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Pharmacokinetic (PK) and Pharmacodynamic Profiles of Artemisinin Derivatives Influence Drug Neurotoxicity in Animals

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1. Introduction

In the past decade, there have been major advances in our knowledge of severe CNS neurotoxicity in mice, rats, dogs, and rhesus monkeys repeatedly administered the oil-soluble artemisinins (ARTs) of arteether (AE) or artemether (AM), and water-soluble artelinic acid (AL). Studies have shown that those drugs are toxic to the central nervous system and induce neuropathologic changes in these animals (Li & Hickman, 2011). Pharmocokinetic and toxicokinetic studies of model animals administered various artemesinin derivitives through different routes have yielded important information that is relevant and useful in predicting possible neurotoxicity in man, particularly as artemesinin drugs are used more widely for other indications beyond malaria treatment such as cancer therapy.

The fat soluble artemesinin derivitives AE and AM are particularly prone to induce neuropathological damage at low doses. For example, in rats, CNS damage was induced by dosing with AE intramuscularly at a daily dose of 12.5 mg/kg for 7 days , while a daily dose of 6 mg/kg for 28 days was required to induce neuropathological changes in dogs, and dosing of 8 mg/kg daily for 14 days was required to induce similar damage in rhesus monkeys (Brewer et al., 1994a; Classen et al., 1999; Genovese et al., 1995, 1998; Kamchonwongpaisan et al., 1997; Li et al., 2007a; Petras et al., 1997). A similar finding was observed for rats treated with oral AL, which was reported to have similar pathological neurotoxicity following an oral dose at 160 mg/kg daily for 9 days (Si et al., 2007).

Despite extensive studies of AE, AM, and AL neurotoxicity, there is no evidence of neurotoxicity in animals related to another water soluble ART derivative, artesunate (AS), the most widely used ART in humans (Li & Weina, 2010). AS was designed for intravenous injection and, up to now, no neurotoxicity (pathologic or behavioural) has been observed in animals following intravenous administration at any repeated doses up to its maximum tolerated doses (MTD). The MTD of AS has been shown to be 240 mg/kg following intravenous injection daily in rats for 3 days, but no neurotoxicity was detected in these animals (Xie et al., 2005). In another study, intravenous AS in sodium bicarbonate dosed daily for 7 days at 120 mg/kg had no effect on neurotoxicity scores (our unpublished data). A third study of AS treatment in rats administered one intramuscular injection of AS at a high dose of 420 mg/kg did not induce any neuronal necrosis. In addition, dosing of up to 200 mg/kg of

AS orally daily for 5-7 days did not result in neuronal changes or any specific clinical signs (Dayan, 1998), and there was little clinical or neuropathological evidence of neurotoxicity found at high dose levels of over 200 mg/kg/day for 28 days in mice treated with oral AS and dihydroartemisinin (DHA), as well as oral AM (Nontprasert et al., 1998, 2000, 2002).

To date, no systematic toxicity has been reported in humans, despite the use of ART and its derivatives in clinical trials designed with special emphasis to detect changes in neurological indicators such as movement, hearing, vestibular, or cerebral abnormalities (Efferth & Kaina 2010). Although ART agents, mainly the oil-soluble derivatives, have been shown to induce fatal neurotoxicity in animal models, a striking fact in treating patients with the ARTs is the lack of any serious adverse events, despite careful monitoring in several clinical studies. Severe malarial infection, particularly cerebral malaria in multi-drug resistant strains in the developing world, often leaves patients with decreases in neural function. This development has made characterization of potential neurotoxic effects of the ART derivatives very difficult to ascertain, even though these drugs are clinically utilized in ever growing numbers (Karbwang et al., 1994; Van Vugt et al., 2000; Angus et al., 2002). In a study of 3500 patients in Thailand, evidence of serious toxicity was not noticed (Price et al., 1999). From other reports, rare neurologic side effects have been noted in humans receiving ART drugs, however, these findings were difficult to dissect from the effects of malaria infection (Li & Hickman 2011).

