Methylene blue usage in the evaluation of traumatic necrosis in a rabbit liver injury model

Tavşan modeli üzerinde deneysel olarak gerçekleştirilen karaciğer hasarındaki travmatik nekrozun değerlendirilmesinde metilen mavisi kullanımı

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BACKGROUND
In this study, the efficacy of methylene blue in the differentiation of devascularized and vital areas in a traumatic liver model in rabbits was investigated.

METHODS
Twenty healthy rabbits were selected and randomly divided into two equal groups. Surgery was performed through an upper abdominal mid-line incision 5 cm in length. After induction of liver injury, diluted methylene blue was injected through the portal vein and staining onset and discoloration times were recorded.

RESULTS
All 20 rabbits survived until the end of the experiment. In cases, non-perfused areas of the liver remained unstained. In control rabbits, liver parenchyma was stained homogeneously. The mean onset time of staining in normal parenchyma was 18.1±1.3 sec in cases and 17.7±1.4 sec in controls (p=NS). The mean discoloration time was 53.1±3.7 sec in cases and 53.1±3.2 sec in controls (p=NS).

CONCLUSION
In major liver injuries requiring surgical intervention, methylene blue injection can be used for detection of non-perfused areas in the liver.

Key Words: Liver necrosis; methylene blue; trauma.

AMAÇ
Bu çalışmada, metilen mavisinin travmatik tavşan karaciğerinin vital ve devaskülarize alanlarının ayırımı konusundaki etkisi araştırıldı.

GEREÇ VE YÖNTEM
Yirmi adet sağlıklı tavşan rastgele iki eşit gruba ayrıldı. Cerrahi işlem, karın üst bölgede orta hattan gerçekleştirilen 5 cm uzunluğunda bir insizyonla uygulandı. Karaciğer hasarı oluşturulduktan sonra, portal ven yoluyla dilüe edilmiş metilen mavisi enjekte edildi ve boyanmaya başlanma zamanları ile boyanın kaybolduğu zamanlar kaydedildi.

BULGULAR
Yirmi tavşanın hepsi de deney bitimine kadar yaşadı. Deneklerde karaciğerin perfüze olmayan alanları boyanmadı. Kontrol tavşanlarında, karaciğer parankimi homojen olarak boyandı. Normal parankimdeki ortalama boyanmaya başlama zamanı, deneklerde 18,1±1,3 sn olurken kontrollerde de 17,7±1,4 sn oldu (p=AD). Ortalama diskolorasyon zamanı, deneklerde 53,1±3,7 sn ve kontrollerde de 53,1±3,2 sn oldu (p=AD).

SONUÇ
Cerrahi girişim gerektiren majör karaciğer yaralanmalarında, karaciğerin perfüze olmayan alanlarının saptanması için metilen mavisi enjeksiyonu kullanılabilir.

Anahtar Sözcükler: Karaciğer nekrozu; metilen mavisi; travma.

The spleen and liver are the organs most commonly injured in blunt abdominal trauma, with each accounting for one-third of the injuries. Injury to the liver is a commonly encountered problem in trauma and a frequent cause of morbidity and mortality. Mortality of grade IV and V injuries has been estimated to range between 35% and 80%.[1,2] The initial hemodynamic status of patients is the main determining factor for treatment. Nonoperative treatment of isolated hepatic injury in stable patients is now

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standard practice.[3-5] The management of unstable patients with liver injury is a multifaceted challenge requiring various techniques and remains a controversial issue.[6-8] Surgical procedures range from packing, to anatomic liver resection, to total hepatectomy with liver transplantation.[9-12]

Liver necrosis resulting from devascularization injuries is an uncommon but life-threatening condition that has been diagnosed either intraoperatively or at autopsy.[13-15] Devascularization injuries to the liver occur when there is disruption of vascular inflow to one or more hepatic segments.[3] In some studies, computed tomography (CT) scanning and magnetic resonance imaging (MRI) were used,[4-12] but at this time, there is no consensus on the precise detection of non-perfused areas in the liver intraoperatively to help in decision-making. The main objective of this study was the detection of non-perfused areas following liver injury in a rabbit model using methylene blue (MB) dye.

MATERIALS AND METHODS

The local research ethics committee approved the study. The experiments were started using 20 New Zealand white rabbits (10 cases, 10 controls) weighing 3.5±1.2 kg. The rabbits were housed in a controlled environment with an ambient room temperature of 24°C and artificial illumination (12-hour light-dark cycle) for at least two days before starting the experiment. They were fasted for four hours before the experiment, but had free access to tap water. General anesthesia was induced with ketamine (35 mg/kg, intramuscularly) and acepromazine (1.2 mg/kg, intramuscularly) and nitrogen oxide/oxygen (50%:50%) plus halothane 3%-4% by means of a cone mask. After endotracheal intubation, halothane concentration was fixed at 1.5%. Lactated Ringer's solution was administered by means of peripheral intravenous access at 2 ml/kg/hr during the operation. The rabbits were placed in the supine position throughout the experiment.

