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Oral administration of stavudine induces hyperalgesia without affecting activity in rats

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Abstract

We have investigated whether long-term oral administration of the nucleoside reverse transcriptase inhibitor (NRTI) stavudine affects nociception in Sprague-Dawley rats, and whether any changes of nociception are accompanied by deterioration in activity and appetite. Stavudine (50 mg kg^{-1}) was administered to rats orally once daily for six weeks in gelatine cubes. Mechanical hyperalgesia of the tail was assessed using a bar algometer, and thermal hyperalgesia by tail immersion in 49 °C water. Withdrawal latencies were compared to those of rats receiving placebo gelatine cubes. Withdrawal latencies to the noxious thermal challenge were not affected by stavudine, but those to the mechanical challenge were significantly decreased in rats receiving stavudine, compared to rats receiving placebo, from week three to week six of drug administration ($P<0.05$, ANCOVA with Newman Keuls post-hoc comparisons). The overall condition of the rats was assessed by recording daily voluntary wheel running distance and maximum running speed, food intake and body mass. Daily stavudine administration did not adversely affect voluntary running activity, appetite or growth. We have shown that long-term daily oral administration of the NRTI stavudine results in mechanical hyperalgesia in rats within three weeks without affecting appetite, growth and physical activity.

Keywords: 2′,3′-didehydro-3′-deoxythimidine (d4T); Nucleoside reverse transcriptase inhibitors; Mechanical hyperalgesia; Voluntary wheel running; Food intake; Growth

1. Introduction

Pain is a common complaint of HIV-positive patients, even in the absence of AIDS-defining diseases, and frequently is underestimated and treated poorly by doctors [3,7,15]. HIV-related pain often is neuropathic in origin, not only because of neural damage caused by the virus, but also because antiretroviral drugs cause toxic neuropathies [4,6]. Although antiretroviral drugs effectively retard the progression of the disease, the prevalence of sensory neuropathy in HIV-positive patients has increased since the introduction of these drugs [4,27]. This increased incidence of neuropathy is particularly related to nucleoside reverse transcriptase inhibitors (NRTIs) [4,16], which form an integral part of Highly Active Antiretroviral Therapy (HAART).

NRTIs cause delayed cell doubling and decreased mitochondrial DNA content [4,17,19], possibly by inhibiting DNA polymerase-γ activity [17]. In HIV-positive patients, administration of NRTIs is associated with axonal degeneration and the loss of small unmyelinated nerve fibres, resulting in decreased peripheral nerve fibre density [4,25,26]. However, not all HIV-positive patients experience pain, even if they have other signs of peripheral neuropathy [16,18]. Our poor understanding of how the mitochondrial toxicity of NRTIs causes pain is partly because of a lack of animal models of the disease process.

In one of the few animal studies focusing on pain caused by antiretroviral drugs, Joseph and colleagues [11] showed that a single intravenous injection of the NRTIs didanosine (ddI), zalcitabine (ddC) and stavudine (d4T) resulted in a dose-dependent hyperalgesia of the hind paw that lasted for twenty days. Subsequent studies showed that disrupting the mitochondrial electron transport chain attenuated zalcitabine-induced hyperalgesia [12]. However, as antiretroviral drugs

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are administered orally to HIV-positive patients, and neuropathy in these patients normally develops only after six to eight weeks of chronic NRTI administration [10], the injection model of NRTI-induced neuropathy may not be entirely suitable to examine the mechanisms of NRTI-induced pain. Joseph and colleagues [11] however did also show that daily oral administration of the NRTI zalcitabine to rats at a dose of 50 mg kg^{-1} for six weeks resulted in hyperalgesia in the hind paw after seven days.

