LETTER TO THE EDITOR

Teriravone Induces Neurotoxicity in Beagle Dogs

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Stroke is the medical emergency and can result in permanent neurological damage, complications, and death [1]. For last three decades, stroke remains the second most common cause of mortality and recently has become the third leading cause of global disease burden estimated using disability-adjusted life years [2,3]. Oxidative stress has been implicated as a pathogenetic mechanism for neurodegeneration in stroke. Edaravone, a potent radical scavenger, has high liposolubility and good permeability through the blood-brain barrier and has been used for acute ischemic stroke in Japan since June 2001 [4]. Its neuroprotective effect has been confirmed by a large number of studies [5,6]. However, edaravone was also reported to induce adverse effects, such as acute renal failure, hepatitis, and disseminated intravascular coagulation [7]. It is necessary to develop a new generation of more effective neuroprotective drugs with lower toxicity to meet the increasing clinical needs. Teriravone, a new compound based on the structure of edaravone, is provided by Tianjing Zhongrui Pharmaceutical Company. The structures of teriravone and edaravone are shown in Figure 1A. In this study, the pre-clinical chronic toxicity and neurotoxic mechanisms of teriravone were evaluated in Beagle dogs to provide data to support its further development.

Twenty-six Beagle dogs (13 male, 13 female), aged 6–8 months and weighted 7.8 ± 1.2 kg, were provided by Shanghai Experimental Animal Co. Ltd. Twenty-six Beagle dogs were randomly divided into four groups: control group and three groups treated with teriravone (5, 15, 45 mg/kg/day). There were eight dogs in high-dose group and six dogs in the other groups, with equal numbers of each sex. Dogs received intravenous teriravone or its pharmaceutical adjuvant once daily for 90 consecutive days followed by a recovery period of 30 days. Clinical symptoms were observed daily throughout the study. Dynamic detections of hematology, serum chemistry, urinalysis, and electrocardiogram were conducted. At the end of the treatment (d 90) and the end of the recovery period (d 120), half of the animals of each

Figure 1 Teriravone-induced neuron degeneration in mesencephalon of the Beagle dogs. (A) chemical structures of edaravone and teriravone. (B–D) hematoxylin- and eosin-stained sections of the dog mesencephalon from control group, high-dose group (d 90) and high-dose group (d 120). (B) The structure of the mesencephalon was normal in control group. (C) At the end of teriravone treatment, the mesencephalon of the high-dose group presented neuron damages such as cell body pyknosis, unclear structure, and even disappearance of the Nissl bodies (arrows). (D) After 30 days' recovery from teriravone treatment, the mesencephalon of the high-dose group showed normal structure.

group were killed, respectively, for histopathological examination. Additionally, mesencephalon of dogs in control and teriravone 45 mg/kg/day groups was collected for the examination of differentially expressed genes by using microarray chip. Real-time PCR was used to validate differentially expressed genes *IL2*, *LAMC2*, and *NRXN1*. At last, protein levels of laminin gamma 2 (*LAMC2*), *IL2*, and neurexin (*NRXN1*) in mesencephalon were determined by Western blot.

Neurotoxic symptoms such as limbs stiff, standing instability, side lying, and opisthotonus appeared during the administration of teriravone at doses of 15 and 45 mg/kg/day. Some animals in

the 45-mg/kg/day group were howling and even developed opisthotonus ($n = 2$). There were no apparent changes in hematology, serum chemistry, urinalysis, and ECG examinations in all groups. Animals in control group showed normal mesencephalon structure (Figure 1B). However, neuron damages, such as cell body pyknosis, Nissl bodies disappearance, and reduction in dendrites amount, were found in dog mesencephalon of 45 mg/kg/day dose group after 90-day treatment (Figure 1C), and the treatment-induced injury was reversible upon discontinuation of treatment (Figure 1D). One of the most important functions of mesencephalon is the regulation of movements and posture, and muscle tone. The neurotoxic symptoms mentioned above might be attributed to the damaged motor function of mesencephalon associated with the injured neurons.

Microarray analysis of the mesencephalon revealed 2085 differentially expressed genes between control and teriravone groups. Real-time PCR for differentially expressed genes *IL2*, *LAMC2*, and *NRXN1*, which exhibited the greatest fold changes, gave results very similar to those from the microarray (Figure 2A). Western blot analysis indicated that expression of *LAMC2* protein was up-regulated, and the level of *NRXN1* protein was reduced in mesencephalon of dogs after teriravone treatment (Figure 2B). Through gene ontology analysis, 110 differentially expressed genes were found involved in different functional classification. A total of 15 signaling pathways were found relevant to teriravone treatment. Among these, *NRXN1* pathway might be an important one associated with the neurotoxicity of teriravone. *NRXN1* is a gene

of the cell adhesion molecules pathway. *NRXN1* is specifically expressed in neurons and is involved in synapse formation and maintenance [8,9]. In this study, reduction in dendrites amount after teriravone treatment had been confirmed by histopathological examination. Reduced *NRXN1* expression might be one of the reasons for this neuron degeneration.

In conclusion, teriravone-induced neuron degeneration in dog mesencephalon and neurotoxic signs appeared during the administration of teriravone at doses of 15 and 45 mg/kg/day in Beagle dogs. The toxic effects were reversible upon discontinuation of treatment. Microarray analysis indicated multiple signaling pathways were likely involved in teriravone-induced neurotoxicity. Down-regulation of *NRXN1* may be an important neurotoxic mechanism of teriravone.

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Conflict of Interest

The authors declare no conflict of interest.

Figure 2 Validation of microarray data with real-time PCR and protein level of differentially expressed genes *LAMC2*, *IL-2*, and *NRXN1* by western blot. (A) *LAMC2* and *IL-2* expression were increased, and *NRXN1* was decreased by 45 mg/kg/day teriravone at the end of the treatment. (B) Expression of *LAMC2* was up-regulated, and *NRXN1* expression was down-regulated in teriravone group. There was no difference in expression of *IL2* between control and teriravone group. $n = 3$ in control group; $n = 4$ in teriravone group. * $P < 0.05$, ** $P < 0.01$ versus control.

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