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Lack of hepatocyte involvement in the genesis of the sinusoidal dilatation related to heroin addiction: a morphometric study

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Summary. A histological and morphometric study demonstrated a relationship between vascular changes in the hepatic lobule and heroin consumption. To establish the role of hepatocytes in the genesis of sinusoidal dilatation, morphometric analysis was performed on ten drug abusers and eight controls. A total of 1800 hepatocytes, in 67 centrilobular areas, were analysed from biopsies from the total patient number. Computerized results of hepatocyte surface area, perimeter, maximum linear dimension and minimum linear dimension demonstrated no statistically significant difference for these variables, particularly for hepatocyte surface area (Controls: 268.66 ± 95.25; drug abusers: 252.00 ± 78.94, $p = 0.24$), when the two groups of patients were compared. Hepatocyte morphology at the time of the biopsy was unaltered, although transaminase values were elevated for all drug abusers. It is, therefore, possible that the hepatocytes were not implicated in the pathogenesis of sinusoidal dilatation. This suggestion supports our previous results, which suggested that heroin was capable of inducing direct vascular hepatotoxicity.

Key words: Hepatocytes – Sinusoids – Heroin – Morphometry

Introduction

Heroin (diacetylmorphine) is one of the most common drugs used by addicts either through smoking or intravenous injection. It exhibits varying degrees of morphine-like pharmacological effects (Lechón-Gomez et al. 1987). After intravenous administration of heroin, the liver metabolizes the drug: 42.5% of the heroin infused into healthy subjects appears in the urine as morphine, 1.31% as 6-monoacetylmorphine and 0.13% as heroin (Elliot et al. 1971). The drug is also quickly converted to these two metabolites after exposure to blood and tissue homogenates of liver, kidney and brain. The liver has the greatest drug-transforming capacity and cholinesterase is the principal enzyme responsible for this reaction (Sawynock 1986; Way et al. 1960).

Liver pathology related to heroin or morphine addiction has been demonstrated experimentally in human or rat hepatocytes in vitro and in mice. After 24 and 72 h of continuous morphine administration, the animals exhibited a two-fold increase in serum glutamate oxalacetate and glutamate pyruvate transaminase activity (Chang and Ho 1979). In vitro studies, hepatic glutathione depletion, loss of cellular protein and lactate dehydrogenase leakage were detected (Eklöw-Låstbom et al. 1986; James et al. 1982; Lechón-Gomez et al. 1987). Clinical hepatic dysfunction in humans has been attributed to addiction or chronic therapy by opioids (Litt et al. 1972; Marks and Chapple 1967). Most authors agree that clinical and biological hepatic abnormalities in addicts are related to viral hepatitis, alcohol abuse, chronic malnutrition or exogenous contaminants administrated with the drug (Gorodetzky et al. 1968; Litt et al. 1972; Potter et al. 1960). In a previous study, we suggested the direct role of heroin on the sinusoidal liver barrier by a semi-quantitative and morphometric analysis. A significant increase was detected in volume sinusoidal density, mainly in the centrilobular zones, associated with sinusoidal and terminal hepatic vein inflammation, followed by a venular and perisinusoidal fibrosis after drug withdrawal (Trigueiro de Araujo et al. 1990).

The interaction between blood cells, sinusoidal wall and hepatocytes, as evidenced by the transfer of blood into space of Disse, seems to be regulated at least in part by the sinusoidal barrier (Wisse et al. 1985). Disturbances in the hepatic circulation in humans, such as thrombosis in a branch of the portal vein and post-sinusoidal hypertension, may disturb the haemodynamic

