Atovaquone/Proguanil-Induced Autoimmune-Like Hepatitis

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We report a novel association between the commonly used antimalarial medication atovaquone/proguanil and drug-induced autoimmune-like hepatitis. The patient developed severe liver disease fulfilling biochemical, immunologic, and histologic criteria for the diagnosis of autoimmune hepatitis after the inadvertent rechallenge with the offending drug, which had caused self-limited hepatitic symptoms a year previously. Over a period of 18 months, the patient underwent two follow-up liver biopsies showing progressive resolution of the liver inflammation and achieved complete biochemical and immunologic remission on steroids. This remission persisted for 20 months following treatment withdrawal. *Conclusion*: This well documented case raises awareness of the potential hepatotoxicity of atovaquone/proguanil. (*Hepatology Communications* 2017;1:293-298)

Introduction

Autoimmune hepatitis (AIH) is a rare immune-mediated liver disorder of unknown etiology. It is characterized by elevated transaminase and immunoglobulin G (IgG) levels, positive auto-antibodies, histologic evidence of interface hepatitis, and good response to steroid treatment.[1,2] Drug-induced liver injury (DILI) can mimic AIH.[3] On the one hand, a drug can unmask or induce classical AIH in a predisposed individual who will often have fibrotic changes on liver biopsy at presentation[4] and who will need chronic treatment with immunosuppressants[1]; on the other hand, a drug can cause a clinical picture indistinguishable from AIH but without fibrosis and without long-term steroid dependence.[3] The International Autoimmune Hepatitis Group (IAIHG) criteria for the diagnosis of definite or probable AIH[1,2] are fulfilled both in classical AIH and in drug-induced autoimmune-like hepatitis (DIAILH). The best documented drugs linked to DIAILH are minocycline and nitrofurantoin.[1,2] DIAILH has a more benign course than classical AIH[3] as steroid therapy can be withdrawn without recurrence. Although rechallenge with the offending drug is not advisable unless it is a life-saving drug, the recurrence of DIAILH after inadvertent rechallenge strongly weighs in favor of a causative link.[4] We report for the first time a well-documented case of atovaquone/proguanil-induced DIAILH that recurred with a more severe liver injury after inadvertent rechallenge with the antimalarial compound.

Case Report

A 65-year-old woman presented with a 10-day history of fatigue, jaundice, and dark urine after returning from Tanzania. She is a retired operating-room nurse and was previously in good health. She was on no other drugs, did not drink alcohol or smoke, and had

Abbreviations: AIH, autoimmune hepatitis; ANA, anti-nuclear antibodies; DIAILH, drug-induced autoimmune-like hepatitis; DILI, drug-induced liver injury; gDNA, genomic DNA; HLA, human leukocyte antigen; IAIHG, International Autoimmune Hepatitis Group; IgG, immunoglobulinG; PCR, polymerase chain reaction; ULN, upper limit of normal.

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TABLE 1. LABORATORY TESTS AT PRESENTATION AND DURING LONG-TERM FOLLOW-UP

	AST IU/L	ALT IU/L	ALP IU/L	Total Bilirubin μmol/L	INR	IgG g/L	ANA	SMA	Anti-LKM	AMA	ANCA
Normal range	<36	<37	35-104	<19.0		7.37-16.04	<1:80	<1:40	<1:20	<1:40	<1:20
At presentation	3,707	2,371	140	404	2.0	20.1	1:320*	1:40	neg	neg	neg
1 week on PDN	191	648		104.7	1.2						
2 weeks on PDN	63	251	107	77.6	1.0						
4 weeks on PDN	27	33	52	27.9	1.0						
3 months on PDN	17	10	39	12.7	1.0		1:80	neg			
6 months on PDN	18	10	40	7.2	0.9	12.4	neg	neg			
18 months on PDN	9	10	54	13.9	1.0	11.4	neg	neg			
1 month off PDN	14	13	59	13.2	0.9		neg	neg			
6 month off PDN	9	10	59	11.8	0.9	11.7	neg	neg			
18 month off PDN	10	<10	51	10.7	1.0	10.9	neg	neg			

*Homogeneous immunofluorescence pattern on HEp2 cells
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, anti-mitochondrial antibody; ANA, anti-nuclear antibody; ANCA, anti-neutrophil cytoplasmic antibody; anti-LKM, anti-liver/kidney microsomal antibody; AST, aspartate aminotransferase; IgG, immunoglobulin G; INR, international normalized ratio; neg, negative; PDN, prednisone; SMA, smooth muscle antibody.

