The Canals of Hering Might Represent a Target of Methotrexate Hepatic Toxicity

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Abstract

Methotrexate treatment for psoriasis is known to cause hepatic fibrosis in some patients, which might progress to cirrhosis. The fine, radiating, fibrous septa developing in this setting have a distribution that is reminiscent of the location of the canals of Hering (coH). To assess the possibility of fibrous obliteration of the coH in patients receiving methotrexate, we developed a staining technique by combining an immunohistochemical stain for cytokeratin 7 with a modified Masson trichrome. Sixteen biopsy specimens from 7 patients were evaluated. The biopsies had a variety of histologic changes, including steatosis, anisonucleosis, multinucleation, chronic inflammation, bile duct damage, and ductular reaction. Fibrosis was present in 13 biopsy specimens (81%) and was mild in 7, moderate in 3, and severe in 3 specimens. Compared with normal (control) liver specimens, biopsy specimens from patients receiving methotrexate had decreased numbers of coH (1.9 ± 0.8 vs 5.2 ± 1.7; P < .025). In specimens with moderate or severe fibrosis, fibrous septa sometimes extended along the coH. These findings suggest that scarring of the coH might be a consequence of the toxic effects of methotrexate.

Methotrexate (amethopterin) is a methyl analog of folic acid, which is widely used in the treatment of various neoplastic and nonneoplastic diseases such as leukemia; lymphoma; osteosarcoma; carcinomas of the head and neck, breast, and lung; choriocarcinoma; hydatidiform mole; rheumatoid arthritis; and psoriasis.1,2 Methotrexate acts as an antimetabolite by inhibiting dihydrofolate reductase and, thus, blocking the reduction of dihydrofolates to tetrahydrofolates, a step that is crucial in the synthesis of purines and pyrimidines. As a result, methotrexate exerts its greatest effect on proliferating cells, such as fetal cells, neoplastic cells, bone marrow cells, epidermal cells, and epithelial cells of the gastrointestinal and genitourinary tracts.

Methotrexate treatment is known to produce hepatic injury in a substantial number of patients.3-7 The histologic features of the toxic effects of methotrexate are nonspecific and include hepatocytic changes such as steatosis, “glycogen” nuclei, multinucleation, anisonucleosis, and lipofuscin accumulation, as well as chronic inflammation of portal tracts, bile duct damage, ductular reaction, and fibrosis.8-10 The pattern of fibrosis is characteristic and comprises thin fibrous septa extending from the portal tracts into the lobules, often in a stellate configuration. Progressive fibrosis over a period of years eventually might lead to cirrhosis. The value of liver biopsies during methotrexate treatment is well established.6,11,12

In the process of examining liver biopsy specimens from patients receiving methotrexate, we noticed that the fibrous septa extending from the portal tracts into the lobules often had a distribution reminiscent of the location of the canals of Hering (coH). This observation suggested that the fibrous septa actually might represent scarring of the coH. We conducted the present study to investigate this possibility.
Materials and Methods

Tissue Samples

We used paraffin-embedded sections of 16 archival liver biopsy specimens, obtained from 7 randomly selected patients during the course of oral methotrexate treatment for psoriasis. All patients were treated and underwent biopsy at New York University Medical Center, New York, NY. Liver disease was ruled out by clinical and laboratory evaluation before the initiation of treatment. The patients were advised to completely abstain from alcohol consumption. Demographic information and the cumulative dose of methotrexate are given in Table 1. Sequential 4-µm-thick sections were stained with H&E and with a method developed specifically for the present study. Six specimens of near-normal liver tissue (donor liver specimens obtained at transplantation) were used as a control group.

Staining Procedure

To best visualize the fibrous septa and the coH on the same slide, we devised a staining technique by combining an immunohistochemical stain for cytokeratin 7 (CK7) with a modified Masson trichrome. The sections were deparaffinized and hydrated through graded alcohols. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide for 20 minutes. The sections then were washed in running tap water, rinsed with distilled water, and incubated in a 1% solution of trypsin for 30 minutes (37°C). The sections then were washed again with tap water, rinsed with distilled water, and washed with phosphate-buffered saline (PBS; pH 7.2). This was followed by incubation with the CK7 monoclonal antibody (DAKO, Glostrup, Denmark) in a 1:200 dilution for 30 minutes (room temperature). The sections then were washed with PBS, incubated with peroxidase-conjugated rabbit antimouse immunoglobulins (DAKO) in a 1:200 dilution for 30 minutes, washed again with PBS, and incubated with peroxidase-conjugated swine antirabbit immunoglobulins (DAKO) in a 1:200 dilution for another 30 minutes. After an additional wash in PBS, the reaction product was visualized with diaminobenzidine.

The sections then were washed in tap water, stained in Weigert iron hematoxylin solution for 1 minute, washed again in tap water, and rinsed in distilled water. They then were stained with Biebrich scarlet-acid fuchsin (9:1 vol/vol) solution for 7 minutes, rinsed in distilled water, and differentiated in 2.5% phosphomolybdic–2.5% phosphotungstic acid.