Five cases have been reported that suggest episodes of neurotoxicity due to multiple courses of ARTs therapy (Elias et al., 1999; Franco-Paredes et al., 2005; Haq et al., 2009; Miller & Panosian 1997; Panossian et al., 2005). These reports may constitute evidence of a causal association that merits further investigation. The possibility exists that repeated and longer duration administration of ART compounds in malaria endemic areas could result in cumulative neuronal damage, especially since these drugs are often freely available for use in tropical countries. Although the authors reported that the toxicity observed in these five cases is drug related, there is considerable disagreement regarding these putative episodes of neurotoxicity, and the evidence of such a causal association may or may not be only related to ART administration (Gachot et al., 1997; Newton et al., 2005; White et al., 2006).

Several perplexities still continue to vex us with the ARTs. Why is neurotoxicity so easily observed in animal models but not seen in humans getting equal or even higher doses of the drug? Why does this neurotoxicity mostly occur in animals treated with intramuscular AE, AM, and oral AL, but not with AS? Why does neurotoxicity mostly take place with the intramuscular and oral dosing routes, but not in intravenous injections? In this chapter, pharmacokinetic characteristics will be evaluated and discussed that may shed light on some of these questions. Principally, we will discuss:

i.   the role of DHA in neurotoxicity and the conversion rates of AM, AE, AL, and AS to DHA;
ii.   the role of drug tissue distribution levels in cerebrospinal fluid (CSF) and brain tissue at the onset of neurotoxicity;
iii.   the role of drug total exposure levels (AUC and C_{max}) in plasma in neurotoxicity; and
iv.   the role of drug half-life in neurotoxicity.

In addition, the drug PK parameters required to induce neurotoxicity in animals after administration of ARTs will be discussed in depth from the available pharmacological literature. We will outline the data that supports the hypothesis that drug accumulation with prolonged exposure time (half-life) demonstrated by PK profiling accompanies the neurological effects shown by Pharmacodynamic (PD) profiling. As will be shown, there is

considerable data to support the view that the drug exposure time is a more important marker to determine and predict ART-driven neurotoxicity than other factors.

2. Key PK parameters associated with artemisinin induced neurotoxicity in animals

2.1 The role of DHA in artemisinin-induced neurotoxicity

DHA is obtained by sodium borohydride reduction of ART, an endoperoxide containing sesquiterpene lactone. Through *in vitro* bioassays, DHA has been shown to have similar antimalarial activity to AS and it is 1.4-5.2 fold more active than other ARTs (Li et al., 2007b). AS has also been shown to be 2-4 fold more toxic than other ARTs *in vitro* and *in vivo* (Li et al., 1998a; 2002; Fishwick et al., 1995; McLean & Ward, 1998; Wesche et al., 1994). Due to its poor solubility in water or oils, DHA has only been formulated as an oral preparation and has been used primarily as a semisynthetic compound for derivatization to other oil soluble and water-soluble ARTs. The effectiveness of AS has been attributed to its rapid and extensive hydrolysis to DHA (Li & Weina 2010). The efficacy of AM and AE has also been credited to their conversion to DHA, but their rates of conversion were significantly lower and less complete than in AS (Li et al., 1998b; Navaratnam et al., 2000). AL is a very stable ART agent *in vitro* and *in vivo*, and AL has almost no biotransformation to DHA in animals (Li et al., 1998c; Lin et al., 1987).

Accordingly, evaluating the conversion rates of various ART drugs is very important for assessing the risk of neurotoxicity for the four compounds that are the focus of this chapter. Conversion evaluations of AM, AE, AS, or AL to DHA in laboratory animals are summarized in Table 1. This summary also illustrates the conversion of ART drugs to DHA in humans, which seems to be much more efficient than in animals. When comparing the data from animals and humans treated with AM or AS in various regimens, one difference that is immediately apparent is that the ratios of AUC_{DHA} to AUC_{AM} or AUC_{AS} are significantly higher in humans (0.41-9.71) than in animals (0.04-2.96), suggesting that the human hydrolysis system is more active and complete (Li et al., 2007a).

