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Differential effects of orally versus parenterally administered qinghaosu derivative artemether in dogs

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With 12 figures and 2 tables

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Summary

Artemether (AM) is an antimalarial drug derived from artemisinin (Qinghaosu), an extract of the herb *Artemisia annua L.*, sweet wormwood. Its antiparasitic effect is that of a schizontocide and is explained by rapid uptake by parasitized erythrocytes and interaction with a component of hemoglobin degradation resulting in formation of free radicals. It has been shown to exhibit a high clinical cure rate. Previous animal safety studies with Qinghaosu derivatives revealed dose-dependent neurotoxicity with movement disturbances and neuropathic changes in the hindbrain of intramuscularly treated dogs, rats and monkeys. Such effects have not been seen in man. The objective of our present studies was to compare the effects of high levels of AM administered to dogs p.o. versus i.m. In a pilot study 20 mg/kg/day of AM was given i.m. to groups of 3 male Beagle dogs for 5 and 30 days, respectively. Clinical signs of neurotoxicity were noted in some individual dogs from test day 23 on. One dog had to be sacrificed pre-term. Hematologic findings indicated a hypochromic, microcytic anemia. Microscopic examination demonstrated neuropathic changes only at 30 days, but not at 5 days. The animals had neuronal and secondary axonal damage, most prominent in the cerebellar roof, pontine and vestibular nuclei, and in the raphe/paralemniscal region. The affected neurons showed loss of Nissl substance, cytoplasmic eosinophilia, shrinkage of the nucleus and in advanced stages scavenging by microglia. In a subsequent experiment, AM was administered to groups of 4 male and 4 female dogs, respectively, at 8 daily doses of 0, 20, 40 and 80 mg/kg i.m., or 0, 50, 150 and 600 mg/kg p.o. Neurologic signs were seen at high i.m. doses only. In most animals they were inconspicuous and consisted of reduced activity with convulsions seen in single dogs shortly before death. Neuronal damage occurred in all animals at 40 and 80 mg/kg following i.m. treatment. At 20 mg/kg minimal effects occurred in 5/8 dogs only, indicating that this level was close to tolerated exposure. No comparable lesions were observed after oral administration. Both i.m. and p.o. exposure at high dose levels was associated with a prolongation of mean QT interval of ECG, suggesting slowing of repolarization of the myocardium. Individual data indicated that in 1 of 4 females at 80 mg/kg i.m. this prolongation was above the 25 % level considered as threshold for concern. After intramuscular administration pharmacokinetics indicated peak plasma levels of AM at 2 to 4 hours postdose, slow elimination and a tendency to accumulate after repeated administration. Only low levels of the major metabolite, dihydroartemisinin (DHA), were found. AM levels in the cerebrospinal fluid (CSF) were < 10 % of plasma levels. After oral administration AM concentrations were considerably lower than after i.m. administration. The concentration of DHA was high on day 1 but almost nil on day 7 indicating its fast inactivation in dogs. Two hours after the 8th oral administration neither AM nor DHA was detected in CSF which may explain the absence of neurotoxicity in dogs after oral administration of AM.

Introduction

Derivatives of artemisinin (Qinghaosu), an extract of the herb *Artemisia annua L.*, or "sweet wormwood" (Qinghao) are promising new antimalarials currently available for the treatment of malaria caused by multiple resistant Plasmodium falciparum, with high clinical cure rate. They are now in widespread use especially in Southeast Asia where resistance to other antimalarials has become common (KAMCHONGWONGPAISAN et al. 1997; KARBWANG et al. 1997). Chemically, artemisinin has a sesquiterpene lactone peroxide structure. Its antiparasitic effect is explained by rapid uptake by parasitized erythrocytes and interaction with a component of hemoglobin degradation resulting in formation of free radicals. Antimalarial activity is increased by reducing the lactone oxygen to yield dihydroartemisinin (DHA), which can be further derivated to ether or ester analogues. Such derivatives include artemether (AM) and arteether (AE) which appear to have similar physical, chemical and antiparasitic properties. These agents have been shown to be effective against blood stages of the malaria

parasites (schizonts) and to produce very little clinical toxicity other than changes in electrocardiogram (ECG). AE was selected by the US Army and the World Health Organization for development as a remedy of severe malaria (BREWER et al. 1994a).

Although no serious adverse effects have been reported in clinical trials in humans (KAMCHONWONGPAISAN et al. 1997), experiments with AE or AM in animals demonstrated neurotoxicity following intramuscular administration to dogs, rats and monkeys (BREWER et al. 1994a, 1994b; PETRAS et al. 1994; GENOVESE et al. 1995). In these animal studies the compound was administered parenterally and over a prolonged time period. In contrast, clinical trials indicated rapid clearing of parasites thereby limiting treatment to few days only. This difference in treatment duration between intramuscular animal studies and the clinical situation greatly impedes risk assessment based on such data. Using a short administration period to compare both clinical and pathologic effects and the pharmacokinetics of oral and intramuscular routes of administration is considered a better basis for extrapolation to man, and for estimating a safety margin for neurotoxic effects of AM when used as intended in clinical trials. Therefore, in the present experiments treatment was limited to 8 days which is closer to the maximum 5 day treatment intended for humans, but long enough for neural lesions to develop (BREWER et al. 1994a). Experiments with AM in dogs described in this report demonstrate characteristic neural lesions after high-level intramuscular treatment and their total absence following oral administration.

Material and methods

Two separate experiments were carried out: experiment I subserved the replication of published data in order to verify the sensitivity of animals and methods used and to characterize neurotoxic effects and systemic toxicity produced by intramuscular (i.m.) administration of AM. In experiment II AM was administered by both the oral and the intramuscular route at various dose levels to test its neurotoxic potential under conditions similar to those intended for clinical use. For both studies healthy, pedigree Beagle dogs (initial age 42 to 50 weeks) were used. The animals underwent antiparasitic therapy and had been vaccinated against rabies, distemper, leptospirosis, infectious canine hepatitis, kennel cough, parvovirus and coronavirus. They were housed in pairs in solid-bottom kennels with available indoor and outdoor runs (total area 10 m^2). Light was provided during at least 12 hours daily and the room temperature was maintained above 15 °C. Certified pelleted standard diet (NAFAG No 9405; Eberle Nafag AG, 9200 Gossau, Switzerland) was given at 350 g per animal daily and tap water was freely available.

