The Effect of Methylene Blue on the Hemodynamic Changes During Ischemia Reperfusion Injury in Orthotopic Liver Transplantation

Heike Koelzow, MD, FRCA*, Jacqueline A. Gedney, MRCP(UK), FRCA†, Janos Baumann, MD, EDICM*, Nicola J. Snook, FRCA*, and Mark C. Bellamy, MA, FRCA*

*St James's University Hospital, Beckett Street, Leeds; and †James Cook University Hospital, Middlesbrough, UK

After graft reperfusion in orthotopic liver transplantation (OLT), ischemia reperfusion syndrome (IRS) is characterized by persistent hypotension with a low systemic vascular resistance. Methylene blue (MB) has been used as a vasopressor in sepsis and acute liver failure. We investigated the effect of MB on IRS during OLT. Thirty-six patients undergoing elective OLT were randomized to receive either a bolus of MB 1.5 mg/kg before graft reperfusion, or normal saline (placebo). We recorded hemodynamic variables, postoperative liver function tests, and time to hospital discharge. Blood samples were analyzed for arterial lactate concentration, cyclic 3',5'-monophosphate, and plasma nitrite/nitrate concentrations. The MB group had higher mean arterial pressure ($P = 0.035$), higher cardiac index ($P = 0.04$), and less epinephrine requirement ($P = 0.02$). There was no difference in systemic vascular resistance or central venous pressure. Serum lactate levels were lower at 1 h after reperfusion in MB patients, suggesting better tissue perfusion ($P = 0.03$). In the presence of MB, there was a reduction in cyclic 3',5'-monophosphate ($P < 0.001$), but not plasma nitrites. Postoperative liver function tests and time to hospital discharge were the same in both groups. MB attenuated the hemodynamic changes of IRS in OLT acting via guanylate cyclase inhibition.

(Anesth Analg 2002;94:824–9)

During orthotopic liver transplantation (OLT), reperfusion of the implanted liver is associated with ischemia reperfusion syndrome (IRS). This syndrome is defined as a decrease in mean arterial blood pressure (MAP) of 30% occurring within 5 min of graft reperfusion and lasting at least 1 min (1). Hypotension associated with a low systemic vascular resistance (SVR) may persist for an hour or more. The severity of the IRS is thought to both result from and contribute to postoperative impaired liver function and primary graft failure (2).

The dye methylene blue (MB) has been used as a vasopressor in sepsis (3–5) and acute liver failure (6,7). When these conditions are associated with low MAP, a bolus of MB increases MAP by an increase in SVR and in some cases cardiac index (CI). Sepsis and IRS display hemodynamic and biochemical similarities, including patterns of increased proinflammatory cytokines (8). In sepsis, these changes are associated with excess nitric oxide (NO) production. MB is believed to act by inhibition of NO synthase, reducing NO production, and by inhibition of guanylate cyclase, the target enzyme for NO (9,10).

The aim of this study was to investigate the role of MB in hypotension after reperfusion during OLT and to evaluate possible modes of action.

Methods

The study was approved by the local research ethics committee and written informed consent was obtained from all subjects. Thirty-six consecutive patients scheduled for elective OLT were studied. All patients were anesthetized using an opioid-volatile anesthetic with veno-venous bypass according to our standard technique (11). Noninvasive monitoring was established and anesthesia induced with midazolam 0.1 mg/kg, alfentanil 0.1 mg/kg, and atracurium 0.6 mg/kg. After tracheal intubation, the lungs were

Financial support was provided by the St. James's Intensive Care Research Fund.

This work was presented in part at the Anaesthetic Research Society meeting at Aberdeen, Scotland, March 25, 1999 and at Plymouth, England, July 8, 1999.

Accepted for publication December 11, 2001.

Address correspondence and reprint requests to Dr. Mark C. Bellamy, Intensive Care Unit, St. James's University Hospital, Beckett St., Leeds LS9 7TF, UK. Address e-mail to mark@livertransplant.org.uk.