The conversion rates of the four ART agents to DHA are different in various animal species. The most extensive hydrolysis of AS to DHA has been shown to be in rats and dogs with a DHA/AS ratio in the range of 0.31-2.74, while the ratio of AM or AE conversion to DHA is less than 0.23. In addition, the calculated ratio of AL conversion to DHA in rats and dogs lies in the range of 0.001-0.03, so it appears that DHA is not contributing as much to the effects of neurotoxicity associated with dosing these three drugs (AM, AE, and AL) while it appears to be a much larger contributor to DHA after AS dosing (Table 1). Data derived from pharmacokinetic studies has demonstrated that the AUC of DHA hydrolyzed from AS is in the range of 236–596 ng·h/ml compared to the AUC of DHA hydrolyzed from AM and AE which lies in the range of 29-71 ng·h/ml at the same dose level in rats (Li et al., 1998b). AM and AE treatment has been shown to induce severe neurotoxicity in animals, AS treatment has not been shown to do so (Genovese et al., 2000). Similar results have been observed in dogs, where the AUC of DHA produced from AS was shown to be 1,109 ng·h/ml at a dose of 10 mg/kg with no neurotoxicity symptoms observed (Li et al., 1999a). In comparison, the AUC of DHA derived from AM and AE dosing in dogs is 312 and 181 ng·h/ml, respectively, at a dose of 20 (AM) and 15 (AE) mg/kg, and those treatments have

been shown to result in fatal neurotoxicity following intramuscular injection (Classen et al., 1999; Li et al., 2000).

Drugs and administrations	Animals		Humans	
	Rats	Dogs	Volunteers	Malaria Patients
Artemether (AM)				
Intravenous	0.04 (65/1857)	-	-	-
Intramuscular	0.07 (71/1007)	0.14 (312/2240)	0.41 – 1.62	1.06
Oral	0.10 (38/366)	2.96 (5592/1887)	1.12 – 7.69	1.42 – 8.55
Arteether (AE)				
Intravenous	0.04 (29/842)	-	-	-
Intramuscular	0.15 (43/286)	0.23 (181/804)	-	0.16 – 0.19
oral	0.17 (50/298	-	-	-
Artesunate (AS)				
Intravenous	0.64 (474/738)	0.50 (778/1533)	-	2.81 – 8.12
Intramuscular	0.31 (236/773)	0.73 (1109/1521)	-	-
Oral	2.74 (595/217)	0.45 (299/660)	4.29 – 5.36	6.35 – 9.71
Rectal	-	-	2.31	-
Artelinic acid (AL)				
Intravenous	0.01 (63/12706)	0.001 (7/11262)	-	-
Intramuscular	0.01 (44/5023)	0.003 (23/10195)	-	-
Oral	0.03 (62/1650)	0.002 (14/8849)	-	-

DHA = dihydroartemisinin

Table 1. Mean ratios of AUC_{DHA} (an active metabolite) / $AUC_{AM, AE, AS, and AL}$, and the concentration values (ng·h/ml) of $AUC_{DHA}/AUC_{AM, AE, AS, and AL}$ as the conversion evaluation of artemisinin derivatives (AM, AE, AS, and AL) to DHA in animals and humans (Li et al., 2007a)

The maximum tolerated dose (MTD) of intravenous AS has been shown to be 240 mg/kg dosed daily for 3 days (Xie et al., 2005). In this study, animals were sacrificed at 24 hrs (day 1) and 192 hrs (day 8) after the last dose. Histopathological evaluation demonstrated mild renal tubular necrosis in uninfected rats treated with AS at 240 mg/kg, however, fewer pathological lesions (minimal tubular necrosis) were observed in malaria infected rats. The renal injury was reversible in all cases by day 8 after cessation of dosing, and also no neurotoxicity was found after any of the intravenous AS dosing regimens (Li et al., 2007c). The AUC of DHA derived from a dose of AS of 240 mg/kg ranged from 6,984–15,768 ng·h/ml. This AUC can be simulated daily in rats using this regimen according to our previous published study (Li et al., 1998c). Although DHA is 2-4 fold more active and more

toxic than other ART derivatives (Fishwick et al., 1995; Li et al., 1998a, 2002; McLean & Ward 1998; Wesche et al., 1994), the high level of DHA generated from AS did not seem to have a key role in the induction of neurotoxicity in these animal studies.

