Liver giant mitochondria revisited

N J Robertson, C H Kendall

Abstract

Aims: To examine the correlation between the severity of alcohol induced liver damage and the presence of intracytoplasmic red bodies (defined as periodic acid-Schiff diastase negative, globular, hyaline cytoplasmic inclusions larger in size than the hepatocyte nucleolus). To investigate the incidence of intracytoplasmic red bodies (ICRBs) in non-alcoholic liver disease.

Methods: Liver biopsy specimens from 53 patients with alcoholic liver disease and 50 patients with a variety of non-alcohol related liver diseases were examined by light microscopy for the presence of ICRBs. For the 53 patients with alcoholic liver disease an assessment of recent alcohol consumption was made indirectly from measurements of red cell volume (MCV) and plasma γ -glutamyl transferase (GGT). In addition, 10 liver biopsies with alcohol induced changes and ICRBs were examined by electron microscopy for the presence of mitochondrial aberrations including enlargement.

Results: ICRBs were detected in 18 of the 53 liver biopsy specimens showing alcohol induced changes, and were more abundant in those showing more advanced changes. Those patients whose liver specimens contained ICRBs were found to have a significantly higher mean plasma GGT activity and mean MCV than those individuals whose liver biopsy specimens did not contain ICRBs. Two of the 50 liver biopsy specimens showing non-alcohol induced changes contained ICRBs. Giant mitochondria were not detected by electron microscopy, but this may reflect sampling.

Conclusions: The results of this study indicate that ICRBs are definitely associated with alcoholic liver disease and are more likely to be found in liver biopsy specimens showing more advanced alcohol induced damage, and when recent alcohol consumption has been high.

Globular intracytoplasmic red bodies (ICRBs) have long been recognised by light microscopy in hepatocytes in liver biopsies, and especially in those liver biopsies from patients with alcoholic liver disease. There is, however, conflicting evidence in the literature as to the correlation between the severity of

alcohol induced liver damage and the presence of ICRBs. In a study by Chedid *et al*¹ in 1986, ICRBs were observed most frequently in liver biopsies exhibiting mild degrees of alcohol induced damage. Junge *et al*² in 1987 obtained similar results. However, in a study by Bruguera *et al*³ in 1977 the frequency of ICRBs in liver biopsies from alcoholic patients was found to be unrelated to the nature of the histological changes present. The significance of ICRBs with respect to non-alcoholic liver disease remains equally unclear. The main purposes of this study are therefore: (1) to examine the correlation between the severity of alcohol induced liver damage and the presence of ICRBs; (2) to investigate the incidence of ICRBs in non-alcoholic liver disease.

Since the advent of transmission electron microscopy giant mitochondria have been identified in hepatocytes in liver biopsies exhibiting both alcohol induced damage and ICRBs.¹⁻⁴ It has therefore followed that ICRBs seen in liver biopsies exhibiting alcohol induced damage have been interpreted as being giant mitochondria, providing they are periodic acid-Schiff diastase negative. There has remained, however, some doubt as to the exact nature of ICRBs, and a further aim of this study is to investigate liver biopsies containing ICRBs ultrastructurally.

Methods

The material consists of 103 liver biopsy specimens from 102 adults and one child; 53 were from patients who either gave, or were strongly suspected of having, a recent history of regular high alcohol consumption. Average daily intakes in excess of 60 g for men and 40 g for women are considered high and are associated with increased morbidity and mortality from a variety of diseases.⁵ And all 53 biopsy specimens exhibited histological changes consistent with an alcoholic aetiology: 50 liver biopsy specimens were from patients having a variety of non-alcohol related liver diseases, and these included the following: primary biliary cirrhosis (nine cases), chronic active hepatitis (seven cases), chronic persistent hepatitis (one case), sarcoidosis (two cases), miliary tuberculosis (one case), Reye's syndrome (one child case), ascending cholangitis (one case), chronic pericholangitis (one case), chronic large bile duct obstruction (one case), α -1-antitrypsin deficiency (one case) and non-specific changes (25 cases). All biopsy specimens were obtained between 1983 and 1989.

Department of
Histopathology,
Leicester Royal
Infirmary, Leicester
LE1 5WW
N Robertson
C H Kendall

Correspondence to:
Dr N J Robertson
Accepted for publication
8 October 1991

Table 1 Histological category and the presence of ICRBs in liver biopsy specimens from patients with and without alcohol induced liver damage

	Histological category	Number of ICRBs				Number of biopsies	Specimens positive for ICRBs
		(-)	(+)	(++)	(+++)		
Group I (n = 53) Patients with alcohol induced liver damage	Fatty change alone	21	5	0	0	26	5 (19.2%)
	Fatty change with alcoholic hepatitis or fibrosis or both	12	5	0	1	18	6 (33.3%)
	Cirrhosis with or without any other alcohol induced changes	2	3	1	3	9	7 (77.7%)
Group II (n = 50) Patients with non-alcohol related liver damage	A variety of non-alcohol related changes of various severity	48	2	0	0	50	2 (4%)

All the liver biopsy specimens exhibiting alcohol induced changes were categorised for subsequent data analysis according to the nature and severity of the histological changes present. The categories were defined as follows: fatty change alone, fatty change with alcoholic hepatitis or fibrosis or both, and cirrhosis with or without any other alcohol induced changes.