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Table 1

Demographic Information for Patients, Cumulative Methotrexate Dose, and Semiquantitative Assessment of Histologic Features in Biopsy Specimens

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An, anisomucleosis; BD, bile duct damage; CD, cumulative methotrexate dose (g) at the time of biopsy; DR, ductular reaction; Fi, fibrosis; GN, “glycogen” nuclei; Mu, multinucleation; Ne, necroinflammatory activity; PI, portal inflammation; PT, portal tracts; St, steatosis.

* The c/p ratio is the number of canals of Hering profiles per number of portal tracts. The following grading scale was used for the histologic features: 0, absent; 1+, mild; 2+, moderate; 3+, marked. Fibrosis was graded as follows: 0, normal amount of fibrous tissue; 1+, mild fibrosis (fibrous expansion of portal tracts without evident fibrous septa); 2+, moderate fibrosis (presence of fibrous septa without distortion of hepatic architecture, corresponding to grade IIIA fibrosis of the classification system by Roenigk et al9); 3+, severe fibrosis (fibrosis with architectural distortion, corresponding to grade IIIB fibrosis of the classification system by Roenigk et al9); 4+, cirrhosis.
solution for 10 minutes. The sections were washed again in tap water, rinsed in distilled water, and stained in aniline blue solution (2.5%) for 2 minutes. Following this, they were rinsed in distilled water and differentiated in 1% acetic acid for 3 minutes. Finally, they were dehydrated, cleared, and coverslipped.

Microscopic Evaluation

The histologic features were assessed blindly by 2 pathologists (P.H., N.D.T.) working independently. Differences in opinion were resolved in conference. The following histologic features were graded semiquantitatively on a 0 to 3+ scale (0, absent; 1+, mild; 2+, moderate; 3+, marked): steatosis, anisonucleosis, multinucleation, “glycogen” nuclei, lobular necroinflammatory activity, portal inflammation, bile duct damage, and ductular reaction.

Fibrosis also was graded on a semiquantitative scale, as follows: 0, normal amount of fibrous tissue; 1+, mild fibrosis (fibrous expansion of portal tracts without evident fibrous septa); 2+, moderate fibrosis (presence of fibrous septa without distortion of hepatic architecture, corresponding to grade IIIA fibrosis of the classification system by Roenigk et al9); 3+, severe fibrosis (fibrosis with architectural distortion, corresponding to grade IIIB fibrosis of the classification system by Roenigk et al9); 4+, cirrhosis.

The coH were recognized as individual cells or small strings of CK7-positive cells within the hepatic lobules, characterized by oval shape and a high nuclear/cytoplasmic ratio Image 1. The total number of coH and the total number of portal tracts were counted in each biopsy specimen. The number of coH profiles per number of portal tracts (c/p ratio) was calculated for each specimen. Only complete or almost complete terminal portal tracts were included in the calculations. CK7-positive circular profiles at the edges of the portal tracts were considered to be ductules and were not counted. The c/p ratios of the patient biopsy specimens were compared with those of the control group by using the $\chi^2$ test.

Results

The biopsy specimens demonstrated a variety of histologic changes, which were graded semiquantitatively as shown in Table 1. Steatosis was present in 13 specimens (81%): mild in 8 cases, moderate in 3, and marked in 2. Anisonucleosis and multinucleation of hepatocytes were seen in all specimens and usually were mild. A small number of glycogen nuclei also were noted in the hepatocytes of most cases. Chronic inflammatory infiltrates were present in the portal tracts of 13 specimens (81%) and were graded as mild in 12 specimens and moderate in 1. In 11 of these 13 specimens, portal inflammation was accompanied by some degree of lobular necroinflammatory activity. Such activity was graded as mild in 10 cases and moderate in 1 (the same case that showed moderate portal inflammation). Epithelial cell changes suggestive of mild bile duct damage were present in the majority of specimens with portal inflammation (12 of 13 cases); in most of these specimens, mild (8 cases) or moderate (3 cases) ductular reaction also was noted in the region of the limiting plate.

Fibrosis was present in 13 specimens (81%) and was mild in 7, moderate in 3, and severe in 3 specimens. No correlation between fibrosis and cumulative methotrexate dose or duration of therapy was noted. In the 6 specimens with more than mild fibrosis, thin fibrous septa extending from the portal tracts into the lobules were present; the coH sometimes were involved by these septa Image 2. The number of coH in biopsy specimens from patients receiving methotrexate was decreased compared with the number in control specimens, and the difference in c/p ratios was statistically significant ($1.9 \pm 0.8$ vs $5.2 \pm 1.7$; $P < .025$) Image 3. No correlation between the degree of fibrosis and the c/p ratio was found in these specimens; although the c/p ratio tended to be higher in specimens with severe fibrosis than in those with less pronounced degrees, the difference was not statistically significant. Ductular reaction was observed only in specimens with fibrosis (11 of 16 biopsy specimens) and usually was mild. Of 3 cases with a moderate ductular reaction, 2 had severe fibrosis and 1 had moderate fibrosis.
Discussion

We evaluated the histologic changes of 16 liver biopsy specimens from 7 patients receiving methotrexate. In addition to changes that are already well described in the literature, such as steatosis, anisonucleosis, multinucleation, glycogen nuclei, portal and lobular inflammation, bile duct damage, ductular reaction, and fibrosis, we found reduced numbers of coH in these specimens compared with control specimens. The decreased number of coH and the topographic distribution of fibrous septa in contiguity with canals suggest that scarring of coH might result from the toxic effects of methotrexate.

