Azathioprine induced nodular regenerative hyperplasia in IBD patients

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SUMMARY
Purine analogues are major drugs in the treatment of inflammatory bowel diseases (IBD). We present four cases of nodular regenerative hyperplasia of the liver (NRH) developed in patients with IBD treated with azathioprine. All patients had either abnormal liver tests and/or low platelet count. Although biochemical and hematological abnormalities regressed after azathioprine withdrawal, the long term evolution of the hepatic lesions (and the risk to develop further complications including portal hypertension) remains to be determined. Male gender seems to be a major risk factor by providing a predisposing pharmacogenetic profile of purine analogue metabolism. Clinicians should be aware of this serious complication which may occur with any of the purine analogues (azathioprine, 6-mercaptopurine, and 6-thioguanine).

RÉSUMÉ
Hyperplasie nodulaire régénérative du foie chez des malades atteints de MICI induite par l’azathioprine
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Introduction
Purine analogues (PA) are commonly used for the treatment of inflammatory bowel disease (IBD), autoimmune hepatitis, organ transplantation and various autoimmune diseases [1]. Among the 3 PA which can be used in clinical practice, azathioprine (aza) and 6-mercaptopurine (6-MP) are the most commonly prescribed. 6-thioguanine (6-TG) has also been proposed and showed to be usually well tolerated in the short term in patients with previous intolerance to aza or 6-MP [2]. However, Dubinsky et al. recently reported an alarming paper showing a high risk of developing nodular regenerative hyperplasia of the liver (NRH) in a series of patients with IBD who received 6-TG because of resistance (and sometimes intolerance) to aza or 6-MP [3]. NRH was described in association with many other diseases but was rarely reported in association with IBD and only in 4 patients treated with aza or 6-MP [4-7]. Furthermore, the natural history once the offending agent is removed is unknown [3]. We report four cases of NRH developed in IBD patients during aza therapy.

Case reports
Case n° 1
A 44 year old man had steroid-dependent ulcerative colitis since January 1999. He was treated with aza 200 mg/day (2.5 mg/kg) starting January 2000. Six months later liver tests abnormalities were noted during monthly follow-up (figure 1): aspartate aminotransferase (ASAT) 1.7 fold the upper limit of normal range (ULNR), alanine aminotransferase (ALAT) 1.1 fold ULNR, gamma-glutamyl transpeptidase (GGT) 1.0 fold ULNR, alkaline phosphatases (AP) 1.5 fold ULNR. Bilirubin, albumin, hemoglobin, white blood cells (WBC) and prothrombin time (PT) were normal. Platelet count was 129000/mm³. Hepatic and biliary magnetic resonance imaging showed normal liver and bile ducts. The patient did not consume alcohol or any other drug; he had no personal or family history of liver diseases. Chronic viral, auto-immune, and metabolic work-up for liver diseases was negative, as was the search for hypercoagulable disorders. Thiopurine methyltransferase (TPMT) activity was 12 nmols/h/mL of red blood cells (RBC) (normal range 8.5-15.5). Percutaneous liver biopsy (figure 2) found areas of distorted architecture with regular alternance of atrophic and regenerative hepatic plates. Mild perisinusoidal and portal fibrosis was noted with no bridging or reticulin staining. A few inflammatory cells could be seen in some portal tracts, but bile ducts had normal epithelia, with no evidence of cholangitis or bile stasis. Hepatic venules and arterioles were normal as were portal venules. Gastrscopy was normal. GGT rose to a maximum value of 10.2 fold ULNR in 12 months time. Aza was withdrawn on May 2001. Biological cholestasis slowly regressed and liver test normalized on
February 2003. However, platelet count remained slightly abnormal: 145,000/mm³ and normalized on March 2004 (figure 1). No other immuno-suppressive agent was introduced and the colitis remained in remission.