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equilibrium of the liver, modify sinusoids and, consequently, the number of functional hepatocytes (Dubuisson et al. 1988). Sinusoidal dilatation and peliosis have also been considered to be responsible for hepatic parenchymal atrophy in patients with the acquired immunodeficiency syndrome (Czapar 1986; Scoazec et al. 1988), tuberculosis, neoplasms and other chronic diseases (Zak et al. 1950). These vascular lesions of liver have been interpreted as having an iatrogenic aetiology involving a toxic mechanism with a direct action either on the sinusoidal barrier (Bagheri and Boyer 1974; Zafrani 1983; Zafrani and Feldmann 1988) or on hepatocytes. Several drugs are able to induce centrolobular necrosis (Dalich and Larson 1985; Davion et al. 1984; Schmidt et al. 1985), hepatitis with bridging necrosis, viral-like hepatitis, cholangitis and centrolobular cholestasis (Kunze et al. 1985; Roschlau 1983). Liver drug cytotoxicity has been demonstrated by the presence of antibodies against hepatocyte membrane (Schmidt et al. 1985) and by histological features suggesting an immunoallergic mechanism for the development of drug hepatitis (Davion et al. 1984; Kunze et al. 1985).

To assess whether there are the morphological alterations in hepatocytes and whether they play a role in the pathogenesis of sinusoidal dilatation, we have carried out a comparative histological and morphometric analysis of these cells in zone III of the hepatic lobule on biopsies from drug abusers (DA) and normal patients (controls).

Materials and methods

Liver samples were obtained by surgical or needle biopsies. Two groups of patients, ten intravenous DA and eight control individuals (mean age 28 ± 6 years), were compared. The DA showed a slight hepatomegaly associated with elevated transaminase values. They were HBs-Ag and anti-HIV negative. Hepatitis C serology was unknown at the time of the study. The control group had no clinical, biological or histological evidence of liver or biliary tract diseases. They were biopsied during abdominal surgery for non-hepatic problems immediately after the initial phase of anaesthesia.

Biopsies were fixed in Bouin's fluid and embedded in paraffin wax. Sections cut at 4 μm for histological and morphometric examination were stained with haematoxylin and eosin, Masson's trichrome, Gordon and Sweet's method and picro-sirius red for collagen.

The automatic image analysis system Biocom 200 was used to provide quantitative data. It consisted of a Biocom histo programme in a Compaq Deskpro 386/25 computer with two colour monitors, Panasonic monochrome television camera with Leitz Orthoplan photomicroscope for light microscopic analysis and a Canon laser printer.

Histological and morphometric data were obtained using a $\times 100$ objective lens. The perimeter of the hepatocytes was traced