0003-2999/02

ventilated to normocapnia with desflurane in oxygen-enriched air. Anesthesia was supplemented with alfentanil at 0.05 $mg \cdot kg^{-1} \cdot h^{-1}$ and muscle relaxation facilitated with atracurium 0.5 $mg \cdot kg^{-1} \cdot h^{-1}$. After the induction of anesthesia, a triple lumen central venous catheter and mixed venous oximetric right-ventricular ejection fraction pulmonary artery flotation catheter were inserted into the right internal jugular vein, and an arterial catheter into the left radial artery. A 21F percutaneous cardiac bypass cannula was inserted in the right internal jugular vein and connected to a rapid infusion system (Level-1; Simms, Rockland, MA) and a second 21F bypass cannula was placed into the right femoral vein to allow veno-venous bypass. Dopamine (3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and 4% glucose in 0.18% saline (1 $mL \cdot kg^{-1} \cdot h^{-1}$) infusions were commenced.

A standard surgical technique was used (12), with an out-of-ice to reperfusion time less than 1 h in all cases. Reperfusion sequence involved removal of the upper cava clamp, portal vein reperfusion, removal of lower cava clamp, and subsequent hepatic arterial anastomosis. No immunosuppression was given before surgery. Methyl-prednisolone 1 g was given during the anhepatic phase.

Patients were randomized to receive a 1% MB solution 1.5 mg/kg or an equivalent volume of saline (placebo) 1 min before graft reperfusion. The dose was given immediately before reperfusion, the period of hemodynamic instability, because the drug has a rapid onset of action in sepsis. In the 5 min after reperfusion, epinephrine was administered to maintain a systolic blood pressure above 70 mm Hg. Thereafter, epinephrine was administered to maintain a systolic blood pressure above 100 mm Hg.

Hemodynamic variables were recorded during the dissection phase (phase I), 5 min before (phase II), and 5, 10, 30, and 60 min after graft reperfusion (phase III) (Baxter Explorer; American Edwards, Irvine CA). Blood samples were collected for lactate analysis at phase I, then 5 and 60 min after reperfusion (EML105; Radiometer Copenhagen, Denmark). Total epinephrine requirements were recorded. Postoperative liver function tests were measured on Days 3 and 7 and the time to hospital discharge was noted.

Blood samples were taken at baseline and 5 and 60 min after reperfusion for measurement of plasma nitrite and cyclic 3',5'-monophosphate (cGMP). Samples for cGMP and nitrite were collected in sterile tubes supplemented with EDTA (Vacutainer® tubes; Becton Dickinson, Rutherford, NJ), centrifuged at 3000*g* for 5 min. Samples for cGMP analysis were stored at −70°C and later analyzed by using an immunoassay technique (cGMP [low pH] Immunoassay; R & D Systems, Abingdon, England). Samples for plasma nitrite analysis were stored at −20°C. These samples were deproteinized before analysis by using 35% sulfosalicylic acid. The supernatant was assayed for nitrite and nitrate. Plasma nitrite and nitrate, the metabolic products of NO, were measured by conversion of nitrate to nitrite and the measurement of plasma nitrite concentration using a Griess reaction (13).

Based on our previous data, this study was powered at 80% to show a difference in MAP at the $P < 0.05$ level assuming a 20% treatment effect. Results were expressed as mean and standard error (SEM). Simple comparisons of continuous variables were made by using Student's *t*-tests. Two-way analysis of variance (ANOVA) was used to compare groups over time and repeated measures ANOVA for comparisons within groups over time. Where *post hoc* testing was applied, a Bonferroni correction was applied. Categorical variables were compared by using Fisher's exact test. Time to hospital discharge was assessed by using Kaplan-Meier analysis and the log-rank test.

Results

The groups were of similar age (MB 48.1 [2.7] yr versus Controls 46.8 [3.6] yr). There was a similar sex distribution (MB 11 males/9 females versus Controls 6 males/12 females; $P = 0.3$). There were 15 patients with cirrhosis in the MB group and 11 patients with cirrhosis in the Control group ($P = 0.3$). No patient died intraoperatively. There were 8 deaths in the first 30 days, 5 in MB and 3 in the Control group ($P = 0.69$). Preoperative liver function tests did not differ between groups (Table 1).