An injectable solution of AM in peanut oil (80 mg/ml; Kunming Pharmaceuticals Corp., China) was used for intramuscular administration; control animals received sterile peanut oil. For oral administration artemether was administered in gelatin capsules as active substance (a white crystalline powder; Novartis Pharma AG, Switzerland); control animals received empty gelatin capsules.

Experiment I

The dose of 20 mg/kg i.m. of AM was selected because it is above the therapeutic dose and it was expected to produce toxicity after prolonged administration. Groups of one control and 3 treated male dogs were dosed for 5 or 30 days (8 dogs in total). The test and control articles were administered once daily in equal volumes of 0.25 ml/kg into gluteal or infraspinous muscles alternating both hind legs and both forelegs. Clinical investigations included observation for mortality, general clinical signs and detailed neurologic examination, body weight, food consumption, ophthalmology, electrocardiography, clinical laboratory investigations (blood biochemistry, hematology and urinalysis) and pharmacokinetics.

For ECG measurements a Cardiovet Cs-6/12 (Schiller-Reomed AG, 8953 Dietikon, Switzerland) six-channel electrocardiograph was used. The dogs were held in right lateral recumbency with forelimbs and hindlimbs perpendicular to the body. Limb leads (Einthoven I, II, III), augmented unipolar leads (Goldberger aVR, aVL, aVF) and the thoracic leads (Wilson v1, v2, v3) were used and heart rate (HR), P-wave (P), PQ-interval (PQ), QRS-complex (QRS), QT-interval (QT), were measured over a period of 10 seconds and averaged by an integrated measurement program. In addition, electrocardiographic tracings were visually compared with the pretest pattern of the same dog. As PQ- and QT-intervals vary with heart rate they were adjusted to an RR-Interval of 500 msec, corresponding to a heart rate of 120 using following equations: $PQ\_500 = PQ - 0.023 * (RR - 500)$ and $QT\_500 = QT - 0.084 * (RR - 500)$.

For pharmacokinetics about 5 ml of blood was drawn from the antebrachial cephalic or jugular vein into heparinized tubes and immediately centrifuged at 3000 rpm at 4 °C for 12 minutes. The plasma was then immediately transferred to 2 ml polypropylene tubes and stored on dry ice. Samples of cerebrospinal fluid were drawn from the cisterna magna and immediately transferred to polypropylene tubes and stored on dry ice. Samples were kept at –80 °C until analysis using a HPLC method (SANDRENAN et al. 1997).

For pathological examination the surviving animals were necropsied 2 hours after the last administration. One moribund dog had to be necropsied prematurely on study day 28. Deep anesthesia was induced by Vetanarcol™ (0.5 ml/kg i.v.) with the addition of 5000 IU heparin and followed by whole-body perfusion with 4 % neutral, phosphate-buffered formalin. A total of 48 different organs and tissues, including brain, optic nerves, peripheral nerves (sciatic and plantar), skeletal muscle (quadriceps femoris and all injection sites), and spinal cord were taken from all animals and postfixed in formalin. The specimens were embedded in paraplast, sectioned at nominally 4 to 6 microns, stained with hematoxylin and eosin and examined by light-microscope. The brain was sectioned at multiple transverse levels to obtain the brain regions reported as susceptible (BREWER et al. 1994b). To improve the definition of the brain lesions, special stains were applied on selected duplicate sections: cresyl violet (Nissl stain), binding of lectin RCA-1 as a marker for microglia cells and Bodian's silver stain for neurofilaments and axons combined with luxol fast blue counterstain for myelin sheaths. Additional samples of the cervical (C1) and thoracic (T1) spinal cord were postfixed in 5 % glutaraldehyde, impregnated with Dalton's solution, embedded in epoxy resin, sectioned at 1 micron and stained with toluidine blue. All preparations were examined under a light microscope.

Experiment II

For this experiment a total of 64 dogs was used. AM was administered once daily for 8 consecutive days to groups of 4 male and 4 female dogs either by the intramuscular or by the oral route. Intramuscular dosages were 0, 20, 40 or 80 mg/kg (injection volumes 0.25, 0.5 and 1.0 ml/kg) with the control group receiving 1.0 ml/kg of sterile peanut oil. The injection sites were the gluteal and infraspinous muscles. Oral administration was performed about 2 hours after feeding at the daily dosages of 0, 50, 150 or 600 mg/kg. Clinical investigations included general clinical signs, detailed neurologic examination, body weight, food consumption, electrocardiography (ECG), and pharmacokinetics, as described with experiment I.

For pathological examination, the animals were deeply anesthetized and perfused with formalin as with experiment I. Following perfusion the brain was removed for postfixation. One high-dose, i.m. treated female died spontaneously on day 8. The brain of this animal was removed and fixed by immersion in formalin. Each brain was divided in two parts by a longitudinal-vertical cut about 2 mm left of the midline. The right, larger part was postfixed in formalin and used for paraffin processing. The left, smaller part, was cut in multiple transverse slices about 5 mm thick and postfixed in 5 % glutaraldehyde for optional embedding in epoxy resin which was performed on selected specimens. Tissues embedded in paraffin were sectioned at nominally 4 to 6 microns and stained with hematoxylin and eosin. Duplicate brain sections of all i.m. treated animals and of high-dose and control animals treated orally were stained with cresyl violet. In the brain the areas identified in experiment I as highly susceptible were examined to establish the dose-effect relationship: the cerebellar roof nuclei, pontine nuclei, vestibular nuclei and paralemniscal / raphe region.

Body weight and food consumption data were compared to the control group by Wilcoxon's two-sample test and tested for increasing or decreasing trends from control up to the respective dose group by Jonckheere's test for ordered alternatives. Electrocardiographic data were analyzed by parametric PC-SAS procedures. Treated groups were compared to the control group by repeated t tests based on the pooled variance estimate. Group means were tested for a linear trend from control to the highest dose group by group number.