The quantity of alcohol consumed around the time of liver biopsy was assessed indirectly from measurements of red cell volume (MCV) and plasma γ -glutamyl transferase (GGT). Both these parameters are used clinically as markers of alcohol consumption. Using upper reference limits of 98 fl for MCV and 50 IU/l for GGT, Chick *et al*⁶ observed macrocytosis in 10 and increased GGT in 15 out of 30 company directors admitting to a daily alcohol intake of more than 65 g.

All liver biopsy specimens were processed for haematoxylin and eosin, chromotrope aniline blue (CAB), Martius scarlet blue (MSB), and periodic acid-Schiff (PAS) before and after diastase digestion. Intracytoplasmic red bodies (ICRBs) were defined as well circumscribed, globular, hyaline, intracytoplasmic bodies which stained bright red with CAB and MSB, and failed to stain with PAS after diastase digestion. Only red bodies larger than the hepatocyte nucleolus were recorded and, in order to avoid confusion with red blood cells, only red bodies seen to be clearly within the hepatocyte cytoplasm and within the same focal plane as the nucleus were recorded. Confusion with red blood cells was minimised with MSB staining as red blood cells appear yellow with this staining method.

The number of ICRBs per unit area of a section of liver biopsy specimen was variable, both within the same biopsy and between different biopsies. ICRBs were subjectively semiquantified as (+++), (++) , (+), and (-), according to their relative frequency in the biopsy specimen. When only occasional ICRBs were detected in the entire biopsy specimen this was denoted (+). Numerous

ICRBs throughout the biopsy specimen were denoted (+++), and (++) denoted an intermediate number of ICRBs. An absence of ICRBs was registered as (-).

For 10 cases with histological evidence of both alcohol induced damage and ICRBs, tiny cubes of paraffin wax embedded tissue were dewaxed in xylene, post fixed in 1% osmium tetroxide and embedded in resin. Semi-thin sections for orientation were stained with 1% toluidine blue, and finally ultra-thin 90 nm sections were stained with saturated uranyl acetate/Reynold's citrate and examined in a Jeol 100 CX transmission electron microscope.

Results

In sections stained with haematoxylin and eosin ICRBs appeared as weakly eosinophilic rounded bodies in otherwise normal hepatocytes and in hepatocytes containing fat vacuoles. Their identification was much enhanced, however, on staining with CAB and MSB where they appeared bright red, and confusion with red blood cells was eliminated as MSB stained these cells yellow.

In all biopsy specimens where ICRBs were identified, they occurred in a minority of hepatocytes and their acinar distribution was entirely random. ICRBs ranged in size between 2 and 10 μ m and their number within hepatocytes varied markedly; some hepatocytes contained only one while others contained two or more.

Table 1 relates the presence of ICRBs to the histological categories defined earlier. Group I comprises 53 liver biopsy specimens from patients with alcohol induced liver damage, and group II comprises 50 patients with a variety of non-alcohol related liver diseases. ICRBs were observed in 18 liver biopsy specimens from group I and in two from group II, the latter being from two cases of primary biliary cirrhosis. ICRBs were seen in five of 26 liver biopsy specimens showing fatty change alone and in seven of nine showing alcoholic

Table 2 Mean plasma GGT activity and mean MCV in relation to the presence and absence of ICRBs

	Mean plasma GGT activity (IU/l) (SD)	Mean MCV (fl) (SD)	Number of liver biopsy specimens
ICRBs present (n = 18)	478.5 (98.2)	105.9 (4.8)	18
ICRBs absent (n = 35)	225.5 (60.3)	94.0 (2.1)	35

cirrhosis. Of the 18 biopsy specimens showing fatty change with alcoholic hepatitis and/or fibrosis six contained ICRBs. ICRBs were more abundant in those biopsies exhibiting more advanced alcohol induced damage.

Table 2 gives the mean plasma GGT activity and the mean MCV in relation to the presence and absence of ICRBs in 18 and 35 liver biopsy specimens respectively. The mean plasma GGT activity for the 18 patients whose liver biopsy specimens were positive for ICRBs was 478.5 (SD 98.2) IU/l. This contrasts with a mean plasma GGT activity of 225.5 (SD 60.3) IU/l for the 35 patients whose specimens were negative for ICRBs. The difference between the mean plasma GGT activities in the two groups is statistically significant ($0.05 > p > 0.02$). The mean MCV was calculated to be 105.9 (SD 4.8) fl for the 18 positive biopsy specimens and 94.0 (SD 2.1) fl for the 35 negative biopsy specimens. Again the difference between the mean values here is statistically significant ($0.05 > p > 0.02$).

