E. E. Douzinás
E. Vamvasakis
K. Rigas
M. Pitaridis
C. Kittas
C. Roussos

Bile canaliculi are defective in hepatic involvement of organ failure and recovery of liver function is due to their secondary regeneration

Abstract  Objective: To investigate the morphological changes in the liver in patients with organ failure and hyperbilirubinemia and to correlate them to the outcome.

Design: A case series prospective study.

Setting: Intensive care units of two general hospitals.

Patients: Twelve patients in organ failure with predominant hepatic involvement, aged 16 to 69 years (mean 56 years).

Interventions: Liver biopsy was performed on all patients 3–15 days after organ failure. A second biopsy was also performed on all four surviving patients, as well as on 3 patients just before death at a mean time of 16 days (6–32) and 31 days (14–55), respectively, after the first biopsy. The samples were studied by electron microscopy and findings were assessed according to Rapapport’s designation.

Measurements and main results: In the first biopsy it was shown that in zone III there was complete degeneration of bile canaliculi and hepatocytes in contrast to zone I. The grade of histological severity for zone III is positively correlated to the bilirubin concentration (p = 0.001). In the specimens from the second biopsy, it was shown that numerous, newly formed secondary bile canaliculi per 20 consecutive hepatocytes had developed in zone III in the surviving patients, whereas there was a complete absence of such canaliculi in the patients who died (mean ± SD: 9.6 ± 3.2 vs 0).

Conclusions: It appears that the destruction of primary bile canaliculi is a striking anatomical defect in patients with organ failure and impaired bilirubin excretion. The restoration of liver function coincides with adequate formation of new secondary bile canaliculi in zone III, giving credence to the hypothesis that this formation is an important structural change responsible for the improvement in liver function.

Key words  Multiple organ failure · Hyperbilirubinemia · Primary bile canaliculi · Secondary bile canaliculi · Cholestasis · Ductular proliferation

Introduction

Jaundice and cholestasis secondary either to infection (i.e. pneumonia [1], and sepsis [2]) or to hypoperfusion (i.e. septic shock [3] and hypovolemia [4, 5]) are well recogni-
the syndrome of multiple organ failure (MOF) is unknown. The effect of endotoxin on the sodium and potassium ATPase pump [8] and the defective exchange of water and electrolytes in the canaliculi [3, 9] have been postulated to play an important role.

Despite the increased bilirubin level, hepatic function, as evidenced by prothrombin time, fibrinogen level and protein synthesis [3], seems to be preserved. This type of cholestasis is in contrast to that of acute viral or drug-induced hepatitis, which is combined with hepatocellular failure. From this discrepancy between bilirubin level and liver function, one may postulate that an anatomical defect cannot be excluded in these patients with impaired bilirubin excretion.

Until now, attempts to show a causative morphological defect have not been instructive; the studies have come mainly from light microscopy, and most of them have referred to autopsies [3, 10, 11]. The aim of this study was to investigate the morphological changes in the liver related to hyperbilirubinemia in patients with MOF and, more importantly, to detect the adaptive changes related to favourable outcome.

Materials and methods

Selection criteria

Among 95 patients hospitalised with MOF [12] from May 1989 to August 1994 in the intensive care units (ICUs) of Evangelismos and Laiko Hospitals in Athens, only 12 fulfilled the following criteria of combined severe hepatic involvement:

1. Progressive hyperbilirubinemia of more than 85.5 µmol/l (5 mg/dl) of conjugated type. Although levels of more than 42.7 µmol/l (2.5 mg/dl) are usually considered indicative of hepatic involvement, it was expected that a higher levels of > 85.5 µmol/l the histological findings in the liver would by typical and fully expressed. For this reason, patients with bilirubin levels of < 85.5 µmol/l were excluded from the study.

2. Lack of evidence on ultrasound of bile duct obstruction.

3. Bile canaliculi were examined in zone I and zone III with electron microscopy. Moreover, the newly formed (secondary) bile canaliculi were counted over the borders of 20 consecutive hepatocytes in each section from three different tissue blocks originating from zone III and the degree of generation was assessed as number of secondary bile canaliculi per 20 hepatocytes.

Liver biopsies

Percutaneous liver biopsy was performed on all patients when the above criteria were met — namely, at 3 to 15 days (mean 8.6 days) from the onset of organ failure. We performed a second biopsy on four patients in recovery, as well as on three of eight patients just before death, at a mean time of 16 days (6–32) and 31 days (14–55), respectively, after the first biopsy in order to evaluate the hepatic morphological changes associated with the outcome. The biopsy specimens were fixed in Bown's solution and in cold glutaraldehyde for study by light and electron microscopy, respectively.

Histological assessment

Two pathologists studied the histological material independently and interpretation of results was carried out according to Rapaport's designation [13] as follows:

1. Portal tract cellularity, the degree of cholestasis and overall hepatocyte degeneration were examined with light microscopy.

2. The grade of histological severity in zone III in the seven patients in whom first and second biopsies had been performed was correlated to the level of bilirubin at the time of biopsy. The grade was assessed with electron microscopy by means of cellular degenerative changes and number of autolysosomes in 20 consecutive hepatocytes in each section of three different tissue blocks from zone III, as follows: grade I: swollen cellular and organelle membranes with up to 10 autolysosomes; grade II: including the above plus swollen mitochondria with loss of cristae and distended cisternae of endoplasmic reticulum with 11–20 autolysosomes; grade III: including all of the above plus rupture of membranes with 21–35 autolysosomes; grade IV: severe cellular disorganisation, presence of amorphous material, extensive rupture of membranes with only 2–4 autolysosomes due to their inefficient reproduction and increased consumption.

3. Bile canaliculi were examined in zone I and zone III with electron microscopy from the first and second biopsies. Moreover, the newly formed bile canaliculi were counted over the borders of 20 consecutive hepatocytes in each section from three different tissue blocks originating from zone III and the degree of generation expressed as number of secondary bile canaliculi per 20 hepatocytes.

Management of organ failure

Organ failure was managed by avoiding systematic treatment differences, particularly as follows: (a) to combat sepsis with antibiotics in accordance with the culture results; (b) to maintain haemodynamic stability by administering fluids and/or inotropes as needed: the patients had to be in a stable haemodynamic state for at least 3 days before the first biopsy could be performed; (c) to feed patients enterally, if possible, providing energy at 1.3 times energy expenditure (formula of Harris-Benedict) with a ratio of non-protein calories to nitrogen of 80–110:1; (d) to use dialysis as indicated.

The protocol was approved by the Research Committees of the hospitals and informed consent was obtained from patients' relatives in each case.

In order to compare the patients' clinical data, the t-test was used. Regression analysis was used to test the trend of bilirubin levels according to the grade of histological severity in zone III using the data from all patients in whom first and second biopsies were performed. Difference of < 0.05 were regarded as significant.

Results

When patients were entered into the study and the first biopsy was performed, there was no statistically significant difference between those who subsequently survived or died with respect to the following: age (46.5 ± 21 vs