Case no 2

A 35 year-old man was referred on October 2001 for biological cholestasis and suspicion of primary sclerosing cholangitis complicating IBD. He had left sided ulcerative colitis since 1995 and was treated since January 2000 by aza 175 mg/day (2.0 mg/kg) because of steroid dependency. TPMT activity was 16 nmoles/h/mL of RBC. Blood counts and liver tests were all normal prior to aza therapy and were monitored during treatment on a weekly basis for the first month, then monthly. On June 2000, the patient was in clinical remission without steroids and abnormal liver tests were noted: ASAT 1.6 fold ULNR, ALAT 1.2 fold ULNR, GGT 3 fold ULNR, PAL 1.4 fold ULNR. The dose of aza was reduced to 100 mg/day (1.2 mg/kg) but further follow-up disclosed fluctuating levels of liver function tests (figure 1) with persistently normal bilirubin and platelets. On February 2002, physical examination was normal. On complete blood count, hemoglobin was 14.6 g/dL, white blood cells was 4800/mm³, erythrocyte sedimentation rate (ESR) was 35 mm on the first hour, and amylase, lipase, protein electrophoresis and PT were all normal. Liver tests were as follow: ASAT 2.1 fold ULNR, ALAT 1.8 fold ULNR, PAL 1.2 fold ULNR, GGT 6 fold ULNR, and normal bilirubin. Abdominal ultrasound was normal. Endoscopic ultrasound (EUS) disclosed an irregular common bile duct, with no wall thickening. Trans-parietal percutaneous liver biopsy showed nodular foci of regenerative hepatocytes, a normal reticulin network with no septal or portal fibrosis. There was no evidence of primary sclerosing cholangitis, steatosis, hepatosiderosis, portal or lobular inflammation, or endothelial injury. Hepatic and portal venules were normal. Few portal tracts enclosed some neoductules without major ductular neogenesis or cholestasis. The patient did not consume alcohol or other drugs. Viral, metabolic, and auto-immune liver work-up was negative, as well as the search for hypercoagulable disorders. Gastroscopy found no evidence of portal hypertension and the colitis was found to be in endoscopic and histological remission. Aza was stopped on March 2002, after 26 months of treatment. On July 2003, IBD was still in remission with normal liver tests and platelet count (250,000/mm³). On February 2004, a mild flare-up was controlled with 5-ASA (oral + enema). Platelet count was 173,000/mm³, GGT rose to 2 fold ULNR, other liver tests remaining within normal range (figure 1).

Case no 3

A 46 year old man presented on January 2003 for biological cholestasis. He had ileo-colonic Crohn’s disease since 1991 with subsequent
pmoles/8

6-Methylmercaptopurine ribonucleotides levels (6MMPR) was 3824 × guanine nucleotides (6-TGN) level was 352 pmoles/8

immune liver work-up was negative, as well as the search for hypercoagulability, hyperhomocysteinemia, and ESR, glycaemia, PT. Viral, metabolic, and autoimmune causes of liver disease were excluded. Albumin, C reactive protein, lipid profile and alpha-foetoprotein were normal. Laboratory tests showed: hemoglobin 12.6 g/dL, white blood cells 108RBC; neutrophils 50% (range 40-70%), platelets 130000/mm³, normal AST, ALT 1.2 fold ULNR, AP 2 fold ULNR, GGT 1.5 fold ULNR. Bilirubin, PT and albumin were normal. These abnormalities persisted despite dose reduction to 100 mg/day and aza was stopped on June 1997 for worsening thrombocytopenia (76,000/mm³). At that time, gastroscopy revealed grade 1 esophageal varices. Abdominal ultrasound found enlarged liver and spleen. The patient did not consume alcohol or other drugs. Search for viral, metabolic, and autoimmune causes of liver disease was negative. Increase in factor VIII was the only potential thrombophilic disorders found. Transjugular liver biopsy disclosed large areas of hyperplastic liver plates surrounding zones of atrophy presenting moderate sinusoidal dilatation and perisinusoidal fibrosis. Only one portal space contained a discrete inflammatory neutrophilic and lymphocytic infiltrate with normal bile ducts. The remaining portal spaces were normal with no widening or fibrosis. There was however some dilated portal venules with images of portal angiomatosis. Hepatic lobules presented normal appearing hepatocytes and centrolobular veins, with no evidence of inflammation, necrosis, fibrosis, steatosis, or sinusoidal inflammation. There was also no cholestasis or hemosiderosis. There was no portal-venous venous gradient during venous pressure recording (2 mmHg). During regular biological follow up, no cholestasis was noted. Slight fluctuations of transaminases levels within the range of 0.8-1.2 fold ULNR were observed. Liver tests and platelets were normal on November 2000. On December 2000, the patient developed ascites associated with a severe flare-up of his Crohn's disease and hypalbuminemia (25 g/L). An intravenous steroid treatment was established after excluding other possible causes of liver disease. Compared to the first cases of IBD patients with aza related NRH published until now, our patients had less clinical signs (none of them had jaundice), a lower cumulative dose of aza and a shorter duration of exposition (range 6-12 months). The earlier diagnosis is probably due to a better systematic biological follow up. We agree with Dubinsky et al. that many patients with slight abnormalities of liver function tests during aza/6-MP therapy do not have liver biopsy and that NRH or other vascular lesions may be underscored [3].