no family or personal history of liver or autoimmune diseases. She had visited Tanzania yearly for 5 years, taking malaria prophylaxis with atovaquone/proguanil from 2 days before until 7 days after returning. After returning the previous year, she developed jaundice and fatigue, which were attributed to azithromycin, which was prescribed for acute bronchitis and was taken for 5 days, beginning 3 days following cessation of the malaria prophylaxis. She presented with malaise and fatigue 21 days after withdrawal of malaria prophylaxis and 14 days after withdrawal of azithromycin. At that time, her bilirubin level reached 240 μmol/L (upper limit of normal [ULN] 17 μmol/L), alanine transaminase was 1,382 IU/L (ULN 37 IU/L), international normalized ratio was 1.2, and blood eosinophil count was normal (205×10^9/L). Acute viral hepatitis A, B, C, and E were ruled out as were acute Epstein-Barr virus and cytomegalovirus infection. Anti-nuclear antibodies (ANAs) were positive at a titer of 1:320, with a homogeneous immunofluorescence pattern on HEp2 cells. A liver biopsy was not performed. She recovered spontaneously within 2 weeks.

At current presentation, she was in poor general condition, deeply jaundiced, with moderate ascites and no fever or rash. Atovaquone/proguanil was stopped 4 days before presentation because of the appearance of jaundice. Laboratory values over time are shown in Table 1. Acute viral hepatitis A, B, C, and E were excluded again. A liver ultrasound showed mild ascites, normal spleen size (9 cm), normal portal vein blood flow (21 cm/second), and absence of focal liver lesions. A liver biopsy, performed with a transjugular approach due to ascites and coagulopathy, showed portal inflammation with plasma cell-rich interface activity, severe zone 3 necrosis, but no significant fibrosis (Fig. 1A-D). Genetic testing for AIH susceptibility showed a heterozygous presence of the human leukocyte antigen (HLA) DRB1*0401 genotype. Based on these clinical,

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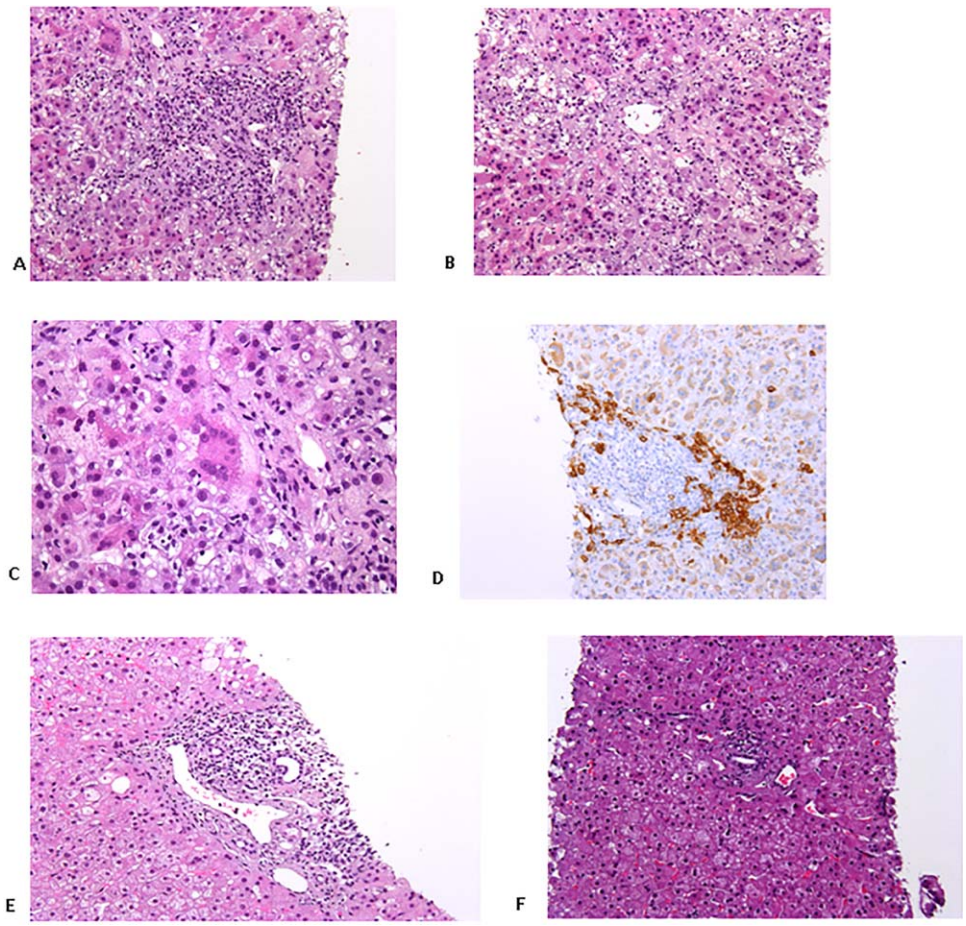