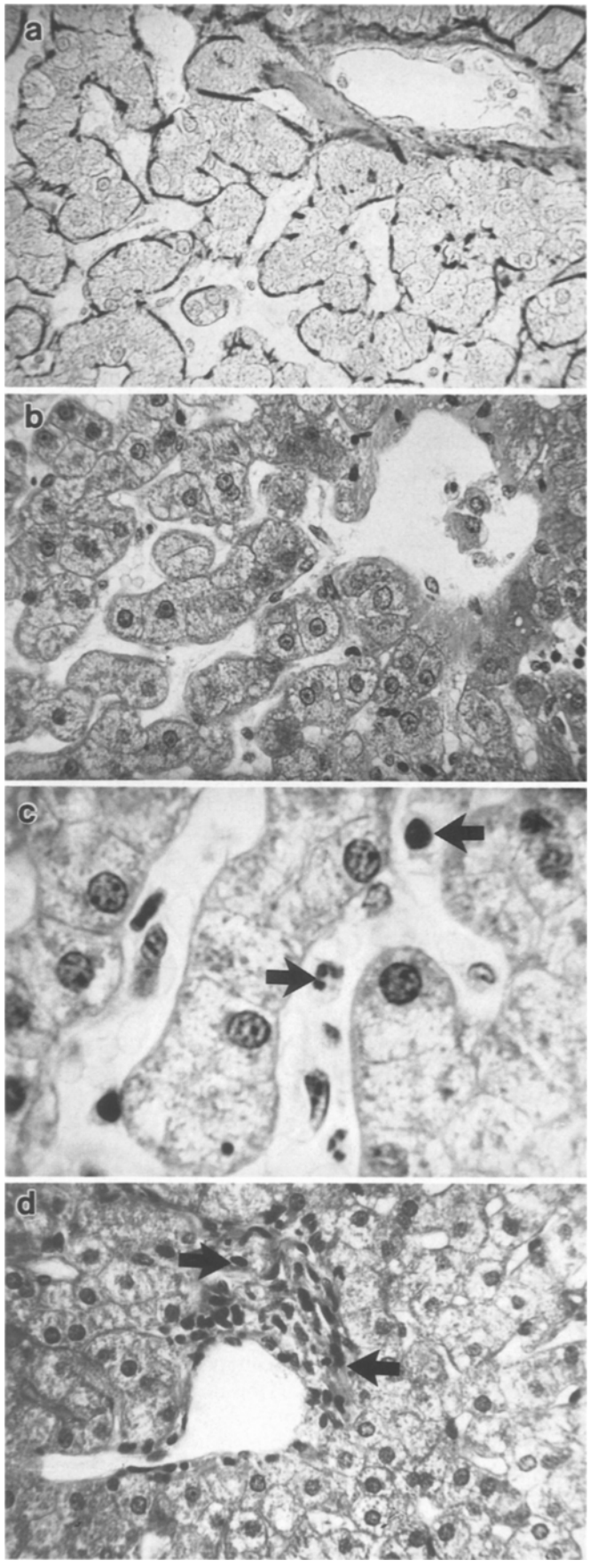

Fig. 1 a–d. Morphologically normal hepatocytes from centrolobular zone with histological vascular lesions. **a, b** Sinusoidal dilatation. Gordon and Sweet's method **a**, Masson's trichrome **b**. **c** Sinusoidal inflammation (*arrows*). Haematoxylin and eosin. **d** Phlebitic reaction in terminal hepatic vein (*arrows*). Masson's trichrome. **a, b, d** $\times 384$; **c** $\times 960$

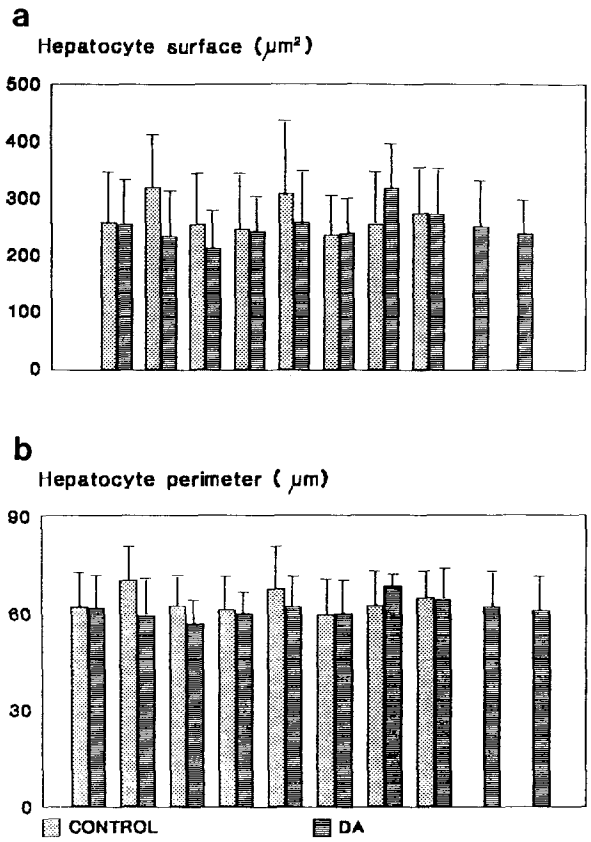

Fig. 2a, b. Morphometric analysis of 1800 hepatocytes from biopsies of controls ($n=8$) and DA ($n=10$) patients. Tested by F-test, non-significantly different, $P=0.24$ for hepatocyte surface area (**a**) and $P=0.18$ for hepatocyte perimeter (**b**)