After preparation was made, lidocaine 2% (1 ml/cm^3) was injected in the skin of the abdomen. Surgery was performed through an upper abdominal mid-line incision 5 cm in length and the liver was exposed. To induce liver injury in cases imitating high-grade injury according to the Liver Injury Scale of the American Association for the Surgery of Trauma, we used a pair of long pliers with blades measuring 4.5 cm in length. After inflicting injury, the portal vein was explored and a disposable sterile 22G catheter was inserted through the vessel into the proximal vein toward the liver for 3-4 cm. Diluted 2% MB solution (urolene blue 2%, Lexi, USA) 1.5 mg/kg (0.2 ml in 2 ml of normal saline) was injected through the catheter. After MB injection, the stained area of the liver was assessed. Staining onset and discoloration times were also recorded. Then catheter was removed and a 1-2 cm^3 liver sample was taken from the unstained area at the end of procedure and was fixed with 4% buffered formaldehyde for pathological examination. In terms of hemostasis, in the presence of uncontrollable bleeding from borders of the injured site, we repaired the liver injury borders by simple sutures and then the abdomen was closed. In the postoperative period, if the general condition of the rabbit was stationary, we re-operated the animal to control the bleeding. These processes between the production of the liver injury and the accomplishment of abdominal closure were all completed within 30 minutes. For laboratory analysis, blood samples were taken from a peripheral vein preoperatively and 48 hours postoperative for comparison of serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, creatinine and urea. Methemoglobinemia was assessed by spectrophotometer method.

All data in the text and tables are presented as mean±SD. Within groups, comparisons of continuous variables were performed using the Wilcoxon test. The nonparametric Mann-Whitney test was used to compare variables between groups. Statistical analyses were performed using SPSS 11 software. The level of statistical significance was set at $p<0.05$

RESULTS

The groups were similar in age and sex (male). There were 10 rabbits with liver injury in the case group and 10 rabbits in the control group. All 20 rabbits survived until the end of the experiment. The mean body weight of rabbits was 3.5±1.2 kg. There was no significant difference between the groups regarding the body weight.

Liver staining data

As shown in Figure 1a, in cases, non-perfused areas of the liver remained unstained (pink areas), but the color of the right liver lobe changed from purple to dark blue. In control rabbits, liver parenchyma was stained homogeneously (Fig. 1b).

Table 1. Staining onset and discoloration times in control rabbits

Rabbit No.	Staining onset time	Discoloration time
1	17	50
2	16	49
3	18	56
4	20	59
5	19	57
6	16	54
7	19	55
8	18	51
9	16	52
10	17	53

Table 2. Preoperative liver and renal function tests in groups

	Cases	Controls	p
ALT (IU/L)	38.1±8.2	37.9±8.5	NS
AST (IU/L)	47.2±6.3	49.1±5.8	NS
Bilirubin (µmol/L)	0.63±0.31	0.65±0.28	NS
Urea (mmol/L)	7.3±1.63	7.1±0.98	NS
Creatinine (µmol/L)	134.1±14.2	138±12.3	NS

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NS: Not significant.

Table 1 shows staining onset and discoloration times in controls. The mean onset time of staining in normal parenchyma was 18.1±1.3 sec in cases and 17.7±1.4 sec in controls (p=NS). The mean discoloration time was 53.1±3.7 sec in cases and 53.1±3.2 sec in controls (p=NS).

Biochemical data

Preoperative liver and renal function tests did not differ between groups (Table 2). In Table 3, biochemical data in the case group were compared pre- and postoperatively. ALT and AST concentrations significantly increased postoperatively but renal function test showed no significant differences. In comparison of the postoperative biochemical data between groups (Table 4), ALT and AST concentrations had significantly increased.

Our findings showed no methemoglobinemia in the groups in postoperative analysis. In evaluation of the biopsy specimens, hypoperfusion was also histologically proven.

DISCUSSION

Despite its relatively protected location, the liver is the most frequently injured intraabdominal organ, although splenic injuries are more common following blunt abdominal trauma.[3]

