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Short communication

Combination chemotherapy with a substance P receptor antagonist (aprepitant) and melarsoprol in a mouse model of human African trypanosomiasis

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Abstract

Drug therapy for late-stage (encephalitic) human African trypanosomiasis (HAT) is currently very unsatisfactory with the most commonly used drug, melarsoprol, having a 5% overall mortality. There is evidence in a mouse model of HAT that Substance P (SP) receptor antagonism reduces the neuroinflammatory reaction to CNS trypanosome infection. In this study we investigated the effects of combination chemotherapy with melarsoprol and a humanised SP receptor antagonist aprepitant (EMEND) in this mouse model. The melarsoprol/aprepitant drug combination did not produce any clinical signs of illness in mice with CNS trypanosome infection. This lack of any additional or unexpected CNS toxicity in the mouse model of CNS HAT provides valuable safety data for the future possible use of this drug combination in patients with late-stage HAT.

Keywords: Human African trypanosomiasis; Substance P receptor antagonist; CNS; Combination chemotherapy; Melarsoprol

Human African trypanosomiasis (HAT), also known as sleeping sickness, continues to be a major threat to human health throughout sub-Saharan Africa [1]. Caused by protozoan parasites of the genus *Trypanosoma*, the disease may present as the West African form, due to *Trypanosoma brucei gambiense* (*T.b.gambiense*), or the East African form due to *T.b. rhodesiense* [1,2]. HAT is invariably fatal if untreated, but the trivalent arsenical drug melarsoprol, the most commonly administered drug for the, encephalitic or late-stage, of the disease, actually kills 5% of patients who receive it because of a severe post-treatment reactive encephalopathy[1,3]. The cause of the PTRE is still unclear [1,2]. While the drug eflornithine (DFMO) is now increasingly used as first line therapy for *gambiense* disease, this drug is not effective for *rhodesiense* patients who, for the foreseeable future, will continue to be treated with highly toxic melarsoprol [1].

We have previously reported the use of a non-peptide Substance P (SP) receptor antagonist in reducing the neuroinflammatory response to experimental trypanosomiasis infection in a highly reproducible mouse model which closely mimics the neuropathological features that are seen in human patients with CNS HAT [4,5]. This finding not only showed the key role of the SP neuropeptide in generating the neuroinflammatory response in experimental HAT, but also suggested that a humanised SP

Table 1
Clinical grading scale

Animal appearance	Numerical score
Normal, healthy	0
Slow, sluggish, starry coat	1
Altered gait, reduced co-ordination of hind limbs	2
Flaccid paralysis of one hind limb	3
Atrophy of muscles and hind quarters, complete flaccid paralysis of both hind limbs	4
Moribund	5
Dead/euthanized	6

The parameters defining the visual assessment scale for the clinical grading scores are shown together with the resulting numerical score.

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Table 2
Neuropathological grading scale

	0	1	2	3	4
Meningitis	None	Mild	Moderate	Severe	Severe
Perivascular cuffing	None	None	Mild cuffing of some vessels	Prominent cuffing of some vessels	Prominent cuffing of most vessels
Encephalitis as defined by cellular activity in the neuropil	None	None	None	Moderate	Severe

This table details the criteria that define the neuropathological grading score associated with specific levels of the CNS inflammatory reaction. The severity scores are given horizontally along the top of the table; the parameters defining the specific score are shown vertically.

receptor antagonist might have therapeutic potential in reducing the incidence and/or severity of the PTRE in patients with late-stage disease who have been treated with melarsoprol. The recent availability of a humanised SP receptor antagonist, aprepitant, (EMEND® Merck & Co. Inc.) now allows this possibility to be tested. EMEND has been shown to be a useful and effective drug in reducing nausea and is registered for use in patients to help prevent chemotherapy induced nausea and vomiting in cancer treatment and post-operative induced nausea and vomiting. However, a pre-requisite for any clinical trial in patients is a clear demonstration that a combination of melarsoprol with this SP receptor antagonist does not produce unexpected CNS toxicity in the mouse model of late-stage HAT. We report here that no such toxicity occurred with this combination.

