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A novel aza-anthrapyrazole blocks the progression of experimental autoimmune encephalomyelitis after the priming of autoimmunity

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Abstract Mitoxantrone is one of the few FDA-approved drugs available to treat rapidly progressing forms of multiple sclerosis; however, its utilization is compromised by a cardiotoxic potential and the risk of mitoxantrone-induced leukemia. BBR3378, a novel aza-anthrapyrazole, is structurally similar to mitoxantrone, but lacks the ring hydroxyls that may contribute to cardiotoxicity. Here, we investigated the therapeutic activity of BBR3378 in a C57BL/6 mouse model of multiple sclerosis. Mice given BBR3378, before or after the priming and expansion of MOG-specific responses, were protected from ascending paralysis. Strikingly, two doses of BBR3378 given a week after EAE induction were sufficient to provide significant protection from clinical symptoms and reduce MOG-specific proinflammatory T cell cytokine production, and serum IgG responses. Furthermore, while mitoxantrone is associated with persistent lymphopenia and cardiotoxicity, no such outcomes were detected in BBR3378-treated mice. Our findings show that BBR3378 can ameliorate encephalitogenic mechanisms in EAE and antagonize underlying autoimmune mechanisms.

Abbreviations: MOG, myelin oligodendrocyte glycoprotein; MFI, mean fluorescence intensity; MNC, mononuclear cells.

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1. Introduction

Multiple sclerosis (MS) is a debilitating neurological disorder with more than 350,000 cases in the US, and an estimated 12,000 new patients identified annually [1,2]. Although the etiology of MS remains unknown, clinical evidence and supporting studies from animal models strongly suggest that it is an autoimmune disease that targets the central nervous system (CNS) [2,3]. MS is characterized by the presence of inflammatory lesions in the CNS that lead to the loss of oligodendrocytes, demyelination, and degradation of neuronal conduction [4,5], with progressive neurological dysfunction and loss of motor function that correlates with axonal loss.

MS is subdivided into four clinical patterns: relapsing–remitting (RRMS), in which patients have episodes of deteriorating function followed by recovery and a stable course between episodes; secondary progressive MS (SPMS), where there is a waning of neurological competence without relapses in an individual who was previously diagnosed as RRMS; progressive-relapsing MS (PRMS), which manifests as a gradual deterioration from the onset with periodic relapses; and, primary progressive MS (PPMS) where there is nearly a continuous deterioration from the onset of diagnosis [6–8]. The majority of individuals with MS are diagnosed as RRMS at the onset, but the condition advances to SPMS in about 50% of untreated patients [4,9]. The event(s) that provoke the transition to more debilitating forms of MS remain to be discovered. Currently, there is a need to develop therapies that can treat the most progressive forms of MS, particularly since treatments for RRMS may be ineffective in rapidly progressing RRMS or secondary-progressive MS [10,11].

Mitoxantrone (Novatrone) is one of the few drugs available for rapidly progressing RRMS and secondary progressive MS [12]. In a rodent model of MS, experimental autoimmune encephalomyelitis (EAE), mitoxantrone was therapeutic in actively-induced and passively-induced disease (reviewed in [12]). Its efficacy in MS was subsequently confirmed in clinical trials [13,14]. Mitoxantrone appears to preferentially induce apoptosis in activated B and T cells [15], but T cell helper and suppressor activities may also be altered [16]. Unfortunately, irreversible and potentially fatal cardiotoxicity is a major complication associated with mitoxantrone [17], limiting its long-term treatment use in MS. The chronic nature of the disease virtually ensures that the threshold-dose for toxicity is eventually reached, usually within 2–3 years of the initiation of the therapy [11]. Recent reports regarding the risk of mitoxantrone-induced leukemia have raised additional concerns for the only FDA-approved chemotherapeutic drug for SPMS, PRMS and worsening RRMS [18–21].

Mitoxantrone, a modified anthracenedione, is structurally related to the anthracycline family of anti-cancer drugs [22,23]. Its mechanism of action is the inhibition of topoisomerase II via the formation of a cleavable complex of enzyme, DNA, and drug [19,23]. An examination of the chemical structures of anthracyclines, like doxorubicin, and anthracenediones having in vivo anti-tumor activity reveals a hydroxylated anthraquinone backbone as a common feature [24]. The proximity of the hydroxyl group and the quinone creates a strong iron-binding site, which contributes to free radical formation and has an adverse impact on cardiac tissue [25]. Converting the carbocyclic backbone to a heterocyclic backbone led to the development of alternative anthracenediones that have excellent activity in animal tumor models [26] with no cardiotoxicity in pre-clinical toxicology tests [25]. The completion of structure activity relationship studies with additional analogs of mitoxantrone revealed that conversion of the carbocyclic anthraquinone backbone to a heterocyclic backbone eliminated the need for the ring hydroxyl groups for anti-tumor activity if the aza-nitrogen is inserted into the appropriate site in the backbone [27]. Secondly, modification of the side arms had a dramatic impact on their biological activity [28], such that drugs having tertiary terminal amines on both side arms, like the novel 9-aza-anthrapyrazole BBR3378, were active in mitoxantrone and doxorubicin-resistant tumor cells [29]. Thus, while similar in structure (Fig. 1), BBR3378 and mitoxantrone differ in several functional and behavioral characteristics [27]. Here we report on the therapeutic potential of BBR3378 in MS using the MOG (myelin oligodendrocyte glycoprotein)-induced model of EAE.

2. Materials and methods

2.1. Mice

C57BL/6 female mice age 5- to 7-weeks were purchased from Taconic Farms (Germantown, NY) and housed in the University of Toledo's Animal Research Facility.

2.2. EAE induction

Peptide MOG35–55 (MEVGWYRSPFSRVVHLYRNGK) was purchased from Ohio Peptide (Powell, OH), while peptides

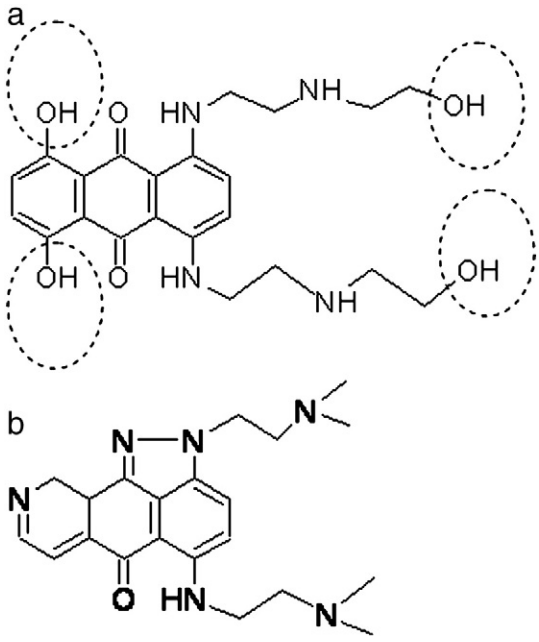

Figure 1 BBR3378 is structurally similar to mitoxantrone, but has distinct features. (a) Mitoxantrone (Novatrone) is a FDA-approved anthracenedione for use in multiple sclerosis. (b) BBR3378, is a 9-aza-anthrapyrazole anti-neoplastic agent. The circles denote ring hydroxyl groups that characterize anthracenediones like mitoxantrone.