Hemodynamic Data

Before drug administration, the groups were hemodynamically similar. The MB group had higher MAP than controls at all time points after MB administration ($P = 0.035$; two-way ANOVA). The MAP was significantly increased in the MB group at time III^{+30} ($P < 0.009$; *post hoc* test) (Fig. 1).

Total epinephrine requirements in the first hour after graft reperfusion were less in the MB group (53 [23] versus 246 [78] μg; $P = 0.02$). Patients receiving MB had higher cardiac indices after reperfusion at 5 min (7.4 [0.3] versus 6.2 [0.4] $l \cdot min^{-1} \cdot m^{-2}$; $P = 0.03$) and at 60 min (7.6 [0.5] versus 6.3 [0.4] $l \cdot min^{-1} \cdot m^{-2}$; $P = 0.04$) (Fig. 2). SVR decreased in both groups but remained similar between MB and Controls at all time points (Fig. 3). There was no difference in central venous pressure between groups at phase I (13.4 [1.0] versus 15.2 [1.1] mm Hg), III_5 (13.7 [0.6] versus 14.6 [0.9] mm Hg) or III_{60} (13.5 [0.6] versus 15.2 [0.7] mm Hg).

Pulmonary artery pressures increased in both groups after reperfusion, but there was no difference between groups. The pulmonary vascular resistance index in the

Table 1. Pre- and Postoperative Days 3 and 7 Liver Function Tests in Methylene Blue and Control Groups, Compared by Using Mann-Whitney *U*-Test

	Control	Methylene blue	*P* value
ALT preop (IU)	112 (46)	79 (31)	0.38
Bilirubin preop (μmol/L)	236 (64)	96 (24)	0.12
AlkPhos preop (IU)	804 (330)	595 (115)	0.95
PT preop (s)	22.9 (2.9)	19.1 (1.5)	0.70
ALT POD 3 (IU)	546 (201)	647 (116)	0.33
Bilirubin POD 3 (μmol/L)	88 (16)	82 (10)	0.98
AlkPhos POD 3 (IU)	262 (51)	379 (62)	0.03
PT POD 3 (s)	17.3 (0.7)	17.9 (0.8)	0.72
ALT POD 7 (IU)	166 (35)	155 (18)	0.74
Bilirubin POD 7 (μmol/L)	108 (22)	125 (24)	0.59
AlkPhos POD 7 (IU)	355 (54)	450 (55)	0.15
PT POD 7 (s)	14.7 (0.5)	15.3 (0.6)	0.39

ALT = aspartate transaminase, preop = preoperative, AlkPhos = alkaline phosphatase, POD = postoperative day, PT = prothrombin time.

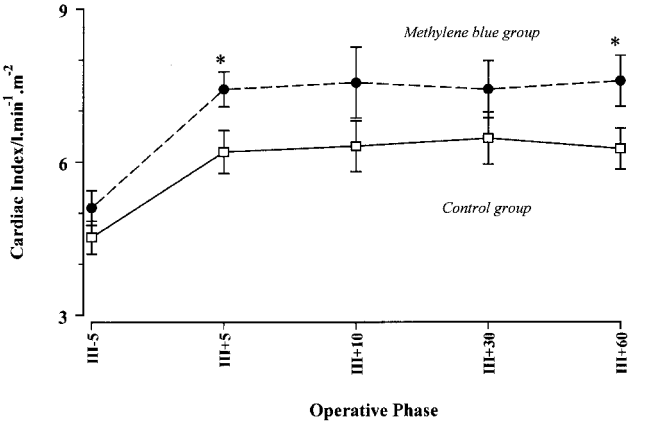

Figure 2. Cardiac index in Methylene Blue and Placebo groups at operative phases III ± *n* (*n* minutes before/after graft reperfusion). The Methylene Blue group is shown by a solid circle and the Placebo group by an open square. Difference between groups, $P = 0.04$ (two-way ANOVA). On individual time point analysis, asterisk denotes $P < 0.05$ (*post hoc* test). Results were expressed as mean ± SEM.