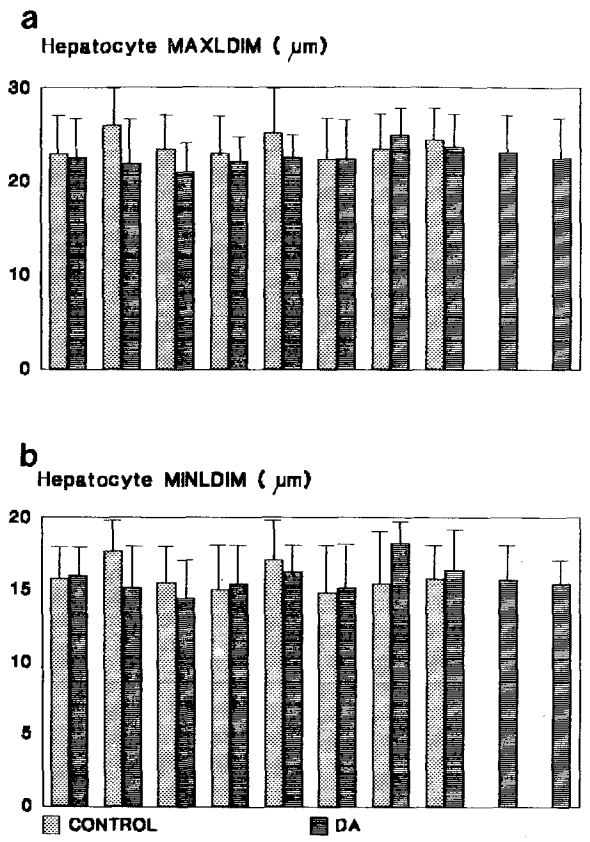

Fig. 3a, b. Histogram of hepatocyte linear dimension **a** maximum linear dimension (*MAXLDIM*); **b** minimum linear dimension (*MINLDIM*)] assessed by morphometric study of 1800 hepatocytes from biopsies of controls ($n=8$) and DA ($n=10$) patients. Tested by F-test, non-significantly different $P>0.125$

with an electronic pen and projected onto the monitor linked to a digitizing table. The study was performed on three to five centrilobular areas (zone III) for each case. Four sections per block were evaluated and a total of 67 perivenous fields were studied for the total number of patients. In each case 100 hepatocytes were measured. In order to work on a homogeneous cellular population, the binucleate and multinucleate hepatocytes were excluded. The measurements were automatically calculated by the computer and variables were obtained: surface area (Surface), perimeter, maximum linear dimension (Maxldim) and minimum linear dimension (Minldim).

The computer software for statistical data evaluation was Manova (SPSS-Chicago). Our study was designed to compare two groups of subjects (controls and DA) following the four morphometric variables mentioned above and two factors which corresponded to measurement variations between the hepatocytes in each group: the first factor with a fixed effect and the second nested under the first one with a random effect. Following that schema a model of variance analysis with two nested factors was used. The F-test performed on the first factor accounted for the random level of the second factor. Values were expressed as mean ± standard error of mean (SEM) and the level of significance was $P<0.125$.

Results

Histological analysis of the hepatocytes in the zone III of hepatic acini from DA showed no cell morphological alterations such as steatosis, necrosis or cholestasis (Fig. 1), although vascular intralobular lesions were present as follows: (1) sinusoidal dilatation; (2) numerous inflammatory cells (polymorphonuclear leucocytes and mononuclear cells) in the sinusoidal lumen; (3) phlebitis of the terminal hepatic veins in association with a thickening of the walls (Trigueiro de Araújo et al. 1990). Figures 2 and 3 and Table 1 show hepatocyte morphometric measurements in controls and DA patients. For the total number of patients (controls and DA) 1800 hepatocytes were analysed. There were no statistically significant differences for the variables of

Table 1. Hepatocyte morphometry in controls and drug addicts (DA): analysis of measured variables

	Controls $n=8$	DA $n=10$	P
Surface	268.66 ± 95.25	252.00 ± 78.94	NS
Perimeter	63.86 ± 11.12	61.62 ± 9.92	NS
Maximum linear dimension	23.84 ± 4.47	22.62 ± 3.89	NS
Minimum linear dimension	15.90 ± 3.09	15.82 ± 2.80	NS

Values expressed in $\mu m^2 \times 10{,}000$ (mean ± SEM) for hepatocyte surface and $\mu m \times 10{,}000$ (mean ± SEM) for perimeter, maximum linear dimension and minimum linear dimension. n = number of patients analysed (1800 hepatocytes measured in the total patient group). Tested by F-test, non-significantly different (NS) $P>0.125$

measurements between the two series of patients mentioned, particularly for the cell surface parameter (controls: 268.66±95.25; DA: 252.00±78.94, $P=0.24$).

