An Iranian Herbal-marine Medicine, MS$_{14}$, Ameliorates Experimental Allergic Encephalomyelitis

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Multiple sclerosis is an inflammatory and demyelinating disease of the central nervous system which mainly affects young adults. To overcome wide spectrum troublesome symptoms of multiple sclerosis which affects the quality of life both in patients and their families, new drugs and remedies have been examined and offered. The preclinical beneficial effects of different medicines have mostly been examined in an animal model of multiple sclerosis called experimental allergic encephalomyelitis (EAE). In this study we have tested a traditionally used natural (herbal-marine) product called MS$_{14}$ in EAE mice. EAE mice were fed with MS$_{14}$ containing diet (30%) on the immunization day and monitored for 20 days. The results show that while clinical scores and therefore severity of the disease was progressive in normal-fed EAE mice, the disease was slowed down in MS$_{14}$-fed EAE mice. Moreover, while there were moderate to severe neuropathological changes in normal fed mice, milder changes were seen in MS$_{14}$ fed mice. Copyright © 2008 John Wiley & Sons, Ltd.

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INTRODUCTION

Inflammatory demyelinating diseases are a heterogeneous group of disorders affecting both the peripheral and central nervous systems (Agius et al., 2003). Close similarities of an animal model of EAE with multiple sclerosis (MS) (Steinman, 2001) has made it to be extensively used for investigations on MS (Ewing and Bernard, 1998). With no available therapies for MS, the use of complementary and alternative medicine (CAM) appears to be high among MS patients. Based on anecdotal evidence found in traditional Iranian medicine, MS$_{14}$ is a natural (herbal-marine) product voluntarily used by MS patients. Preliminary studies indicate that MS$_{14}$ may have some benefits on both the quality of life and symptoms of the patients (Gharegozli, 2004; unpublished data). MS$_{14}$ has been patented by the Invention and Patent Registration Office of I.R. of Iran with no observable adverse effects (Hajhashemi et al., 2004), according to the criteria classified by Klaassen (2001).

MS$_{14}$ contained 90% *Penaeus latisculatus* (king prawn), 5% *Apium graveolens* (Umbelliferae) and 5% *Hypericum perforatum* L. (St John’s wort). To investigate the potential therapeutic effects of MS$_{14}$, EAE mice were fed with MS$_{14}$ and examined for both the progressiveness of the disease and neuropathology of CNS.

MATERIALS AND METHODS

**Animals, treatment and assessment of the disease.** Female C57/BL6 mice (12 weeks old, 20 g) were purchased from Pasteur Institute (Tehran, Iran), housed in groups of seven per Plexiglass cage (15 x 20 x 30 cm) and allowed free access to food and water. They were maintained under standard conditions approved by Animal Ethic Committee of Tehran University. MS$_{14}$ was supplied by the Iranian Center of Neurological Research (ICNR) and Shahed Medicinal Plants Research Center.

To induce EAE, the mice were immunized with 200 μg of MOG peptide (35-55) emulsified in complete Freund’s adjuvant supplemented with 4 mg/mL of killed mycobacterium tuberculosis. A volume of 0.1 mL of the mixture was injected at the base of the tail in each mouse. Pertussis toxin (500 ng/mouse) was injected immediately after and 48 h later (Lando et al., 1980; Fritz and Zhao, 2001).

Mice were grouped into two groups of nine: normal diet treated EAE and MS$_{14}$ containing (30%) diet treated EAE. On day 20 post-immunization, the mice were anaesthetized with chloral hydrate 5% with their
brains taken out and soaked in Bouin’s fixative. Mice from both groups were weighed before and after intervention and assessed for clinical manifestation of the disease on a daily basis. Clinical assessment was performed on the basis of the following scores: 0: normal, 1: limp tail or mild hind limb weakness, 2: limp tail and moderate hind limb weakness, 3: moderately severe hind limb weakness, 4: limp tail and severe hind limb weakness or moderate ataxia, 5: paraplegia with no more than moderate forelimb weakness, 6: limp tail and paraplegia with severe forelimb weakness or severe ataxia followed by death (Devaux et al., 1997; Lassmann and Wisniewski, 1979).