Results

Experiment I

Clinical investigations and pharmacokinetics: On test day 23 a single animal showed tremors and restlessness. Signs improved over the day and the dog appeared perfectly normal the next morning. On test day 27 this dog showed a swaying gait and was aggressive and the next morning, despite discontinuation of treatment, was found recumbent with dyspnea, poor general condition and was thus sacrificed pre-term (day 28). The animal may have had convulsions overnight. Shortly before planned sacrifice on test day 30 another animal showed tonic-clonic convulsions ending in opistothonus. No other toxicologically relevant clinical signs or changes in behavior were observed at any time during the experiment. Although food consumption was reduced during weeks 3 and 4, body weight development was not affected by treatment. Hematological findings at day 29 indicated a hypochromic, microcytic anemia. No relevant changes were observed at opthalmoscopic and neurologic examinations of the animals. Electrocardiographic examinations indicated a trend toward prolonged QT intervals. As this effect was only minimal and observed in individual animals only it was considered of equivocal relevance. Pharmacokinetics indicated a plasma profile comparable to that produced in humans after intramuscular administration (ZHOU et al. 1988).

Pathology: Post-mortem examination at 5 and at 30 days of treatment did not reveal any treatment-related gross changes. Microscopic examination revealed treatment-related pathologic findings only at 30 days. All three treated dogs had increased hemosiderosis and extramedullary hematopoietic activity in the spleen. Two treated dogs also had hemosiderin-like pigment in the hepatic Kupffer cells. The debilitated dog sacrificed prematurely had a moderate acute bronchopneumonia.

Characteristic, bilaterally distributed changes were found in the central nervous system of all three treated animals at 30 days of treatment. Most frequently affected and therefore obviously the most susceptible areas were the cerebellar roof nuclei, pontine nuclei, vestibular nuclei and the paralemniscal / raphe region. Most extensive lesions occurred in the prematurely sacrificed dog: they involved also the regions of the lateral geniculate body, substantia nigra, nucleus ruber and even the neurons in gray matter of the lumbar spinal cord. The changes affected the nerve cell bodies. There were only few occasional degenerating nerve fibers probably representing secondary degeneration of processes of damaged neurons. The affected neurons were characterized by loss of basophilic staining, probably reflecting loss or dissolution of Nissl bodies. Their perikarya were eosinophilic in hematoxylin and eosin and pale in cresyl violet stain. Such chromatolytic neurons showed occasional nuclear eccentricity, a feature resembling "axonal reaction" (figs. 1 and 2). The advanced lesions were characterized by shrinkage of the nuclei and fragmentation of the cytoplasm. Silver stain demonstrated clumping and break-down of the perikaryal neurofilaments. Such necrotic neurons were surrounded by scavenging microglia cells, forming characteristic microglial nodules, positive for RCA-1 lectin binding reaction (figs. 3 to 6). In the peripheral nerves, no lesions were found. The brain of the prematurely sacrificed animal showed additional lesions in the cortex and hippocampus with features of ischemic neuronal change, which probably were non-specific and associated with the debilitated condition of this animal.

Experiment II

Clinical investigations: After intramuscular administration animals showed reduced activity observed in high-dose males on day 8, high-dose females on days 7 and 8 and in one mid-dose male on day 8. In addition, some high-dose group animals showed salivation and vomiting on day 7 or 8 and one female of mid-dose group a single episode of salivation on day 8. Detailed neurologic examination did not reveal any other findings. Mean body weights were reduced dose-dependently in all treated groups but the difference to controls was statistically significant for high-dose groups only. Food consumption was decreased in females of the high-dose group. Quantitative evaluation of the ECG recorded at day 7 indicated prolonged mean QT intervals adjusted for heart rate (QT_500) in males and females of mid- and high-dose groups, and in males of the low-dose group (see fig. 9).