The evolution of hepatic lesions, portal hypertension, biologic signs, and complications after stopping the offending PA has not been reported in literature. We observed in our patients (cases 1, 2, and 4) a progressive normalization within several months. We report here four cases of NRH in IBD patients treated with aza. Hepatotoxicity was revealed by liver function test and/or blood platelet count abnormalities and the diagnosis of NRH was made on histological basis. This diagnosis may be difficult to establish based on findings in small needle biopsies. Reticulin stains are especially useful in identifying the unique structural features of nodular regenerative hyperplasia. The responsibility of aza was established after excluding other possible causes of liver disease. Compared to the first cases of IBD patients with aza related NRH published until now, our patients had less clinical signs (none of them had jaundice), a lower cumulative dose of aza and a shorter duration of exposition (range 6-12 months). The earlier diagnosis is probably due to a better systematic biological follow up. We agree with Dubinsky et al. that many patients with slight abnormalities of liver function tests during aza/6-MP therapy do not have liver biopsy and that NRH or other vascular lesions may be underscored [3].

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months of AST, GGT and AP as well as the platelet count (figure 1). However this does not prove that lesions will improve and it remains important to follow such patients for the potential development of portal hypertension. Russman et al. described a case of an aza-induced NRH occurring in a patient with ulcerative colitis and which was complicated by hepatocarcinoma [6]. Regular surveillance of hepatic ultrasound and alpha-fetoprotein seems therefore warranted.

PA metabolism is complex and variable and this is probably why the precise toxic metabolite(s) from PA responsible for these hepatic vascular lesions has not been identified yet [8, 9]. This metabolite may differ from the metabolite(s) responsible for other hepatic side effects such as immunological hepatitis and cholestasis [10, 11]. There seems to be three risk factors for PA-induced NRH: male gender, preferential 6-MMPR metabolism, and direct use of 6-TG. While NRH usually occurs without gender preference [12], male gender has been recognized as a major risk factor in all series of PA-induced NRH [4-7]. The only gender related change concerning PA metabolism reported to our knowledge is TPMT. Blood TPMT does not differ between women and men but Anthony et al. showed that TPMT is 1.46 higher in male human liver biopsies than those from females [13]. Noticeably, it has also been reported that boys need a higher dose of 6-MP than girls for leukemia treatment [13]. Noticeably, it has also been reported that boys need a higher dose of 6-MP than girls for leukemia treatment [13]. Blood TPMT was in the normal range in all of our 4 patients, but the enzyme activity was not assessed in their liver. The high risk linked to preferential 6-MMPR activity and direct use of 6-TG have recently been recognized by Dubinsky et al. [14]. In her series, the odds ratio for 6-TG-induced NRH was 2.97 in patients with preferential 6-MMPR production (which is even higher than the odds ratio for males, i.e. 2.86). We measured blood 6-MMPR in one of our patients with aza-induced NRH and found it in the normal range. The relative risk of 6-TG vs. aza or 6-MP to induce hepatic vascular lesions has not been properly measured but seems obviously high. This recently lead Dubinsky et al. to express their major concerns on the further use of 6-TG in patients with IBD [3]. The cumulative dose of 6-TG (which was not studied until now) may be important. Interestingly 6-TG is often used at higher doses in USA (40-80 mg/d) [2] than in Europe (20 mg/d) [15].

NRH can be severe, especially because of portal hypertension and may even need hepatic transplantation [16]. We conclude that severe vascular lesions of the liver, especially NRH can be seen in patients treated with PA for IBD. This does not occur only with 6-TG (which has at least at high daily doses a very high and unacceptable risk) but also with aza/6-MP. PA are major drugs in the treatment of IBD, especially in prevention of relapse and steroid sparing; their tolerance is usually very good. The present challenge is to identify sensitive risk factors or markers with a good positive and/or negative predictive value to better adapt indication and monitoring strategies. Clinicians should know that NRH may occur with only slight or even without biological abnormalities and that the diagnosis (which may be often underscored) presently relies on hepatic biopsy.

REFERENCES