FIG. 1. Liver biopsy at presentation. (A) Marked portal inflammation with interface activity (HE stain, ×200 original magnification). (B) Severe zone 3 necrosis, loss of hepatocytes around the central vein (HE stain, ×200). (C) Syncytial giant cell transformation of hepatocytes (HE stain, ×400) and (D) numerous plasma cells at the interface highlighted by immunohistochemical staining for CD38 (×200). (E) Liver biopsy after 6 months of prednisone treatment (HE stain, ×200 original magnification) with mild interface hepatitis; (F) liver biopsy after 18 months of prednisone treatment (HE stain, ×100) showing absence of inflammatory infiltrates and a normal portal tract. Abbeviation: HE, hematoxylin and eosin.

biochemical, serologic, genetic, and histologic findings as well as an IAIHG scoring of 8, indicating definite AIH, a tentative diagnosis of type 1 AIH was made. Intravenous steroid therapy was started (prednisone 60 mg/day), and a rapid biochemical response followed. The prednisone dose was gradually tapered over 3 months to 5 mg/day. During a 6-month follow-up on prednisone 5 mg/day, transaminase levels reached and remained within the normal range, IgG normalized, and ANAs and anti-smooth muscle antibodies became negative (Table 1). Nevertheless, a liver biopsy continued to show mild lymphocytic portal infiltrates with a few plasma cells but no interface hepatitis (Fig. 1E). Because of the severity of the original presentation, the genetic background, and the persistence of portal hepatitis, prednisone 5 mg/day was continued for a further year despite persistently normal transaminase and IgG levels and negative auto-antibodies. At the end of the year, a further liver biopsy showed normal histology (Fig. 1F). Prednisone was then slowly withdrawn (1 mg/month) with no biochemical alterations. The patient is well with normal transaminase and IgG levels and negative auto-antibodies 20 months after stopping steroid treatment (Table 1).

The patient was investigated for genetic variations known to alter the function of CYP2C19 (*1A, *2, *3, *4, *5, *6, *7, *8, *9, *12, and *17), the enzyme involved in proguanil metabolism. Briefly, whole blood was collected in ethylene diamine tetraacetic acid K Monovettes by venipuncture. Genomic DNA (gDNA) was isolated from ∼350 μL blood with the EZ1 DNA Blood 350 μL Kit (Qiagen) on the EZ1 Advanced XL platform (Qiagen) according to the manufacturer's instructions. gDNA was quantified with the QIAxpert (Qiagen) spectrophotometer and had an absorbance $(A)_{260}/A_{280}$ between 1.7 and 1.9. Equal volumes of

gDNA (~50 ng/μL) and 2X TaqMan OpenArray real-time polymerase chain reaction (PCR) master mix were loaded onto the TaqMan OpenArray PGx panel with the OpenArray AccuFill system and run on the QuantStudio 12K Flex real-time PCR system according to the manufacturer's instructions. Results were analyzed by TaqMan Genotyper Software version 1.3 and verified by visual inspection of the real-time PCR amplification traces. All reagents, analysis software, and specialized equipment were from Thermo Fisher Scientific. The principle of this methodology is based on genotyping of single nucleotide polymorphisms by allele-specific hydrolysis probes, using endpoint fluorescence values generated by real-time PCR.[5]

Discussion

We describe for the first time a case of DIAILH related to the intake of the combined antimalaria drugs atovaquone and proguanil, followed up biochemically and histologically for 3.5 years until complete resolution of the liver pathology and absence of recurrence after treatment withdrawal.

DIAILH has been mostly reported in association with nitrofurantoin and minocycline treatment,[1-3] and more recently with statins,[6] antitumour necrosis factor α agents,[7] and black cohosh (*Cimicifuga racemosa*, *Actaea racemosa*), an herbal remedy widely used to treat menopause symptoms.[8-10]

Atovaquone/proguanil is a double-compound medication widely used for both prevention and treatment of malaria, with no DILI-resembling AIH having been reported. Mild transaminase elevation occurs in a small proportion of treated patients. A single case of acute hepatitis has been reported[11] with spontaneous recovery within 8 weeks after the last intake of the drug. A liver biopsy was not performed, and serum auto-antibodies (ANA, anti-mitochondrial antibody, anti-liver/kidney microsome antibody, anti-double-stranded DNA) were absent. Because atovaquone/proguanil is a double-compound medication, it is unclear which of the two drugs is responsible for DIAILH. In this context, the report of a patient who developed hepatotoxicity in association with the repeated use of a combination of chloroquine and proguanil is of interest[12] as it suggests a mechanism of sensitization similar to that observed in our case and a possible causative role for proguanil. This latter patient, however, was auto-antibody negative, did not undergo a liver biopsy, and recovered spontaneously within 1 month of stopping the drug.