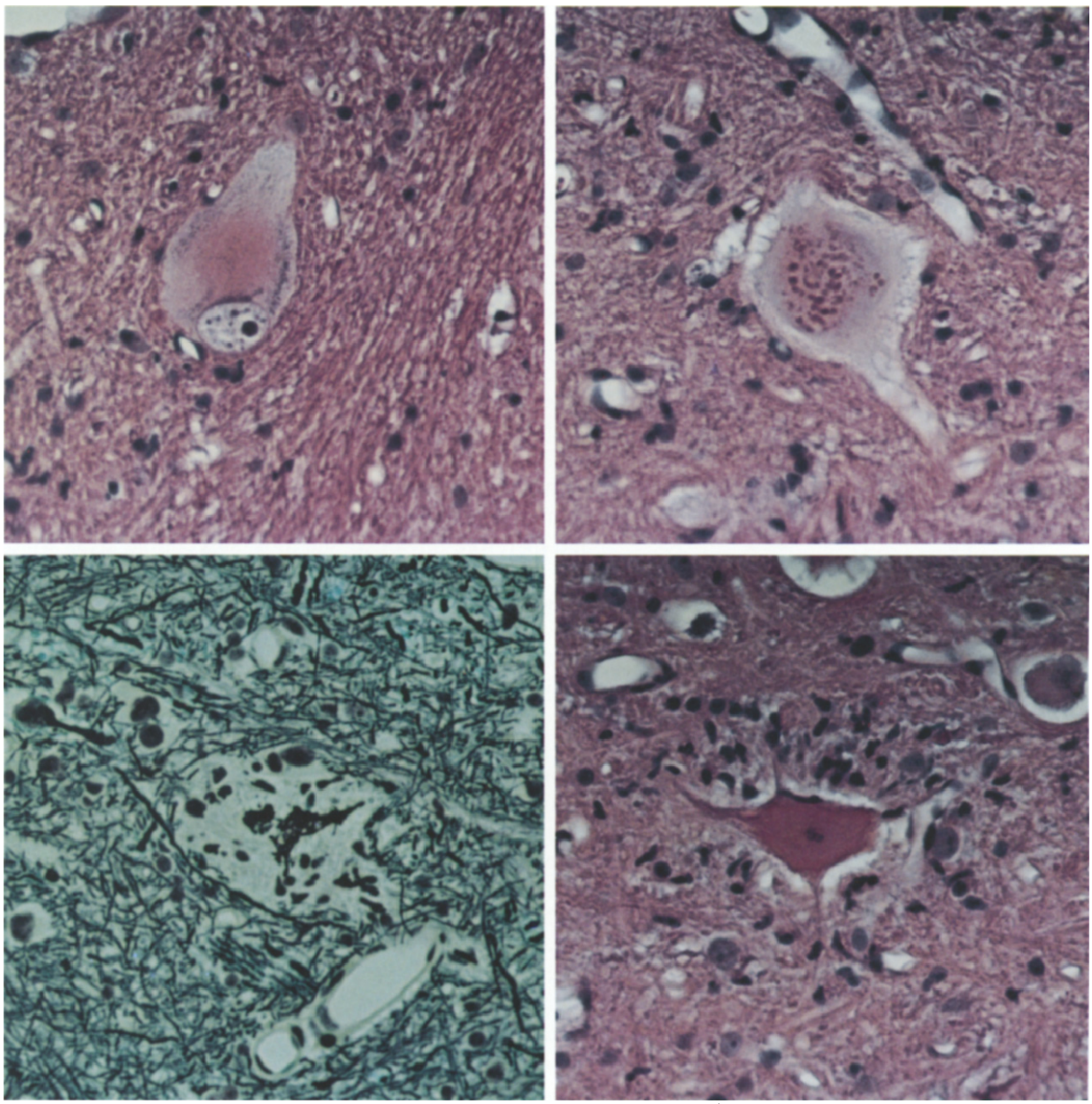


Fig. 1. A neuron in the cerebellar roof has eccentric nucleus and eosinophilic central cytoplasm. Male dog treated with 20 mg/kg i.m. for 30 days, HE, x 360.

Fig. 2. A neuron in the cerebellar roof with pale cytoplasm containing eosinophilic fragments in the center. Same animal as in Fig. 1, HE, x 360.

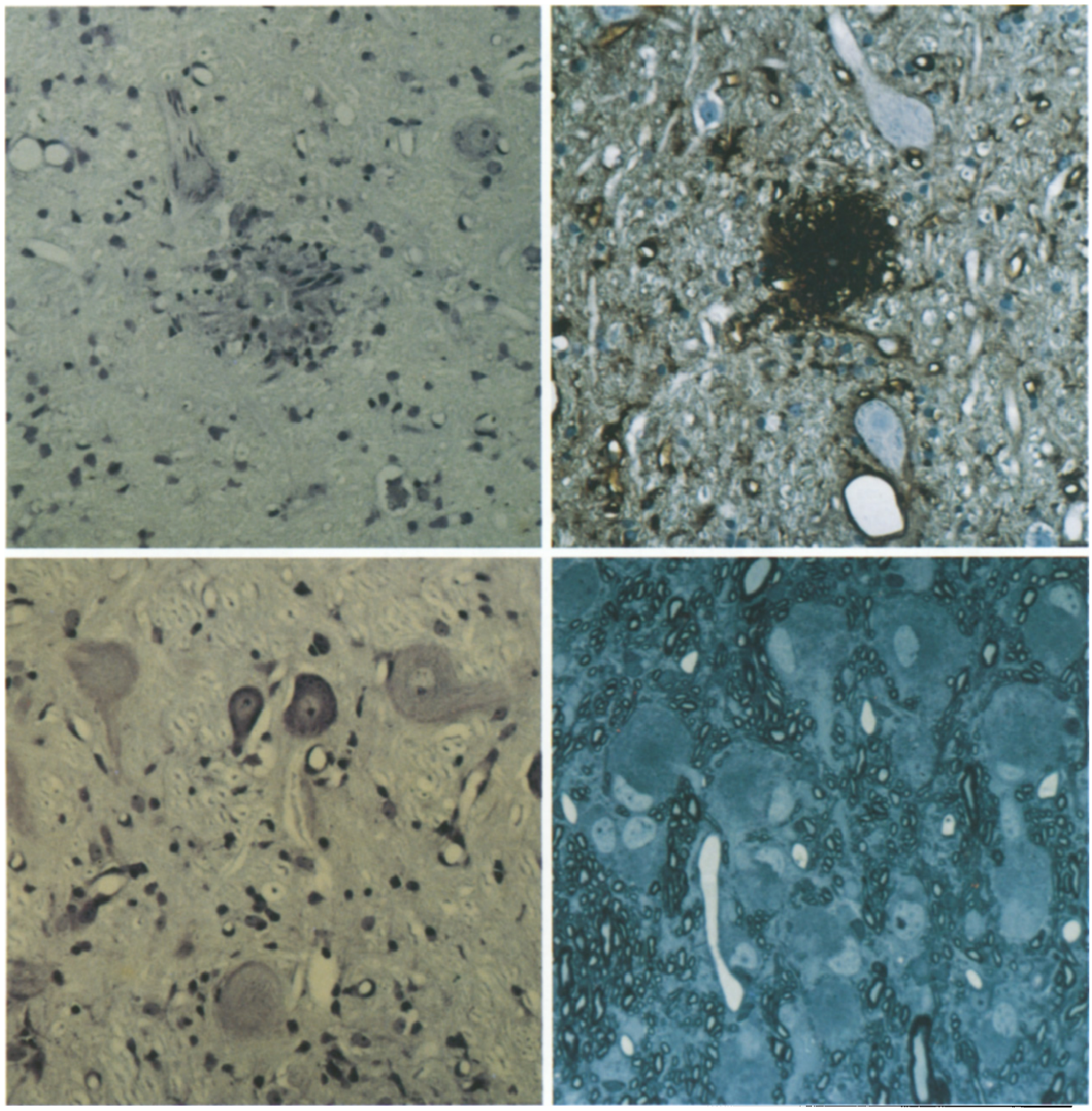
Fig. 3. A neuron in the cerebellar roof shows clumping on neurofilament remnants in silver stain. Same animal as in Fig. 1, Bodian's silver with luxol blue, x 360.

Fig. 4. A neuron in the cerebellar roof shows nuclear shrinkage and deep eosinophilia of cytoplasm. It is surrounded by microglia cells. Same animal as in Fig. 1, HE, x 360.

Heart rate, P-wave, PQ-interval and QRS-complex were not affected.

Following oral administration all high-dose males and females vomited some hours after almost each administration. Detailed neurological examination conducted on day 7 did not reveal any treatment-related findings. Male and female animals of high-dose group and females of mid-dose group had decreased mean body weights. Food consumption was significantly decreased in females of high-dose group. Quantitative examination of ECGs recorded at day 7 indicated prolonged mean QT_500 intervals in males and females of the high-dose group (fig. 9). There were no effects on any of the other ECG parameters.

