Several artemisinin (qinghaosu) derivatives have been developed and are in use as antimalarial drugs but scant animal or human toxicity data are available. We noted a progressive syndrome of clinical neurological defects with cardio-respiratory collapse and death in 5/6 dogs daily for 8d with intramuscular (IM) arteether (AE) at 20 mg/kg/d in a pharmacokinetic study. Neurologic findings included gait disturbances, loss of spinal reflexes, pain response reflexes and prominent loss of brain stem and eye reflexes. Electrocardiography showed prolongation of the QT interval corrected for rate (QTc). Prominent neuropathic lesions were sharply limited to the pons and medulla. Neurologic injury, graded by a pathologist blinded to dose group, showed a dose-related region-specific injury which was most pronounced in the pons and medulla in all animals. Rats treated with AE and artemether (AM) at 12.5 to 50 mg/kg/day for 28 d confirmed clinical neurologic abnormalities with high doses (25 mg/kg/day) after 6-14d. Neuropathological examination of rat brain sections at 5 levels from the rostral cerebrum to the caudal medulla showed a dose-related pattern of injury characterized by hyalinized neuron cell bodies and loss of Nissl substance changes congruent with those noted in dogs.
Neurotoxicity in animals due to arteether and artemether

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Abstract

Several artemisinin (qinghaosu) derivatives have been developed and are in use as antimalarial drugs but scant animal or human toxicity data are available. We noted a progressive syndrome of clinical neurological defects with cardio-respiratory collapse and death in 5/6 dogs dosed daily for 8 d with intramuscular arteether (AE) at 20 mg/kg in a pharmacokinetic study. Neurological findings included gait disturbances, loss of spinal reflexes, pain response reflexes and prominent loss of brain-stem and eye reflexes. Electrocardiography showed prolongation of the QT interval corrected for rate (QTc). Prominent neurogenic lesions were sharply limited to the pons and medulla. Neurological injury, graded by a pathologist 'blinded' to dose group, showed a dose-related region-specific injury which was most pronounced in the pons and medulla in all animals. Rats treated with AE and artemether (AM) at 12.5 to 50 mg/kg/d for 28 d confirmed clinical neurological abnormalities with high doses (>25 mg/kg/d) after 6-14 d.

Neuropathological examination of rat brain sections at 5 levels from the rostral cerebrum to the caudal medulla showed a dose-related pattern of injury characterized by hyalinized neuron cell bodies and loss of Nissl substance; changes congruent with those noted in dogs. No significant difference was noted in the extent type, or distribution of lesions in the brains of rats treated with equivalent doses of AE or AM. We conclude that (i) a neurological syndrome with central nervous system neuropathological changes occurred in dogs, rats, and a related, and an unrelated species of both dogs and rats gave similar results at high doses of AM, artemether or arteether; (ii) prolonged QTc interval was a preterminal clinical finding in dogs and rats treated with high dose AE; (iii) the mechanism and aetiology of these lesions was not determined in this study but a long-lived toxic drug metabolite is suggested.

Introduction

The discovery of qinghaosu (artemisinin) by the Chinese and identification of its unique sesquiterpene lactone endoperoxide structure was an important milestone in antimalarial chemotherapy. Antimalarial activity is increased by reducing the lactone oxygen to yield dihydroartemisinin which can be further derivatized to ether or ester analogues (Klayman, 1985). Two derivatives of artemisinin include methyl ether (artemether) and ethyl ether (arteether) forms, which appear to have similar physical/chemical and antiparasitic properties (Shmulskovsky et al., 1993). Artemisinin and arteether have been widely reported by the Chinese and others to have been successfully used as a blood schizontocide, formulated as an intramuscular (i.m.) peanut oil solution or oral capsules (Hien & White, 1993). Very little clinical toxicity other than occasional changes in the electrocardiogram has been noted in the relatively high daily doses used in published animal toxicity studies (Hien & White, 1993).

The rapid onset of action, activity against drug resistant malaria, and low reported toxicity have led to considerable interest in this group of drugs. Arteether was selected by the training program of the World Health Organization (WHO) for development as an i.m. sesame oil solution for emergency treatment of severe malaria—especially cerebral malaria.

Early animal toxicology studies showed occasional unexplained deaths in dogs receiving high doses of the compounds. Abnormalities in the electrocardiogram (ECG) with prolongation of the QT interval had been noted in animal and human studies, so the unexplained deaths were presumed to be cardiovascular. During the course of multiple-dose pharmacokinetic studies in our laboratory, sudden unexplained neurological findings and death were noted in several animals given high doses of i.m. arteether. These observations led to the investigations described below. Our studies demonstrated a delayed onset, dose-dependent central nervous system toxicity with a unique region-specific distribution in rats and dogs after repeated dosing with either arteether or arteether. This report describes the nature and extent of these neuropathic lesions and associated observations.