That atovaquone/proguanil is the likely cause of the liver pathology in our case is corroborated by the severe relapse of liver disease after an inadvertent rechallenge with the drug due to the fact that the original hepatopathy had been attributed to azithromycin, a macrolide antibiotic reported to induce a hepatitic picture in 1% to 2% of patients treated for short periods.[13] Using the Council of International Organizations of Medical Sciences classification system, called the Roussel Uclaf Causality Assessment Method,[14] our case has a score of 10 for atovaquone/proguanil, which suggests a highly probable adverse drug reaction, and has a score of 7 for azithromycin, which suggests a probable adverse drug reaction. The relative high value of the score for azithromycin is due to its well-recognized hepatotoxicity, which is not the case for atovaquone/proguanil. Although the first hepatitis episode was reasonably attributed to azithromycin, the subsequent clinical course indicates that atovaquone/proguanil was probably responsible for the liver injury because acute hepatitis recurred after rechallenge with the latter drug combination and without exposure to azithromycin. Positive rechallenge is considered as the most reliable evidence of DILI.[4] Nevertheless, because this is the first report of DIAILH related to atovaquone/proguanil, we cannot exclude that the hepatitic episode and the exposure to the double-compound medication was just coincidental until at least a second similar convincing case is reported.

We note that the first symptomatic episode of liver toxicity was mild and resolved spontaneously after withdrawal of the offending drug in our patient; asymptomatic episodes during the previous exposures cannot be excluded. This could suggest that an immune-mediated sensitization occurred, as suggested for proguanil in the above-mentioned case report,[12] and for other drugs, such as halothane.[15] Halothane is metabolized by the CYP2E1 enzyme; its metabolite trifluoroacetyl can covalently bind to CYP2E1 to form protein adducts, which act as neo-antigens and are targeted by auto-antibodies, triggering immune-mediated hepatotoxicity.[16] Proguanil is metabolized by the CYP2C19 enzyme. The analysis of genetic variations known to alter CYP2C19 function (*1A, *2, *3, *4, *5, *6, *7, *8, *9, *12, and *17) showed that the patient is heterozygous for both *2 and *17 and is classified as an intermediate metabolizer with reduced capacity for metabolizing drugs by means of CYP2C19. It is therefore possible that the patient was exposed to an excessive proguanil concentration that could have led to the formation of protein adducts, giving rise to neo-

antigens that subsequently were recognized by the immune system, leading to the observed DILI. Possible mechanisms could include but are not limited to formation of hapten–carrier complexes or the pharmacological interaction with immune receptors, also known as the p-i-concept, both increasing the risk of immune-mediated hepatotoxicity.[17]

Differentiation between DIAILH and classical AIH is difficult and not facilitated by the application of the IAIHG scoring system, as confirmed in our case with a definite AIH score. Clinical presentation, biochemical and immunologic profiles, as well as liver histology in our patient were indistinguishable from those found in classical AIH. In addition, as in AIH, there was a swift response to corticosteroids. The fact that the first follow-up liver biopsy, performed 6 months after starting treatment, still showed some evidence of portal tract inflammation despite normalization of biochemical and immunologic indices was compatible with either diagnosis. The third biopsy, however, showing normal liver histology and the long-term follow-up without relapse after discontinuing steroids supports the diagnosis of DIAILH. Current guidelines[1] suggest treating cases of AIH-like disease after drug ingestion with steroids alone to be gradually tapered and stopped after normalization of liver function; if the disease does not relapse, a diagnosis of DIAILH is made. In our case, liver inflammation took a long time to resolve even in the presence of normal liver function tests, allowing a firm diagnosis of DIAILH only 3 years after presentation and underscoring the difficulty in clinical practice to assign a case to one or the other category.

To add complexity to the diagnostic workup, our patient is positive for HLA-DRB1*0401, an allele predisposing to adult-onset AIH type I without concomitant autoimmune disorders,[18] the association being strong enough to be considered of diagnostic relevance by the IAIHG.[19] Although it could be argued that this genetic background has played a predisposing role in the development of DIAILH, a recent paper on a large number of DILI with features of AIH could not demonstrate an association with possession of specific genes in the HLA region.[20]

In conclusion, we report a well-documented association between exposure to atovaquone/proguanil and DIAILH. As this is the first case description, the reporting of at least a second convincing case is needed to confirm the rare but potentially severe hepatotoxicity of this commonly used antimalarial prophylaxis/treatment. In our patient, the hepatotoxicity was possibly related to a CYP2C19 polymorphism.

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