Pharmacokinetics: After intramuscular administration determination of the plasma profile of AM indicated peak levels around 2 to 4 hours post-dose, slow elimination and a tendency to accumulate with repeated administra-



5	6
7	8

Fig. 5. A cluster of microglia cells surrounding a shrunken, necrotic neuron in the cerebellar roof. Same animal as in Fig. 1, cresyl violet, x 240.

Fig. 6. Same situation as in Fig. 5: the microglia cells are distinctly positive with lectin marker RCA-1, x 240.

Fig. 7. The vestibular area contains two smaller, still regularly staining neurons, several pale, chromatolytic perikarya, and some diffusely distributed microglia cells. Female dog treated with 40 mg/kg i.m. for 8 days, cresyl violet, x 360.

Fig. 8. The nerve cell bodies in pontine nuclei frequently show nuclear eccentricity and absence of Nissl bodies. Same animal as in Fig. 7, epoxy resin, toluidine blue, x 360.

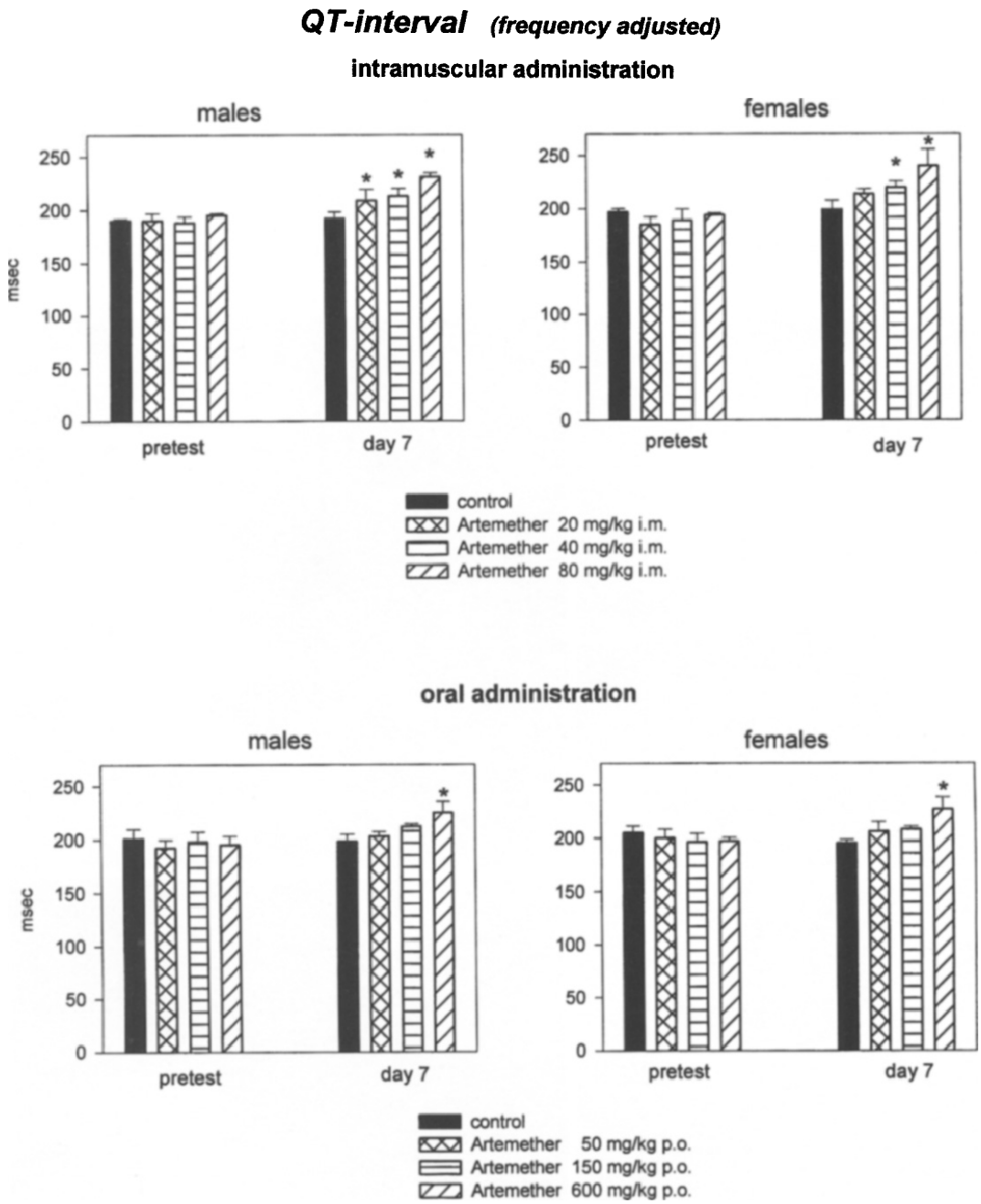


Fig. 9. QT-interval adjusted for a heart rate of 120 beats per minute (QT_500). Group means (± S.D.) are given for male and female dogs receiving AM either by the oral or intramuscular route.

tion (figs. 10–12). Two hours after the 8th administration only low levels of AM were found in the cerebrospinal fluid, while DHA concentration was below the level of quantification (table 2). After oral administration AM is very rapidly and extensively metabolized in dogs resulting in a similar but considerably lower plasma profile of AM than observed after i.m. administration. In contrast, high plasma concentrations of the major metabolite, dihydroartemisinin (DHA), were observed on day 1 showing peak levels around 2 to 4 hours post-dose. At day 7 peak plasma AM levels reached only about 15 % of those of day 1 while plasma DHA concentrations were almost nil throughout the entire sampling period (figs. 10–12). This indicates that in dogs repeated oral administration may induce liver enzymes resulting in a fast inactivation of DHA. In the cerebrospinal fluid neither AM nor DHA were found (table 2).

Pathology: Examination of the brain of dogs treated orally did not reveal any treatment-related changes, whereas the brains of dogs treated intramuscularly had neuropathic lesions with both incidence and grading reflecting a dose-effect relationship. In the low-dose i.m. groups only some

Table 1. Cumulative grading of findings in affected brain areas of Beagle dogs intramuscularly treated with AM (experiment II).
Based on individual gradings of the 4 brain areas affected (cerebellar roof nuclei, pontine nuclei, raphe / paralemniscal nuclei, and vestibular nuclei) cumulative gradings are calculated for the different lesion types (maximum possible score: 80 (max. grade 5 x 4 brain areas x 4 animals); data for oral administration are 0).
Findings were graded as 0 (absent), 1 (minimal), 2 (slight), 3 (moderate), 4 (marked), and 5 (severe).