Methotrexate-induced damage to bile ducts and coH was reported in an ultrastructural study by Hopwood and Nyfors\textsuperscript{13} more than 25 years ago. Pathologic changes included the presence of atrophic cells and luminal debris, accumulation of lipofuscin, widespread damage to mitochondria, foci of intracellular edema, hypertrophy and dilatation of the Golgi apparatus, decreased number of microvilli with presence of damaged forms, dilatation of the lateral intercellular spaces with presence of debris, and zones of duplication

Image 2 A, Occasional thin fibrous septa connect a portal tract with nearby canals of Hering. B, In this area of marked periportal fibrosis, several fibrous septa extend along canals of Hering (A and B, combined cytokeratin 7–Masson stain, ×200).

Image 3 A, The hepatic parenchyma around this portal tract is largely devoid of canals of Hering (combined cytokeratin 7–Masson stain, ×100). B, On higher power examination, parts of a single canal are seen (arrows) (combined cytokeratin 7–Masson stain, ×240).
of basement membranes. These changes might lead to coH loss, as documented in the present study.

The coH have received little attention until recently, being widely thought of simply as tubes that transport bile from the lobules to the portal tracts. This is largely because the coH are not visualized easily on histologic sections with routine stains, leading to an underestimation of their importance in liver physiology and pathology. However, Theise et al. demonstrated that the coH contain hepatic progenitor cells with the capacity to regenerate hepatocytes and bile duct cells in livers with massive hepatic necrosis. Saxena et al. have shown that the coH, along with small and medium-sized bile ducts, are the target of immune attack in primary biliary cirrhosis, leading to a statistically significant reduction in the number of coH (and, therefore, the c/p ratio) in liver biopsy specimens from patients with this disease. Furthermore, we have noticed a proliferative attempt to restore the numbers of coH in stage 2 primary biliary cirrhosis. This is commensurate with the ductular reaction characteristic of this stage of the disease.

The findings of the present study indicate that a similar pattern of changes involves the coH in methotrexate toxicity. Significant loss of coH seems to be an early alteration in these livers. Continued administration of methotrexate might lead to scarring, which is accompanied by an increase in the numbers of coH and bile ductules (ductular reaction). Similar to our findings in primary biliary cirrhosis, the increase in the number of coH might be a delayed attempt at compensatory regeneration. However, as in primary biliary cirrhosis, this response fails to restore coH to normal levels. The histologic similarity of the changes occurring in primary biliary cirrhosis and methotrexate toxicity and their timing are consistent with damage to a progenitor cell population with a long renewal time.

The special stain we devised for the study provides the opportunity to visualize fibrous septa and CK7-positive structures (bile ducts, ductules, coH, and progenitor cells) on the same histologic section. Therefore, this method might be useful in future studies of epithelial-mesenchymal interactions in a variety of liver diseases. However, in this biopsy material, we were unable to demonstrate a correlation between the c/p ratio and the degree of fibrosis. This may be due to the small number of cases assessed as well as to sampling error; larger studies are needed to definitively elucidate such correlations, if any. Furthermore, the detection of fibrous septa on follow-up biopsy specimens from these patients was an indication to discontinue methotrexate treatment.

Our findings should not be interpreted as indicating that the cells of the coH are the only hepatic cell type damaged by methotrexate. The hepatocytes are a documented target of toxicity, as evidenced by the wide range of changes observed early during methotrexate treatment. Such changes extend from the light microscopic (steatosis, anisonucleosis, multinucleation, glycogen nuclei, lipofuscinosis) to the ultrastructural level (pleomorphic mitochondria, increased number and size of lysosomes).

The question then arises about what is the cause of fibrosis in these patients. Although our findings suggest that the fibrous septa follow the course of the coH, elucidation of the mechanisms responsible for fibrosis, including the possible roles of inflammatory cell infiltrates, activated stellate cells, and proliferating ductular and progenitor cells, will require further study. From a clinical standpoint, the reversibility of fibrosis observed in a significant number of patients emphasizes the need for close follow-up with liver biopsies during treatment. Future research in this area is needed to elucidate the mechanisms of methotrexate toxicity to hepatocytes and coH, improving our understanding of the destruction and regeneration of coH in chronic liver disease and of any possible role in hepatic fibrogenesis.

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References