Although zalcitabine is effective at treating HIV, stavudine is prescribed more commonly, and is recommended by the World Health Organisation (WHO) as part of first-line antiretroviral drug regimens [31]. Combinations of antiretroviral drugs that include stavudine produce greater increases in CD4 cell count than do other antiretroviral drug combinations not including stavudine [20]. In addition, while neuropathy is the most common reason for patients discontinuing the use of stavudine, stavudine is less neurotoxic than is zalcitabine [6]. Therefore, the aim of our study was to investigate how long-term daily oral administration of the NRTI stavudine affects nociception in rats. As the drug has been associated with other side effects such as hepatitis, pancreatitis and gastrointestinal disturbances [22], we also wanted to expand the work of Joseph et al. [11] by examining whether long-term daily stavudine administration affects the overall condition of the rats, and, particularly, produces deficits resulting from neural malfunction. Consequently we also investigated the effect of daily stavudine administration on food intake and voluntary running activity.

2. Methods

2.1. Animals

Experiments were performed on female Sprague-Dawley rats that were housed individually and had free access to standard rat chow and water. All procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance no. 2004/20/3).

2.2. Drug administration

Stavudine (Zerit, Bristol-Myers Squibb, Johannesburg, South Africa) was administered orally once daily, at a dose of 50 mg kg^{-1}, as a suspension set in a flavoured gelatine cube. Gelatine cubes were made by adding 7 ml savoury bread spread (Bovril, Unilever, Johannesburg, South Africa), 20 g cane sugar and 12 g unflavoured gelatine powder (Davis Gelatine, Johannesburg, South Africa) to 100 ml warm water [13]. The solution was aliquoted into 3 ml moulds and allowed to set. Stavudine-containing gelatine cubes were made by adding powdered stavudine to each aliquot, and mixing thoroughly before the gelatine set. Placebo gelatine cubes did not contain stavudine. Rats were fed placebo gelatine cubes, once daily, for one week before the start of experimentation, by which time they ate the entire cube within 15 min of it being placed in their cage. This method of administering a drug allows a precise dose to be administered orally, without the rat being handled.

2.3. Nociceptive testing

We tested for hyperalgesia by recording the withdrawal latency to a noxious mechanical challenge and a noxious thermal challenge applied to the tail of rats placed in clear plastic restrainers, which restricted trunk movement but allowed free movement of the tail. The rats were familiarized with the restrainers for three hours a day for three consecutive days before measurements began. All measurements were made by the same observer between 09:00 and 12:00 in the morning. The withdrawal latency was recorded only when the rat displayed a clear tail withdrawal from the noxious challenge or the rat tried to turn around in the restrainer to get at the noxious challenge being applied to the tail. Other nondescript end-points, such as the rat starting to fidget were ignored.

2.3.1. Noxious mechanical challenge

A bar algometer with a 1 mm diameter probe (Haldex AB, Halmstad, Sweden), was placed across the dorsal surface of the middle of the tail and a static force of 4 N was applied [29]. The time taken for the rat to withdraw its tail was recorded with a stopwatch. The test was repeated three times for each rat at slightly displaced sites, with at least one minute between each measurement, and the average of the three measurements was recorded as the withdrawal latency for each rat. The algometer was removed from the tail if the rat had not reacted after 30 s.

2.3.2. Noxious thermal challenge

The tail of each rat was submerged in 29 °C water for 30 min before testing began. Thereafter, the whole tail of each rat was submerged in 49 °C water [9]. The time taken for the rat to show a characteristic tail flick response was recorded with a stopwatch. The test was repeated three times for each rat, with at least one minute between each measurement, and the average of the three measurements was recorded as the withdrawal latency for each rat. The tail was removed from the water if the rat had not reacted after 30 s.

2.4. Voluntary activity, body mass and food intake

To assess the general health status of the rats and possible motor defects, we recorded voluntary running activity, body mass and food intake. Rats were weighed daily and food containers were filled daily with 60 g of standard pelleted rat chow. Daily food intake was measured by subtracting the amount of food remaining in the food container and on the cage floor every morning from the amount of food given the preceding day. Because we wanted to monitor whether stavudine affects voluntary exercise, we selected rats that ran spontaneously on running wheels attached to their cages. To select the rats, we recorded the distance 30 rats ran each night using odometers (Cateye Tomo XC, Cyclocomputer, Model CC-ST200) attached to the running wheels, and then selected the 20 rats that ran the furthest over 12 consecutive nights for subsequent nociceptive testing. Running distance and maximum running speed then were measured daily for each rat for the remainder of the study.