Dose	Finding	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg
MALES	Neuronal chromatolysis	0	12	47	57
	Neuronal necrosis	0	0	5	7
	Microgliosis	0	6	10	15
FEMALES	Neuronal chromatolysis	0	2	47	56
	Neuronal necrosis	0	0	6	3
	Microgliosis	0	2	15	9

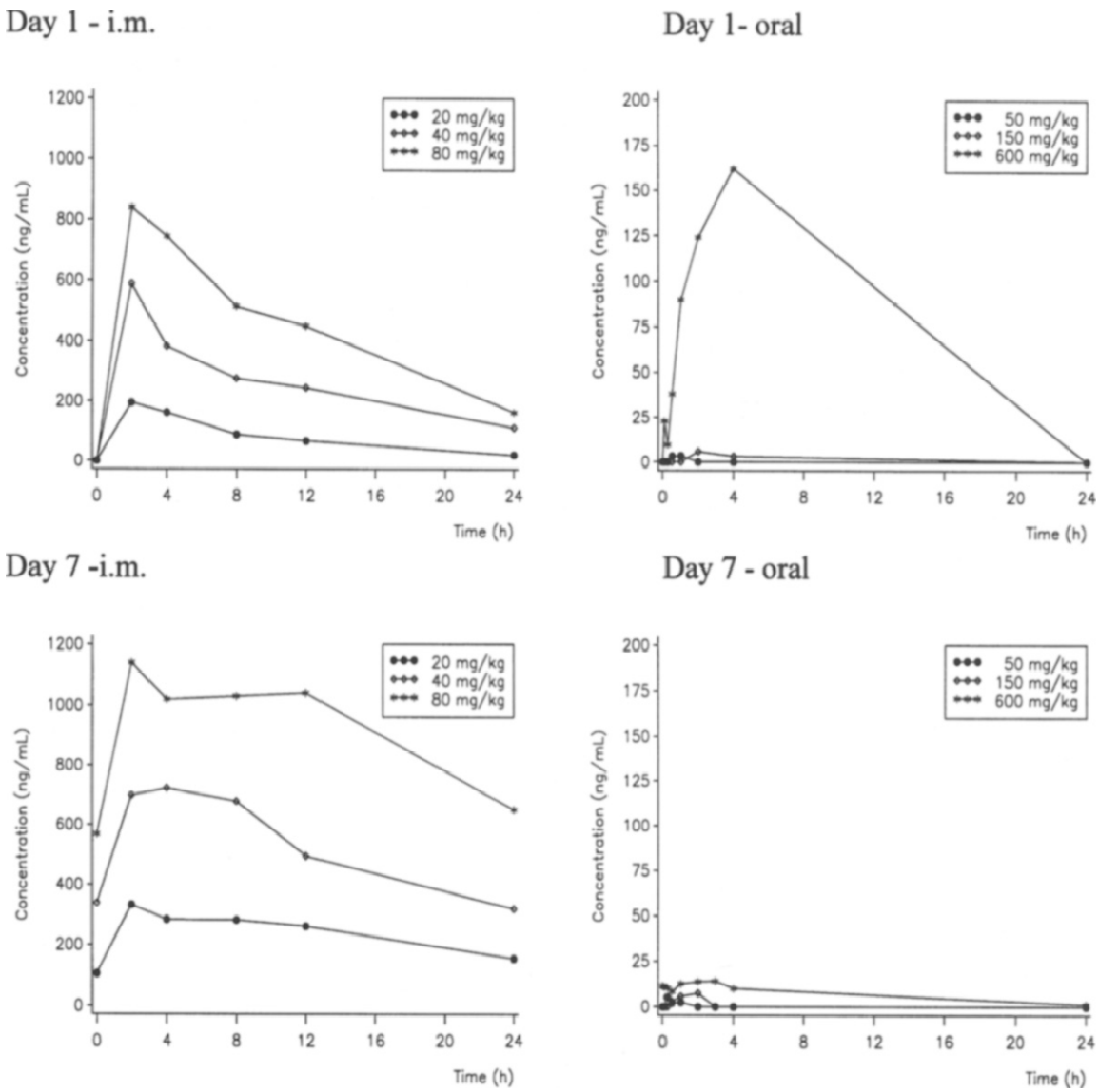


Fig. 10. Mean plasma profiles of artemether in healthy Beagle dogs after single or repeated intramuscular administration of artemether.