Fig. 1. a) Schematic sagittal outline of dog brain with section levels indicated by numbered vertical lines. Level 1, immediately anterior to union of optic nerve; level 2, through the center of the obex cerebrum; level 3, immediately anterior to the pontal protuberance; level 4, through the posterior portion of the protuberance at the base of the trigeminal nerve; and level 5, distal to the hypoglossal nerve. b) Outline of sagittal rat brain showing section levels by numbered lines. Brain figures modified from Petras et al., 1979; Lim et al., 1965.
Clinical neurological findings in multiple-dosed dogs

Plasma levels of arteether were determined by high performance liquid chromatography using reductive electrochemical detection (Melendez et al., 1991) and showed a dose related increase in plasma level until day 5 (data not shown). No neurological finding was noted in low dose groups (5 and 10 mg/kg) in either study, but animals in high dose groups demonstrated depressed sensorium with abnormal neurological findings (see below and progressive ataxia, with death of 6 of the 10 high dose animals in both studies. ECGs of animals with neurological findings exhibited a prolonged QTc interval (QT interval corrected for rate) (Gallagher, 1992; Todt et al., 1992) without arrhythmias or ectopy.

Both high-dosed and low-dosed animals were examined neurologically. No significant, reproducible neurological deficit was noted in low-dosed animals, while all high-dosed dogs showed neurological deficits, albeit to different degrees. In low-dosed animals, all tested reflexes, including cranial nerve function by menace reflex, vestibular nystagmus (doll's eyes), and pupillary light reflex were normal. Spinal reflexes (patellar, biceps, triceps, perineal, panniculus and deep pain reflexes) were intact. Postural reactions including 'wheel-barrowing', hopping, extensor postural thrust, hemi-stand, hemi-walk, proprioceptive and optic/tactile placing, were also intact. The most severely affected high-dosed animals were unable to stand or walk and, despite their having eyes open, did not spontaneously track objects or have a menace reflex or response to visual auditory environmental stimuli. Normal vestibular nystagmus was absent but the pupillary light reflexes were normal bilaterally. All spinal reflexes were present and deep pain reflexes were normal. All postural reflexes examined were absent. Animals demonstrated rapid shallow respiration, which became progressively depressed until respiratory arrest supervened.

Clinical and neurological changes in multiple dosed rats

Clinical findings in rats comprised dose-related changes in growth and weight gain and mortality rate between the controls and the animals receiving the 3 dose levels of arteether or artemether. A number of animals had neurological changes, which included abrupt onset of ataxia as well as spontaneous myoclonic-like activity, in the 1-2 d before death.

Electrocardiographic changes

Serial ECG recordings from rats showed marked changes in the ST-T wave morphology, inversion of the
T wave and increased QTC duration \( P < 0.05 \); 72-96 h before the animal exhibited clinical abnormalities (data not shown).

Neuropathological description

**Dogs.** Brain lesions in all dogs from both studies were similar in type and distribution but of varying severity, consisting of scattered neuronal degeneration and necrosis, characterized by swelling and rounding of nerve cell bodies, increased eosinophilia, vacuolation of cytoplasm with a loss of Nissl substance (central chromatolysis), swelling and fading of nuclei, and separation and clumping of fibrous and granular nucleolus. Only small focal changes were seen in the anterior sections, levels 1 and 2, even in high dosed animals. There was a striking distribution of lesions in the central nervous system: they were almost exclusively limited to levels 3, 4 and 5, corresponding to the pons and medulla. Cytotoxic changes in injury were particularly marked in the paralemniscal nucleus, nucleus dorsalis raphae, nucleus pontis, nucleus vestibularis superior, the principal sensory nucleus \( V \), nucleus cochlearis dorsalis, nucleus cochlearis ventralis, and nucleus olivaris superior. Changes in the caudal medulla and thoracic spinal cord showed scattered axonal degeneration and necrosis characterized by swelling of axonal processes and spheroid formation. These lesions were most prominent in animals receiving 15 mg kg\(^{-1}\) or more. However, scattered neuropathic changes were also seen in the lowest dosed animals (5 mg kg\(^{-1}\) for 28 d; but in sections 4 and 5 only).

No such lesion was seen in any reference control brain.

**Rats.** Histological evaluation of rat brains showed degeneration of cell bodies in the central nervous system and spinal cord, morphologically and anatomically similar to those seen in dogs. Injury was predominantly at the level of the red nucleus and areas caudal to that structure in the brain-stem. Damage appeared to have a highly selective distribution within the hind brain with specific involvement of certain nuclei and cell groups. Involved nuclei in rats receiving arteether and arteether included the following. In the midbrain: red nucleus, dorsal cochlear, ventral cochlear, superior olive, trapezoid nucleus, ventral lateral lemniscus, dorsal lateral lemniscus, inferior colliculus, degenerated fibres to medial geniculate nucleus; in the hindbrain: reticular formation neuronal injury nucleus (reticular), pontis oralis nucleus (reticular), pontis caudalis nucleus (reticular), gigantocellularis nucleus, magnocellularis dorsal nucleus, medulla oblongata centrals, ventral nucleus medulla oblongata centrals; in the cerebellum: paramedian nucleus, lateral reticular nucleus, deep cerebellar nuclei: fascicular cuneatus nucleus and cuneatus externus vestibular nucleus, 'deep nuclei' (nucleus fastigii nucleus intermedius and lateralis) (PETRAS et al., 1993).

Moderate to severe damage was consistently noted in the 25 and 50 mg kg\(^{-1}\) groups treated with both arteether and arteether. Silver staining confirmed prominent fibre degeneration in several areas, especially in the region of the superior olivary nucleus and fibre tracts to the medial geniculate nucleus (J.M.P.). Severity scores from sections at 5 levels showed significant differences in neuropathic scores by region with sections

**References**