Female CD-1 mice were infected with 1×10^4 parasites of *Trypanosoma brucei brucei*, cloned stabilate GVR35/C1.8, by intraperitoneal injection. The infection was allowed to progress naturally until the CNS-stage of the disease was attained. On day 21 post-infection the animals were divided into 3 separate treatment groups. The first group of animals was treated with a curative topical melarsoprol regimen where 0.1 ml of melarsoprol gel formulation (approximately 100 mg/kg melarsoprol) was administered between the shoulders of the mice and gently massaged into the skin on days 21, 22 and 23 post-infection [6]. The second group of mice was treated with 1.78 mg/kg aprepitant on day 21 post-infection and 1.14 mg/kg aprepitant on days 22 and 23 post-infection. Aprepitant is largely insoluble therefore a fine suspension of the drug was prepared in sterile water and the appropriate dose administered by oral gavage. The remaining animals were treated, on days 21, 22 and 23 post-infection, with the topical melarsoprol and aprepitant protocols simultaneously. Uninfected groups of mice treated with identical drug regimens to those described were run in parallel with the infected animals to act as controls.

Parasitaemia was monitored by microscopic examination of fresh blood smears before and after administration of the drug

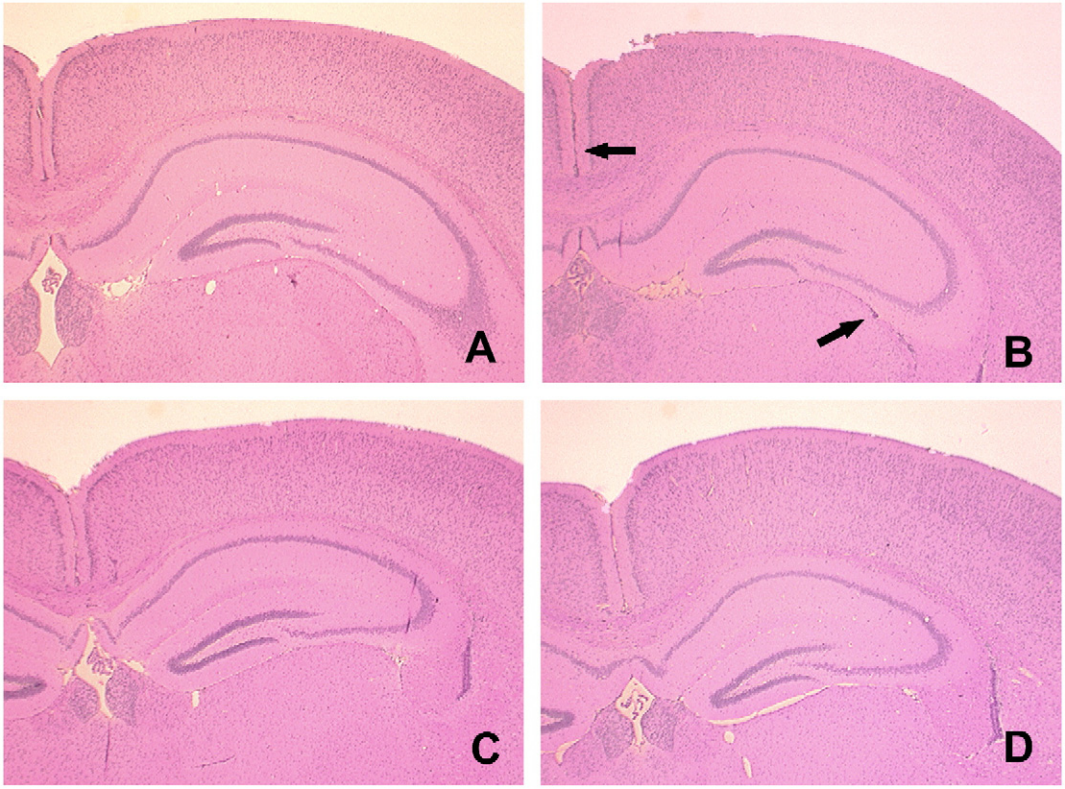

Fig. 1. Coronal brain sections stained with H&E from infected mice treated with melarsoprol (A), aprepitant (B), melarsoprol and aprepitant (C), and an uninfected mouse given melarsoprol and aprepitant (D). An inflammatory infiltrate can be seen in the meninges and lateral ventricles in all of the infected animals (A–C), Arrows indicate an increased presence of inflammatory cells in the infected mouse treated with aprepitant alone (B) compared to the other infected mice. No signs of inflammation are apparent in the uninfected mouse treated with the melarsoprol/aprepitant combination (D).(original magnification × 12.5).