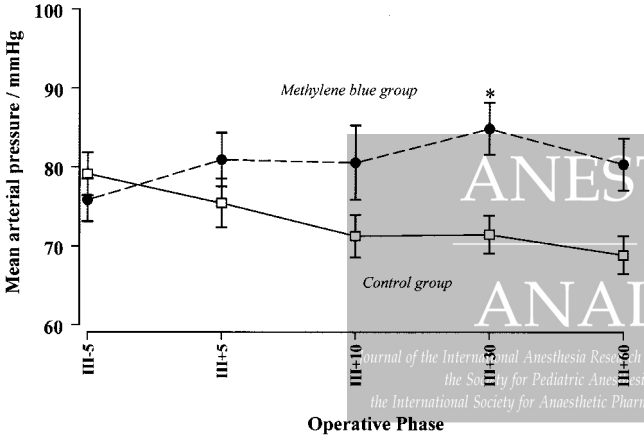

Figure 1. Mean arterial pressure in Methylene Blue and Placebo groups at operative phases III ± *n* (*n* minutes before/after graft reperfusion). The Methylene Blue group is shown by a solid circle and the Placebo group by an open square. Difference between groups, $P = 0.035$ (two-way ANOVA). On individual time point analysis, asterisk denotes $P < 0.05$ (*post hoc* test). Results were expressed as mean ± SEM.

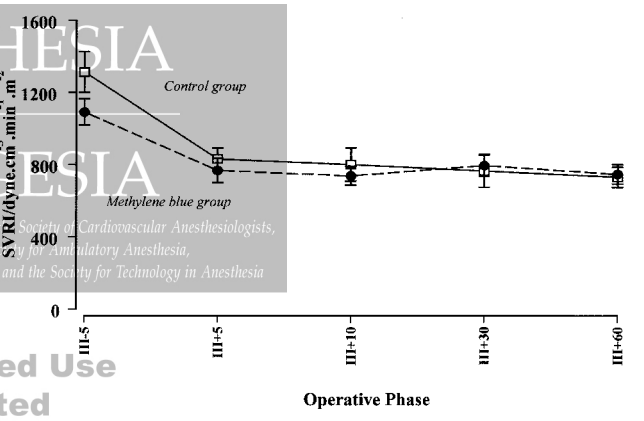

Figure 3. Systemic vascular resistance (SVR) in Methylene Blue and Placebo groups at operative phases III ± *n* (*n* minutes before/after graft reperfusion). The Methylene Blue group is shown by a solid circle and the Placebo group by an open square. Difference between groups not significant. Results were expressed as mean ± SEM.

MB group was 487 (44) dynes · cm^{-5} · min^{-1} · m^{-2} and in the Control group 389 (24) dynes · cm^{-5} · min^{-1} · m^{-2} ($P = 0.057$). However, there was no difference between groups in the change in pulmonary vascular resistance index expressed as a percentage of baseline at all subsequent time points ($P > 0.4$). There was no difference in right ventricular ejection fraction between groups at any time point.

Biochemical Data

Serum lactate was less at 1 h in the MB Group (5.5 [0.4] versus 6.7 [0.4] $mmol^{-1}$; $P = 0.03$). The coefficient of variation of measurement of serum lactate was 1.4%. Postoperative liver function tests and time to hospital discharge (Table 1) did not differ between groups.

Across all patients, there was correlation between nitrite concentrations and cGMP concentrations ($r = 0.4$, $P = 0.002$). Plasma nitrite concentrations (Fig. 4) were comparable between groups at all time points. The coefficient of variance of nitrite measurement was 3.7%. Nitrite concentrations showed a progressive reduction with time in the Control group ($P < 0.01$), though not in the MB group (not significant, repeated measures ANOVA).

