats by Acetaminophen (APAD) 250 mg/kg, DMSO has been found to be effective if it's given before and after 4 hours of APAD administration, but after 8 hours ıt was noneffective if measured by the quantity of SGPT activity. The mechanism of this protective effect is unknown. However free radicals form during APAD metabolism. The quantity of plasma transaminases (SGOT and SGPT) are lower than the control groups in a study using cyclosporine in rats (16,23).

DMSO (%2) increases the life period of human hepatocytes invitro. However the reason for this cannot be explained. After DMSO is given an increase in calcium ions is seen. It's thought that the increased calcium ion concentration may have role in longer hepatocyte survival invitro because intracelluler calcium is an important second messenger (21,24). The quantities of SGOT are found to be higher than the control group on the 2 nd, 21 th and 24 th days and has a statistically significant difference in a study where verapamil is used as a calcium channel bloker (18).

In our study when compared with the control group the serum SGOT quantities were lower on the 2 nd day indicating a statistical significance. On the 2 nd day the other parameters were of lower values in the experimental group but the difference was not statistically significant. On the 14 th day the values of LDH, SGPT, and AP were found to be lower with a statistically significance but the SGOT values showed no signifanct statistical difference These results suggest that liver cell injury were to a lower extent in the experiment group on the 2 nd and the 14 th day.

The ultrastructural changes due to ischemia reported in the literature are found in both two groups (25).

In control groups both the initiation of reversible changes and irreversible changes were seen macromolecularly because of hypoxemia in hepatocytes and stromal elements. Protection of mitochondria and other organelles of hepatocytes hardly shows that these are compensatory changes. As disorders; in mitochondria showed a decrease in functional synthesis of Adenosine Triphosphate, smooth ER showed insufficiency of transport and cell membrane pore mechanisms and, rough ER showed a decrease in protein synthesis.

The changes due to ischemia in the experimental group effected by DMSO were less irreversible and less severe than the control groups. Although destructive changes on all organelles in small amount could be seen in these groups, they were less prominent than the control group. The great amount of the organelles without a destructive change showed that there was an increase in compensatory reactions. On the contrary in this group protection of granules of glycogen was bounded to DMSO's positive effect and for this reason ischemia stopped in a reversible step. That no edema in stromal elements, either no destruction or disorder was observed indicated that the metabolic functions were normal in liver tissue. Hydrolase activity increases during ischemia (26). In control group the changes seen in Iysosomes prove this fact. In experimental group there was an increase in primary Iysosomes and decrease in other Iysosomes indicating that the activity of hydrolases was at normal levels.

As a result; DMSO decreases the effect of ischemia as shown electron microscopically.

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