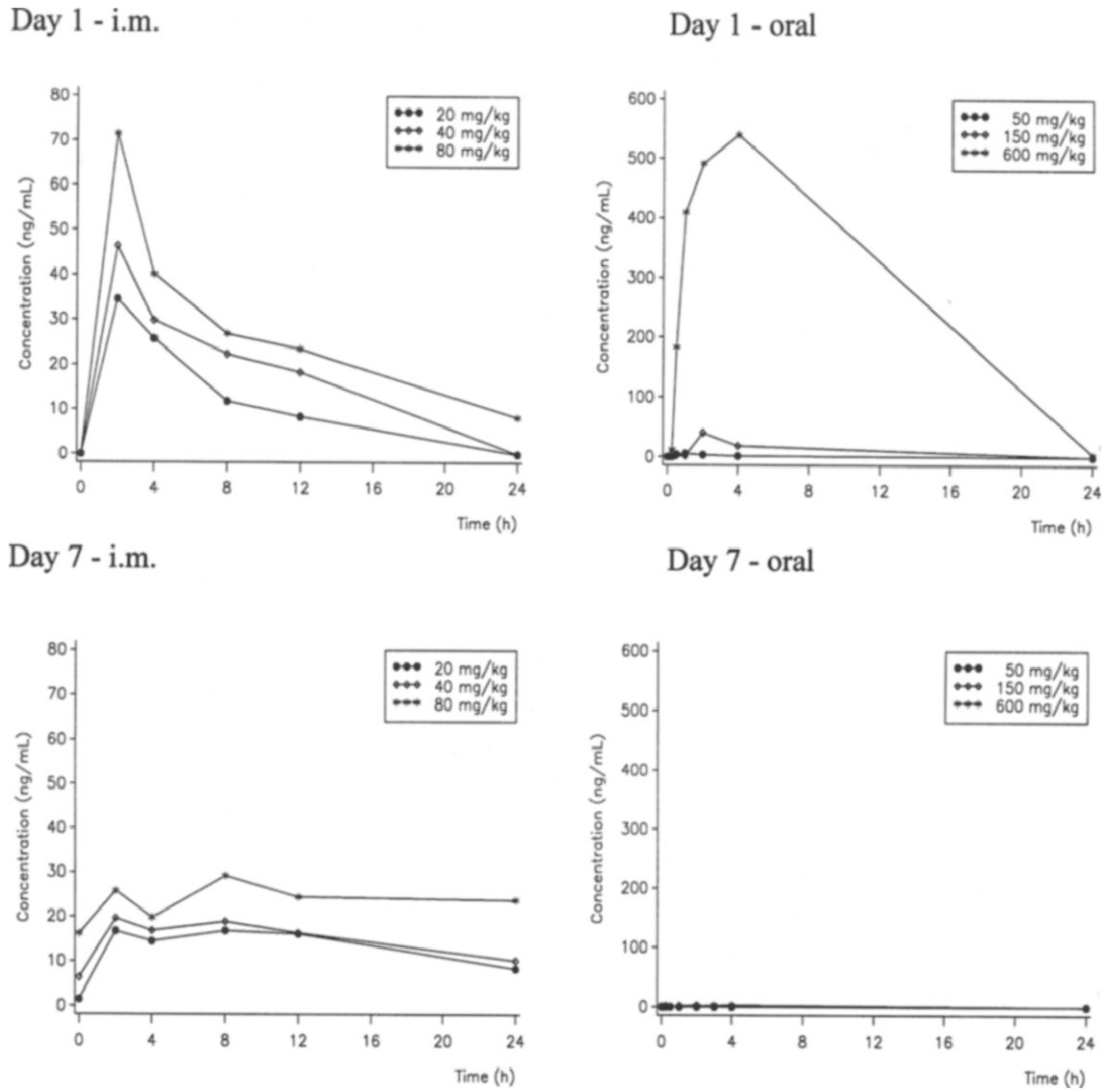


Fig. 11. Mean plasma profiles of dihydroartemisinin in healthy Beagle dogs after single or repeated oral administration of artemether.

Table 2. Concentration of artemether and dihydroartemisinin in cerebrospinal fluid.
Mean concentration of artemether and its metabolite dihydroartemisinin in cerebrospinal fluid (in ng/ml). Samples were taken 2 hours after the 8th administration.

	intramuscular dose (mg/kg)			oral dose (mg/kg)		
	20	40	80	50	150	600
Artemether	25.2 ± 6.3	60.0 ± 41.8	71.1 ± 21.2	n. d.	n. d.	n. d.
Dihydroartemisinin	blq	blq	blq	n. d.	n. d.	n. d.

blq: below level of quantification (< 10 ng/ml); n.d.: not detectable (< 1 ng/ml)

(5 out of 8) animals showed slight changes, whereas in the mid-dose and high-dose i.m. groups all animals were affected and the lesions were prominent (table 1). The characteristic changes in the affected neurons were again, loss of cytoplasmic basophilia, occasional nuclear eccentricity (figs. 7 and 8) with occasional fragmentation and shrinkage of the nerve cell body and microglial reaction.

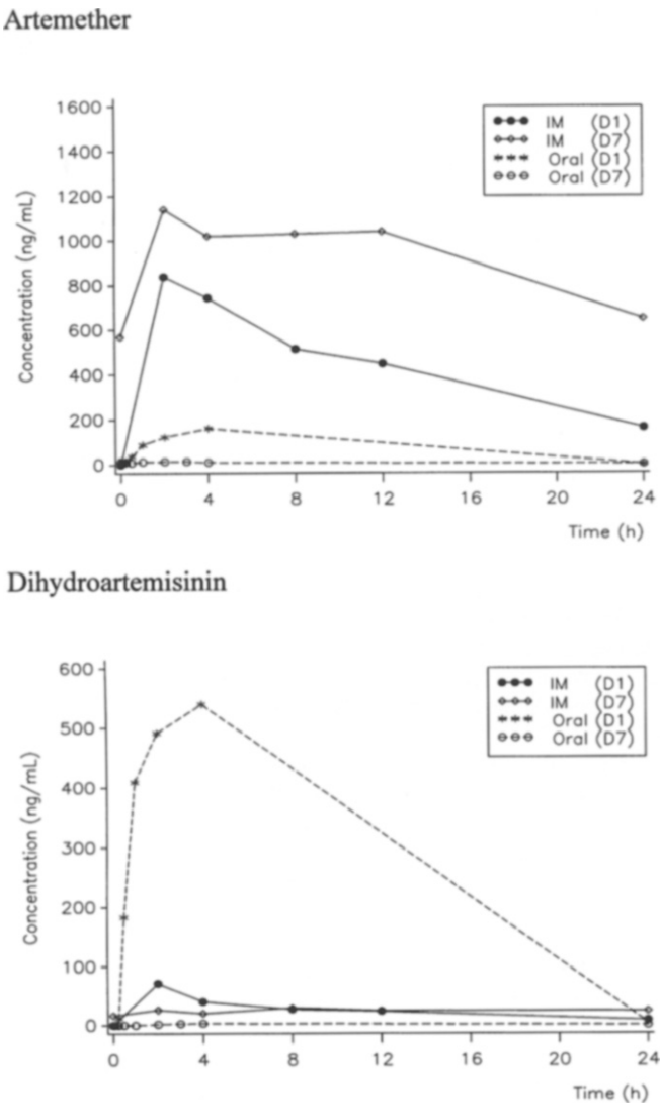


Fig. 12. Comparison of mean plasma profiles of artemether and dihydroartemisinin in healthy Beagle dogs after single or repeated administration of artemether at 80 mg/kg i.m. or 600 mg/kg p.o.

Discussion

The results of *experiment I* demonstrated the spectrum of toxic changes evoked by excessive exposure for 30 days and simultaneously the absence of such changes after a shorter exposure period of 5 days at the same exposure level of 20 mg/kg i.m. The findings specifically associated with AM effects were decreased motility, recumbency, convulsions, a trend towards a prolonged QT interval (ECG), hypochromic, microcytic anemia correlating with increased hemosiderosis in the spleen and liver and hematopoietic activity in the spleen, and neuropathic changes in the central nervous system.