A *post hoc* analysis found that baseline nitrite levels were significantly increased in cirrhotic patients compared with noncirrhotic patients (68.9 [13.0] versus 38.3 [3.8] μmol/L; $P = 0.033$). The nitrite levels for cirrhotic patients were 75% (6%) and 75% (5%) of baseline at 5 min and 1 h after reperfusion ($P = 0.01$ and $P = 0.007$, respectively). There was no change in the nitrite levels in the noncirrhotic patients.

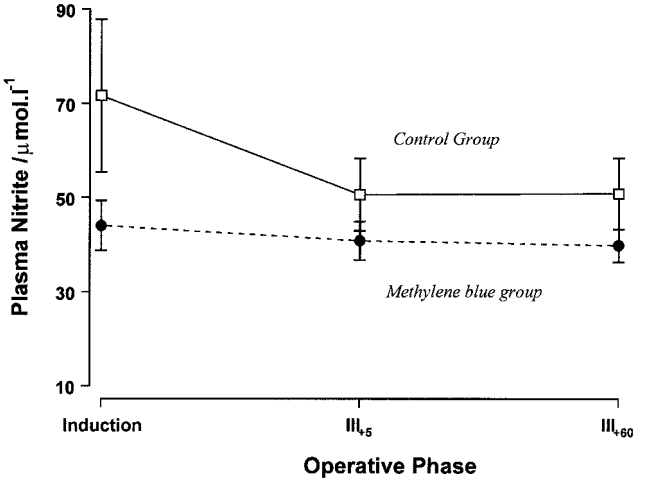

Figure 4. Plasma combined nitrate/nitrite concentrations in the Methylene Blue and Placebo groups at operative phases III ± *n* (*n* minutes before/after graft reperfusion). The Methylene Blue group is shown by a solid circle and the Placebo group by an open square. Difference between groups not significant. Results were expressed as mean ± SEM.

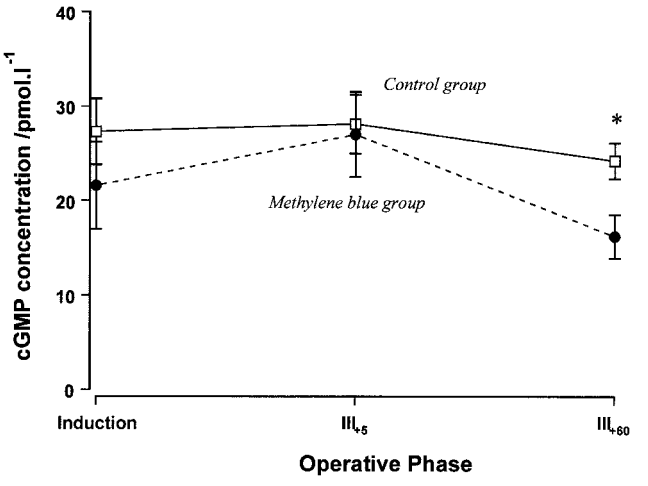

Figure 5. Cyclic 3',5'-monophosphate (cGMP) concentration against operative phase in the Methylene Blue and Control groups at operative phases III ± *n* (*n* minutes before/after graft reperfusion). The Methylene Blue group is shown by a solid circle and the Placebo group by an open square. cGMP concentrations were similar between groups at baseline, but decreased in MB ($P < 0.001$) but not in Controls ($P = 0.2$). On individual time point analysis, asterisk denotes $P < 0.05$ (*post hoc* test). Results were expressed as mean ± SEM.

cGMP concentrations were similar between groups at baseline, but changed over time in the MB group ($P < 0.003$) but not in Controls ($P = 0.4$, repeated measures ANOVA) (Fig. 5). The intra-assay coefficient of variance of cGMP was 5.6% and the inter-assay coefficient of variance was 6.9%. One hour after reperfusion, cGMP was significantly less in the MB group than Controls, 16.3 (2.5) versus 23.7 (1.9) pmol/L ($P < 0.01$, Student's *t*-tests). There was no significant difference between the cirrhotic and noncirrhotic patients at any time point.

Time to Hospital Discharge

The time to hospital discharge was a median 22 days in each group. There was no difference between groups ($P = 0.78$) when assessed by the log rank test. The data were censored for mortality.