Tissue preparation. Bouin’s fixed brains were dehydrated in graded series of ethanol 70%, 90%, 96%, 100% (2 × 1 h), cleared in ethanol/toluene (2:1, 30–45 min) ethanol/toluene (1:1), toluene 100% (2 × 30–45 min). For paraffin embedding the brains were first soaked in toluene/paraffin (1:1, 30 min), followed by paraffin baths (2 × 1 h) which were then blocked in fresh paraffin. Blocks were allowed to be cooled and sectioned by using a rotary microtome at 7 μm. Sections were stained by hematoxylin and eosin and mounted by depex.

Histopathology. Samples of the EAE brains (control and MS14-fed groups) were studied for inflammation, demyelination, hemorrhage, necrosis, etc. using a light microscope. Quantification of histological changes were scored blindly by using + to +++ for mild, moderate and severe symptoms respectively (Haeri et al., 2006; Mahdavi et al., 2006; Soltani et al., 2005; Shirazi et al., 2005).

Statistical analysis. All statistical analyses were performed by means of SPSS software. Values of \( p < 0.05 \) were considered statistically significant. Statistical evaluation was performed using Friedman, parametric repeated measures and Mann-Whitney tests.

RESULTS

Clinical assessment

In contrast to normal-fed EAE mice in which clinical scores and therefore the severity of the disease was progressive (from 0 on the immunization day to 4.6 on day 20 post-immunization; \( p = 0.004 \)), the disease was slowed down in the MS14-fed EAE mice (Fig. 1; from 0 on the immunization day to 2.66 on day 20 post-immunization; \( p = 0.584 \)). No difference was seen in body weight changes of MS14-fed EAE mice compared with those of normal-fed EAE mice (Fig. 2; \( p = 0.477 \)).

Neuropathology of the brain

In the control group, there was inflammation, multilocal leukoencephalopathy with focal demyelination in the meninges and nervous tissue. In the MS14 fed group, however, only the meninges were inflamed in all individuals, with a very mild injury in just two of the samples (Fig. 3). Mann-Whitney analysis of the mean neuropathology scores indicated that abnormal changes seen in the control group were more robust compared with those in the MS14 treated group (Table 1).

DISCUSSION

The use of alternative medicine is increasing all over the world, however, little is known about the effect of these remedies on neuropathology of the CNS in...
neurodegenerative diseases such as multiple sclerosis. Besides, many effective therapies in the animal model (EAE) are either ineffective in MS or actually make MS worse (Pender and Wolfe, 2002). A variety of alternative medicines has been examined for the therapy of MS in recent years elsewhere (Aktas et al., 2004; Seiwa et al., 2007). Based on the available data within the libraries of complementary and traditional Iranian medicine, an herbal-marine compound called MS14 has been prescribed as a remedy for the treatment of MS resulting in a reduction of clinical complications. MS14 contained 90% Penaeus latisculatus (king prawn), 5% Apium graveolens (Umbelliferae) and 5% Hypericum perforatum L. (St John’s wort). This compound contains many organic salts, complexes and also trace elements such as Br, Sr, Va, Ti, Ni and Zn (Ahmadi, 2004). Although positive effects have been seen from this traditional medicine, no considerable or official neurological information is available so far. This study showed that oral treatment of the EAE mice with MS14 not only halted the progression of the disease but also attenuated the inflammation in CNS indicating that this herbal-marine compound has antiinflammatory effects. Toxicological and pharmacological studies on rats have shown that MS14 is an antioxidant agent with no observed adverse effects and is safe in chronic long term oral use (Hajhashemi et al., 2004). Overall, the alleviation of clinical and neurological symptoms in EAE mice by MS14 explains the beneficial effects of traditionally used MS14 in MS patients. Further experiments on different combinations of the MS14 active compounds as well as on the clarification of their mechanisms may lead to the development of new pharmacotherapeutic agents for multiple sclerosis.

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REFERENCES