Individual animals showed a short and dramatic clinical deterioration ending in convulsions and death. Neuropathologic changes were found in these animals as well as in surviving animals without severe signs. This is in accordance with the findings of GENOVESE et al. (1995) who observed normal behavioral performance in AE treated rats as measured by an operant conditioning task despite substantial neuronal damage. Similarly BREWER et al. (1994a) described that in dogs treated with AE neurological changes appeared only shortly before death. There are several factors that may contribute to the apparently normal function despite the presence of neuronal damage: 1) neuronal reserve capacity and/or the capability of the nervous system to compensate for lost neurons and 2) some areas affected are involved mainly in auxilliary functions. In the absence of early clinical signs that could act as warning signals, neurotoxicity induced by intramuscular AM has to be evaluated with great caution.

The neurotoxic changes were restricted to the central nervous system and affected primarily the nerve cell bodies, so that the lesion can be classified in terms of cellular targets as "neuronopathy". The early change, characterized by dissolution of Nissl bodies and occasional nuclear eccentricity resembled "axonal reaction" known to occur in response to damage of axons. However, whereas in axonal reaction the rough endoplasmic reticulum rearranges at the neuronal periphery in order to produce cytoskeleton needed for reconstitution of the damaged axon (KRINKE et al. 1985; KRINKE and FITZGERALD 1988), such rearrangement did not occur in AM toxicity. It is quite possible that an initial partial neuronal damage in AM toxicity triggers some of the signals involved in axonal reaction and that transduction of such signals results in similar remodeling of neuronal soma. The features of neuronal changes resembled pathology known to occur in spontaneous motor neuron diseases such as amyotropic lateral sclerosis (ALS) in man and a variety of similar diseases in animals (SUMMERS et al. 1995). Interestingly, oxidative stress has been proposed to play a role in the etiology of ALS, specifically a malfunction of superoxide dismutase, resulting in surplus of hydrogen peroxide and hydroxyl radicals or peroxynitrite damaging the neurons (CEBALLOS-PICOT 1997). Since oxidative mechanisms are involved in the antiparasitic effect of AM, it is conceivable that the toxic effect of AM could employ similar mechanisms acting on the neurons after excessive exposure. The reasons for selective distribution of the lesion in the brain, affecting the lower brain stem and preserving the upper brain stem and the cortex, are unclear. It may reflect selective vulnerability of the affected neurons, uneven distribution of AM in the brain, or both. The explanations of the mechanisms involved in neuronal damage due to oxidative stress usually consider triggering of apoptosis. In our studies however, nuclear shrinkage occurred rather than karyorrhexis which is known to result form apoptosis. In AM neuropathy the early microscopic changes appear to affect the cytoplasm and apoptosis does not seem to play any significant role. Therefore, to mitigate an overdose of AM, scavengers of free radicals could be relevant antidotes rather than agents inhibiting apoptosis.

Quantitative evaluation of the ECG in *experiment II* indicated slight prolongation of mean QT_500 interval in

high-dose animals dosed by the oral route and after i.m. administration in mid- and high-dose groups and males of the low-dose group. In contrast to earlier reports (BREWER et al. 1994a) no inversion of the T-wave was observed. Prolongation of the QT interval in the absence of any effect on QRS-interval indicates that the ST-interval is prolonged selectively which reflects slowing of ventricular repolarization. Similar effects are known from other pharmaceuticals such as quinidine or procaine. For these two compounds a prolongation of the QT interval of 50 % and more is associated with the danger to provoke syncopes or sudden death while a 25 % increase is considered as threshold for concern. Compared to their pretest values QT_500 interval was prolonged by 25 % or more in 1 of 4 females each at 80 mg/kg i.m. (137 %) and at 40 mg/kg i.m. (125 %), while after oral administration prolongation was below 25 % in all animals. Using a 25 % prolongation as criterion for adversity, only the increase in the QT_500 interval observed after i.m. administration of 80 mg/kg of artemether is considered to be of toxicological relevance. Although available data do not allow to elucidate the mechanism underlying these ECG changes, it is interesting to note that prolongation of QT intervals occurred both after intramuscular and oral administration and therefore appears unrelated to neurotoxicity which is observed after i.m. administration only.

Pharmacokinetic evaluation showed that in orally treated dogs, in contrast to intramuscular treatment, AM was rapidly metabolized to the pharmacologically active DHA resulting in low plasma levels of the parent compound but high plasma levels of this metabolite. As indicated by low plasma levels of DHA after the 8th administration, repeated administration accelerated its further metabolism and inactivation. Unlike in dogs, in humans the pharmacokinetic profile of AM and DHA were similar when administered by the oral or i.m. route. A single oral dose of 300 mg (5 mg/kg bw) resulted in maximum plasma concentrations of AM slightly lower and in higher DHA levels than after i.m. administration of the same dose (KARBWANG et al. 1997, TEJA-ISAVADHARM et al. 1996) and repeated oral dosing in humans did not lead to a similar rapid metabolic inactivation of AM as in dogs (NA BANGCHANG et al. 1994). In dogs intramuscular administration resulted in plasma profiles of AM and DHA comparable to those observed in humans after either route of administration.

Taken together, the results of our experiments in dogs indicate that oral administration of AM did not produce neuropathy at very high exposure levels. Repeated intramuscular treatment with 20 mg/kg did not induce neurotoxicity after 5 days, while marginal effects occurred in some animals dosed for 8 days. Based on the differences in pharmacokinetics between humans and dogs it could be questioned if results obtained with oral administration in dogs can be directly extrapolated to humans dosed orally. A comparison based on pharmacokinetics profiles shows that in healthy animals and patients dosed orally initially with 200 mg/kg plus 100 mg/kg followed by daily 100 mg/kg for 4 days, plasma concentrations of AM were similar to those observed in this study in dogs treated with 20 mg/kg i.m. (NA BANGCHANG et al., 1994). In dogs, this dose was tolerated for 5 days, while the effects at 8 days represented a level of neurotoxicity. However, in humans neurotoxicity was neither observed in clinical trials (KAMCHONWONGPAISAN et al. 1997) nor reported in the published literature generally.

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