Discussion

In this study, the administration of MB resulted in less hypotension, reduced inotrope requirement, and better cardiac performance after hepatic graft reperfusion as compared with patients receiving placebo. We have demonstrated a progressive reduction in nitrite concentration suggestive of decreasing plasma NO during OLT. cGMP levels correlated with nitrite levels at all time points, consistent with NO exerting its action through guanylate cyclase activation in this patient group. In the presence of MB, there was a reduction in cGMP, but not in plasma nitrites.

IRS resulting in intraoperative hypotension and increased transaminases postoperatively is observed in the majority of patients undergoing OLT; in severe cases, this can lead to graft failure and death (2). Ischemia in the transplanted liver results in breakdown of adenosine triphosphate to hypoxanthine and conversion of xanthine dehydrogenase to xanthine oxidase. During reperfusion, xanthine oxidase catalyzes the reaction between hypoxanthine and oxygen to produce xanthine and superoxide radicals. This leads to production of further oxygen radicals such as hydroxyl radicals and hypochlorous acid. These reactive oxygen species disrupt cell membranes, and activate monocytes and neutrophils with the production of inflammatory mediators. Characteristic hemodynamic and proinflammatory changes ensue (1,8,14). After graft reperfusion, there is a reduction in MAP and SVR and an increase in CI which often persists for one to two hours (1). Some patients show reduced myocardial contractility (14). The precise mechanisms underlying these hemodynamic responses are still not fully elucidated.

In the present study, MB administration was associated with less hypotension and inotrope requirement after graft reperfusion than placebo. The MB group had significantly smaller serum lactate concentrations at one hour after reperfusion, which may indicate either improved tissue perfusion or better lactate clearance, suggesting improved liver function. In this study, patients receiving MB had a higher CI after reperfusion, whereas SVR and central venous pressure were similar in MB and Placebo groups. Our findings suggest that the effects of MB could be mediated through preservation of myocardial function rather than prevention of vasodilatation.

In several previous studies, MB at doses of 1 to 3 mg/kg improved hemodynamics in sepsis and acute liver failure (3–7). The dose of 1.5 mg/kg was chosen for this study because it was in this range and the midpoint of the British National Formulary dose range of 1 to 2 mg/kg. In contrast to our results, studies in sepsis have suggested that MB acts primarily by increasing SVR. Most clinical studies in sepsis have failed to show a change in CI. However, in *in vitro* experiments, using tissue from endotoxin-treated animals, MB increased ventricular myocyte contractility (15). Two case reports in liver failure in humans have also demonstrated improved cardiac output (6,7). Therefore, the changes seen in SVR and cardiac output may depend on the clinical situation.

Several modes of action of MB have been postulated. Different mechanisms may predominate in different clinical situations. MB has properties as a NO blocking agent, both by inhibition of guanylate cyclase and possible inhibition of NO synthase (10,16,17). It also acts as an antioxidant, a prooxidant, inhibits prostacyclin synthesis, and accelerates reductive processes in the cell.

The hemodynamic effects of MB we have seen may be explained in part through interaction with the NO pathway. Septic shock and reperfusion syndrome share the cardiovascular characteristics of low SVR and high CI. The massive production of NO by the inducible isoform of NO synthase is held responsible for the profound vasodilatation and myocardial dysfunction in septic shock. The role of NO in IRS in OLT is not known.

NO stimulates guanylate cyclase in endothelial and vascular smooth muscle cells to produce intracellular cGMP causing vascular relaxation. MB acts as an inhibitor of the soluble guanylate cyclase through binding to the heme moiety of the enzyme in competition with NO (9). Evidence from *in vitro* (10) and *in vivo* (18) studies suggests that MB might also directly inhibit NO synthase, which also contains a protein bound heme moiety. The production of inducible NO synthase is regulated at the transcriptional level and thus dependent on new protein synthesis. It is therefore unlikely to be responsible for the immediate decrease in MAP and SVR and increase in CI observed after graft reperfusion. However, there is some *in vitro* and *in vivo* evidence that the rapid development of hypotension in response to endotoxin and tumor necrosis factor (occurring in less than five minutes) may also be mediated by an increased release of NO (19,20). Plasma nitrite and nitrate are the stable endproducts of NO metabolism and reflect NO levels. They are eliminated by the kidney. There is no hepatic metabolism.