Ultra-thin sections from 10 liver biopsy specimens, all of which exhibited alcohol induced changes and ICRBs by light microscopy (including the four (+ + +) cases shown in table 1), were carefully examined by transmission electron microscopy. The integrity of cellular organelles was not optimal in any of the sections examined because the liver biopsy specimens had initially been embedded in paraffin wax. Nevertheless, mitochondria were easily identified and in the majority of cases their internal membranous structure was satisfactorily preserved. In none of the sections examined were giant mitochondria detected, although occasional mitochondria showing slight enlargement or abnormal shape were found.

Discussion

Intracytoplasmic red bodies, as defined by the strict criteria detailed elsewhere in this paper, can readily be differentiated from a number of similar eosinophilic intracytoplasmic bodies including Mallory's hyalin, α -1-antitrypsin globules and phagocytosed red blood cells. Invariably Mallory's hyalin has a clumped appearance with an irregular indistinct margin and stains blue/grey with CAB. α -1-Antitrypsin globules are easily differentiated as they are PAS positive after diastase digestion. Phagocytosed and misplaced red blood cells stain yellow with MSB and can also be compared morphologically with red blood cells within sinusoids.

ICRBs are clearly an important finding in liver biopsy specimens exhibiting alcohol induced damage. They were observed in 18 of 53 liver biopsy specimens from patients with alcoholic liver disease, in contrast to only two of 50 from patients with other types of liver disease. The histological diagnosis in each of the two positive biopsy specimens from patients with non-alcoholic liver disease was primary biliary cirrhosis. Both patients were female and their sera were positive for anti-mitochondrial antibodies; neither was suspec-

ted of abusing alcohol. Notwithstanding this, the evidence strongly suggests that ICRBs are generated mainly by processes which induce damage in alcohol exposed livers in contrast to processes which give rise to other liver diseases.

This study also shows that ICRBs are found more often in liver biopsy specimens exhibiting more advanced alcoholic damage; seven of nine specimens exhibiting cirrhosis contained ICRBs in contrast to only five of 26 exhibiting fatty change alone. This is at variance with the findings of Chedid *et al*¹ and Junge *et al*² who observed ICRBs most frequently in liver biopsies exhibiting mild degrees of alcohol induced damage.

Rosalki introduced and popularised the measurement of plasma GGT activity as an important biochemical index of alcohol consumption.^{7,8} The MCV is also known to correlate positively with average daily alcohol intake.^{9,10,11} Therefore, as an indirect measure of alcohol consumption the plasma GGT activity and the MCV were examined in each of the 53 cases with alcohol induced liver damage, and these parameters were then related to the presence or absence of ICRBs. Those individuals whose liver biopsy specimens contained ICRBs were found to have a significantly higher mean plasma GGT activity and mean MCV than those individuals whose liver biopsy specimens did not contain ICRBs. It can be inferred from this that ICRBs are more likely to be found when recent alcohol consumption has been high. It is also reasonable to suggest that individuals with a high alcohol consumption are likely to have more advanced alcoholic liver damage. More ICRBs would therefore be expected in cirrhotic livers than in livers exhibiting fatty change alone, as was found.

What is the ultrastructural nature of ICRBs? As stated earlier, giant mitochondria have been found by electron microscopy in alcoholic livers which contained ICRBs at the light microscope level.^{1,2,3,4} Mitochondrial changes, including enlargement, have been observed in livers exhibiting alcoholic hepatitis^{12,13,14} and alcoholic fatty change without inflammation.¹⁵ Apparently giant mitochondria are not exclusive to alcoholic liver disease, having been reported in a number of dietary¹⁶ and toxic conditions,¹⁷ and after starvation.¹⁸ Giant mitochondria reaching the size of the hepatocyte nucleus are said to have been induced in experimental animals by various conditions such as the administration of isonicotinic acid derivatives¹⁹ and dietary riboflavin deficiency.²⁰ In this current study sections from 10 liver biopsies containing ICRBs were examined carefully by transmission electron microscopy for giant mitochondria, although none were found. This may simply reflect sampling problems, although occasional mitochondria showing slight enlargement or abnormal shape were apparent. The concept that ICRBs seen in alcoholic liver disease are giant mitochondria has largely gained acceptance in the literature, but could not be further confirmed in this study.

On the basis of this study the evidence indicates that ICRBs are definitely associated with alcoholic liver disease, and are more likely

to be found when recent alcohol consumption has been high and in liver biopsy specimens showing more advanced alcohol induced damage.

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