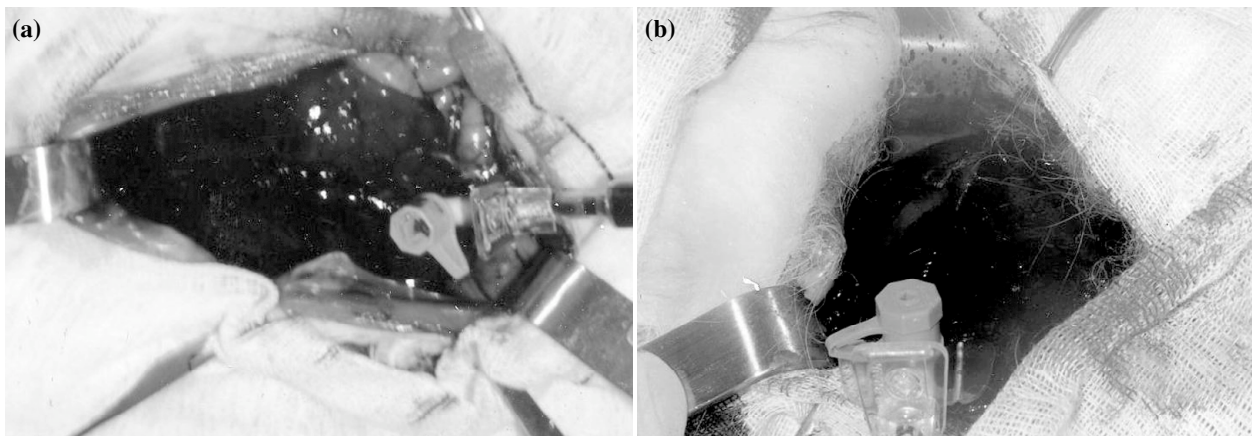

Fig. 1. **(a)** Methylene blue staining in a case rabbit. **(b)** Methylene blue staining in a control rabbit.

Table 3. Pre and postoperative liver and renal function tests in the case group

	Cases	Controls	p
ALT (IU/L)	38.1± 8.2	64.76±13.4	0.02
AST (IU/L)	47.2± 6.3	97.1±32.1	0.03
Bilirubin (µmol/L)	0.63±0.31	0.65±0.28	NS
Urea (mmol/L)	7.3±1.63	7.1±0.98	NS
Creatinine (µmol/L)	134.1±14.2	138±12.3	NS

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NS: Not significant.

Table 4. Postoperative liver and renal function tests between groups

	Cases	Controls	p
ALT (IU/L)	64.76±13.4	39.3±6.1	0.02
AST (IU/L)	97.1±32.1	49.7±6.8	0.02
Bilirubin (µmol/L)	0.63±0.31	0.65±0.28	NS
Urea (mmol/L)	7.3±1.63	7.1±0.98	NS
Creatinine (µmol/L)	134.1±14.2	138±12.3	NS

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NS: Not significant.

Associated injuries to other organs, uncontrolled hemorrhage and septic complications contribute significantly to morbidity and mortality. Although mortality rates from liver trauma have reduced over the past few decades, the surgical management of the liver remains a challenging problem for surgeons.[6-8]

In 1966, Mays[16] recognized hepatic necrosis as a potential source of mortality in bursting injuries of the liver. In his report, 22 of 24 patients died. Smadja et al.[17] reported on seven patients with major liver injury initially managed at a referral hospital. Three of the seven patients died after resection. The author concluded that patients with extensive liver necrosis should be brought for definitive surgical resection before the development of secondary complications. Several other authors studied the effect of liver necrosis on mortality,[7,18-20] but only a few reports have studied its definitive diagnosis.

Hamill et al.[12] showed that MRI of extensive hepatic necrosis in the cardiovascularly stable trauma patient provides compelling evidence in favor of hepatic resection.

Anderson et al.[2] proposed a diagnostic and treatment algorithm. In their algorithm, the hemodynamically stable patient would have augmented CT scans obtained on admission. If significant parenchymal disruption was determined, the CT scan would be repeated in 48 hours. If a devascularization injury was confirmed and there was a worsening or no improvement in the clinical situation, the patient would undergo resection after appropriate preparation. However, the extent of the liver necrosis could not be assessed definitively during operation and therefore surgeons would overestimate the boundaries of necrosis and extend the liver resection.

Although initially ischemic areas may recover by developing collateral portal and arterial circulation, this is a time-consuming process and the surgeon can not rely on this process for initial liver resection intraoperatively.

Our study demonstrates that it is possible and safe to inject MB into the liver intraoperatively. It also demonstrates that MB is retained in the viable liver parenchyma and demarcates its necrotic areas. MB is an antidote in the therapeutic category for cyanide poisoning and drug-induced methemoglobinemia.[21] It is also a genitourinary antiseptic. It has been used as a vasopressor in sepsis and acute liver failure. Koelzow et al.[22] reported the effect of MB on the hemodynamic changes during ischemia-reperfusion injury in orthotopic liver transplantation. In his study, no adverse effects were observed using MB. Although MB is a safe solution, it is contraindicated in history of hypersensitivity to MB, renal insufficiency or pregnancy. Its adverse reactions are hypertension, headache, and dizziness, staining of the skin, nausea and vomiting.[23-26]

To our knowledge, this is the first animal study

with severe liver injuries to investigate the benefit of a new method of necrosis assessment. None of the rabbits showed complications of MB injection. Increase in the postoperative ALT and AST concentrations in the case group was due to the traumatic injury to the liver. It is a safe method that rapidly demarcates necrotic areas of the liver parenchyma and helps surgeons in the decision-making process.

Although the liver parenchyma is perfused by the hepatic artery and portal vein, no perfusion occurred in this area since we occluded all sides of the injured site.

Therefore, we suggest that MB injection can be performed in patients with major liver injuries requiring surgical intervention in suspected cases of liver necrosis.

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