The prereperfusion pretreatment nitrite levels in both groups were comparable with levels found in liver disease and septic patients and higher than those in healthy volunteers (15.5–28.9 μmol/L), indicating increased NO levels in this population (21,22). In our study, there was no increase in NO levels after reperfusion to explain the hemodynamic changes seen in IRS. In both MB and non-MB groups, nitrite levels decreased one hour after reperfusion. However, there was no difference between the two groups, indicating that MB did not increase MAP by inhibition of NO production.

Previous authors have reported an increase in nitrite levels in patients with cirrhosis, but not in noncirrhotic patients (23). A *post hoc* analysis based on these reports demonstrated that nitrite levels were significantly increased in patients with cirrhosis. There was a decrease in nitrite level after MB in the cirrhosis group, but not in the noncirrhotic group. This suggests that different pathways for NO production could apply in cirrhotic and noncirrhotic patients.

Prereperfusion cGMP concentrations were also increased. These results are more than those found by Schneider et al. (24) in septic patients, and much greater than nonseptic patients (1.77 [0.18] pmol/L). Schneider et al. also previously reported increased cGMP levels comparable with those they found in sepsis in patients with fulminant liver failure (25). This study confirms that there are increased cGMP levels in liver failure. One hour after reperfusion, cGMP was significantly lower in the MB group than Controls. These results suggest that MB acts via inhibition of guanylate cyclase. A *post hoc* analysis of cirrhosis patients versus noncirrhotic patients failed to show any difference in the cGMP response to MB.

Although nitrite and cGMP levels were increased in OLT before reperfusion, indicating increased NO production in liver failure, we found no evidence of an increase in NO production after reperfusion to implicate NO in IRS. However, the measurement of plasma nitrite and cGMP concentrations in samples from the radial artery reflects global changes and could miss localized areas of increased production. It is possible that areas of increased production may exist to explain the decrease in SVR and CI observed after reperfusion. MB may also act via different pathways to produce some of its effects.

In this study, MB attenuated the hypotension of IRS in OLT, apparently through an effect on myocardial function. This effect is mediated predominantly through guanylate cyclase inhibition. The dye MB has been used for many years in diagnostic procedures and to treat methemoglobinemia without major side effects. In this study, no adverse effects were observed using MB. We did not show differences in postoperative liver function tests or hospital stay; however, this was a small study not powered to detect changes in these endpoints. In selected cases, MB may be useful in the control of hypotension after reperfusion of the

transplanted liver. This pilot study provides promising results; however, larger studies are required before the drug can be recommended for routine use.

We acknowledge the help of Sarah Perry, University Department of Surgery, Leeds, and Lorna Smith, Department of Molecular Medicine, University of Aberdeen. The Leeds Liver Unit comprises: M. C. Bellamy, M. H. Davies, J. P. A. Lodge, C. E. Millson, S. G. Pollard, N. J. Snook, G. Toogood, and Y. Young.