Discussion

The pathogenesis of liver dysfunction among DA has been a source of controversy for many years. Attempts to explain the clinical and morphological lesions in DA led some authors to conclude that these hepatic alterations are due to the use of contaminated hypodermic needles (Litt et al. 1972; Potter et al. 1960). In other reports, liver damage is seen as a direct hepatotoxic effect of the drug (Chang and Ho 1979; Marks and Chapple 1967).

In man and in animals it has been demonstrated that elevated serum transaminases, as well as the depletion of hepatic glutathione, are important biochemical consequences of liver injury after morphine, cocaine and heroin administration. Glutathione provides cell protection against reactive metabolites in the liver (Chang and Ho 1979; James et al. 1982; Eklöw-Låstbom et al. 1986; Lechón-Gomez et al. 1987; Marks and Chapple 1967; Nagamatsu et al. 1986; Skoulis et al. 1989). Studies have indicated that the most pronounced toxic effects are seen in heroin exposure. Its biotransformation to monoacetylmorphine and morphine by deacetylation was found to occur most rapidly and to the greatest extent in the liver (Way et al. 1960). Morphine can also directly induce several hepatocellular lesions and a marked loss of cell viability through its metabolic conversion to morphinone and morphinone-glutathione (Nagamatsu et al. 1986). It has been reported that morphine metabolization leads to lipid accumulation in hepatocytes (steatosis), cholestasis or hepatic necrosis with an overall abnormal liver function (Litt et al. 1972; Nagamatsu et al. 1986; Thuresen-Klein et al. 1978). Thus, clinical and histopathological lesions can be used to explain the direct hepatotoxicity inherent in metabolic intermediate drugs (James et al. 1982). In a previous study, we demonstrated a significant increase in the volume sinusoidal density accompanied by terminal hepatic vein inflammation, mainly in zone III of the hepatic acini, related to heroin consumption. A perisinusoidal and venular fibrosis, associated with a thickening of the terminal hepatic vein wall, was observed after drug withdrawal (Trigueiro de Araujo et al. 1990). Reports indicate that several other drugs are implicated in the direct mechanism of hepatic injury, represented by necrosis, cholestasis, drug-induced hepatitis and morphological features of hypersensitivity, with changes of hepatocyte structure (Dalich and Larson 1985; Davion et al. 1984; Kunze et al. 1985; Roschlau 1983; Schmidt et al. 1985).

The present study is an attempt to determine whether the heroin consumption is responsible for hepatocytic atrophy and the consequences of this in the pathogenesis of sinusoidal liver dilatation, using histological and morphometric analysis. A study was carried out on ten needle biopsies from patients who had administered heroin parenterally and who had elevated serum transaminases. They were HBs-Ag and anti-HIV negative and they represented a subgroup of patients included in our previous study (Trigueiro de Araújo et al. 1990). They were compared with eight surgical biopsies from control patients showing no clinical, biological or histological liver alterations. Histological analysis of DA biopsies showed lobular vascular lesions, predominantly in centrilobular areas. Cholestasis, steatosis and cellular necrosis were absent. On the basis of morphometric data, hepatocyte surface area, diameter and perimeter showed statistically non-significant differences, when controls and DA were compared. Consequently, at the time of biopsy, changes in hepatocyte morphology were not seen in DA, although there was elevation of serum transaminase. These findings are in accordance with previous observations by Borgia et al. (1982). In an experimental study, they showed an elevation of serum glutamate oxalacetate transaminase and serum glutamate pyruvate transaminase values in morphine- and heroin-dependent mice. However, no light microscopic changes in liver histology were found and ultrastructural lesions were only detected morphometrically. For our patients, although biological liver function was abnormal, no structural lesion was found in hepatocytes, by light microscopic quantitative analysis. These morphological data support the results obtained in our previous study (Trigueiro de Araújo et al. 1990), in which we suggested that the drug is directly responsible for sinusoidal and terminal hepatic vein damage in heroin users. On the basis of these new findings, therefore, we were able to conclude that the hepatocyte size is not implicated in the pathogenesis of lobular vascular hepatic injury related to heroin addiction.

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