References

1. Aggarwal S, Kang Y, Freeman JA, et al. Postreperfusion syndrome: cardiovascular collapse following hepatic reperfusion during liver transplantation. Transplant Proc 1987;19:54–5.
2. Williams JW, Vera S, Peters TG, et al. Cholestatic jaundice after hepatic transplantation: a nonimmunologically mediated event. Am J Surg 1986;151:65–70.
3. Preiser JC, Lejeune P, Roman A, et al. Methylene blue administration in septic shock: a clinical trial. Crit Care Med 1995;23: 259–64.
4. Daemen-Gubbels CRGH, Groeneveld PHP, Groeneveld ABJ, et al. Methylene blue increases myocardial function in septic shock. Crit Care Med 1995;23:1363–70.
5. Zhang H, Rogiers P, Preiser JC, et al. Effects of methylene blue on oxygen availability and regional blood flow during endotoxic shock. Crit Care Med 1995;23:1711–21.
6. Midgley S, Grant IS, Haynes WG, Webb DJ. Nitric oxide in liver failure. Lancet 1991;338:1590.
7. McGinn PV. Reversal of the haemodynamic features of acute liver failure by methylene blue. Intensive Care Med 1996;22:612.
8. Bellamy MC, Galley HF, Webster NR. Changes in inflammatory mediators during orthotopic liver transplantation. Br J Anaesth 1997;79:338–41.
9. Gruetter CA, Gruetter DY, Lyon JE, et al. Relationship between cyclic guanosine 3'5'-monophoshate formation and relaxation of coronary smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: effects of methylene blue and hemoglobin. J Pharmacol Exp Ther 1981;219:181–6.
10. Mayer B, Brunner F, Schmidt K. Inhibition of nitric oxide synthesis by methylene blue. Biochem Pharmacol 1993;45:367–74.
11. Bellamy MC, Valentine JMJ, Whiteley SM. Anaesthesia for hepatic transplantation. Aether 1996;2:16–9.
12. Starzl TE, Marchioro TL, von Kaulla KN, et al. Homotransplantation of the liver in humans. Surg Gynecol Obstet 1963;117: 659–76.
13. Green LC, Wagner DA, Glogowski J, et al. Analysis of nitrate, nitrite, and ^{15}N nitrate in biological fluids. Anal Biochem 1982; 126:131–8.
14. Webster NR, Bellamy MC, Lodge JPA, Sadek SA. Haemodynamics of liver reperfusion: comparison of two anesthetic techniques. Br J Anaesth 1994;72:418–21.
15. Brady AJB, Poole-Wilson PA, Harding SE, Warren JB. Nitric oxide production within cardiac myocytes reduces their contractility in endotoxemia. Am J Physiol 1992;263:H1963–6.
16. Paya D, Gray GA, Stoclet JC. Effects of methylene blue on blood pressure and reactivity to norepinephrine in endotoxemic rats. J Cardiovasc Pharmacol 1993;21:926–30.
17. Salaris SC, Babbs CF, Voorhees WD. Methylene blue as an inhibitor of superoxide generation by xanthine oxidase: a potential new drug for the attenuation of ischemia/reperfusion injury. Biochem Pharmacol 1991;42:499–506.
18. Keaney JF, Puyana JC, Francis S, et al. Methylene blue reverses endotoxin-induced hypotension. Circ Res 1994;74:1121–5.
19. Salvemini D, Korbut R, Änggard E, Vane J. Immediate release of a nitric oxide -like factor from bovine aortic endothelial cells by *Escherichia coli* lipopolysaccharide. Proc Natl Acad Sci USA 1990;87:2593–7.
20. Szabo C, Mitchell JA, Thiemermann C, Vane JR. Nitric oxide-mediated hyporeactivity to norepinephrine precedes the induction of nitric oxide synthase in endotoxin shock. Br J Pharmacol 1993;108:786–92.
21. El-Newihi HM, Kanji VK, Mihas AA. Activity of gastric mucosal nitric oxide synthase in partal hypertensive gastropathy. Am J Gastroenterol 1996;91:535–8.
22. Ochoa JB, Udekwu AO, Billiar TR, et al. Nitrogen oxide levels in patients after trauma and during sepsis. Ann Surg 1991;214: 621–6.
23. Hori N, Okanoue T, Mori T, et al. Endogenous nitric oxide production is augmented as the severity advances in patients with liver cirrhosis. Clin Exp Pharmacol Physiol 1996;23:30–5.
24. Schneider F, Lutun P, Couchot A, et al. Plasma cyclic guanosine 3'-5' monophosphate concentrations and low vascular resistance in human septic shock. Intensive Care Med 1993;19:99–104.
25. Schneider F, Lutun P, Boudjema K, et al. *In vivo* evidence of enhanced guanylyl cyclase activation during the hyperdynamic circulation of acute liver failure. Hepatology 1994;19:38–44.