In another comparative PK study of DHA hydrolysis, AL and AS were administered to malaria-infected rats using 3 daily equimolar doses (96 µmoles/kg, which equals 36.7 mg/kg for AS and 40.6 mg/kg for AL) via intravenous administration. The plasma concentration of AS observed was one-third less on day 3 than on day 1, similar to the plasma concentrations of its active metabolite, DHA (Li et al., 2005a). The results were similar for other ART drugs with an auto-induction metabolic profile (van Agtmael et al., 1999; Ashton et al., 1996, 1998; Khanh et al., 1999), but not for AL. The PK parameters of AL were very comparable from day 1 to day 3 at the same molecular doses of AS (40.6 mg/kg daily). AS seems to be the pro-drug of DHA with a DHA/AS ratio of 5.26 (AUC_{DHA} of 13,051 ng·h/ml) compared to the ratio of 0.01 (AUC_{DHA} of 524 ng·h/ml) for DHA/AL. In this study, dosing with the two drugs did not induce any CNS toxicity (Li et al., 2005a), but the general toxicity of AL was 3-fold greater than that of AS in malaria-infected rats (Xie et al., 2005). In conclusion, we believe the conclusions drawn from our data and from other published work support the hypothesis that DHA is not an important factor in induction of neurotoxicity in animals.

2.2 The impact of ARTs distributed in the CNS on neurotoxicity

There are only a few studies of tissue distribution of ART derivatives in the animal brain, and these published studies used a variety of methods that differ significantly. Therefore, there are no comparative published results for quantitative evaluation of the ART drugs distributed in the central nervous system (CNS).

The first quantitative determination study of ARTs in the CNS using the thin-layer chromatography densitometry method found that ART, AM, and AS can cross the blood-brain barrier (BBB) and the blood-placenta barrier in rats (China Cooperative Research Group, 1982). ART has been noted to be more readily detected in the brain after oral administration than after intravenous injection (Niu et al., 1985). By radioimmunoassay, the highest level of AS was found in the rat intestine 10 minutes after intravenous administration, followed by the brain, liver, kidney, and other organs, in decreasing order. After one hour, the AS drug levels dropped significantly but not uniformly in all tissues, with high levels still remaining in the brain, fat, intestine and serum. The highest levels were found in rat brains 5 minutes after intravenous injection (Zhao & Song, 1989). Moderate levels of AM were also found in the heart, lung and skeletal muscles, while AM levels in liver and kidney were low (Jiang et al., 1989).

Using the tissue homogenate method, intramuscular injection of ^{14}C-arteether resulted in higher levels of radioactivity detected 24h after dosing in the intestinal tract, liver, kidneys, and spleen, while lower amounts of drug were found in the brown fat and in the brain . High-performance liquid chromatographic data from this work indicated that three metabolites of arteether, but not arteether itself, could cross the BBB. The calculated apparent concentration in the whole brain was 0.89% of the total radioactivity after intramuscular injection of 25 mg/kg AE (Li et al., 2007a).

Using the whole body autoradiograph method, administration of ^{14}C-AL showed widespread distribution of the radiolabeled drug in rats one hour following dosing. The

relative activity of ^{14}C-AL found in various body tissues (as density per unit area) was, from highest to lowest, in the intestinal tract followed by the liver, kidney, bone marrow, spleen, brown fat and salivary glands, heart & testes, and lastly by the brain. At 48 h, the relative activity of ^{14}C-AL found in various body tissues (as density per unit area) was, from highest to lowest, in the intestinal tract, followed by the spleen, kidney, liver, salivary glands, brown fat and bone marrow, testes, and lastly by the brain. The low residual activity found in the brain appeared to be uniformly distributed. Autoradiographic evidence confirmed the presence of ^{14}C-AL in the brain from 1 to 192h. However, the presence of ^{14}C-AL in the brain was at a lower level than the presence of ^{14}C-AL in most other tissues. There was no evidence of a label concentration in any area of the brain that was visible in the whole body sections. Penetration of the BBB by ^{14}C-labelled AL was shown to be very poor. An apparent concentration of 0.1% of total radioactivity/brain was calculated from an intravenous and oral administration of 10 mg/kg AL (Li et al., 2005a).

Another neurotoxic dose range study showed that neuronal damage occurred in all beagle dogs dosed with multiple daily intramuscular treatments of AM at 40 and 80 mg/kg. After dosing with AM at 20 mg/kg administered through multiple daily treatments, minimal neuronal effects were observed in 5 out of 8 dogs. Two hours after the eighth administration (on day 8), only low levels of AM (25, 60, and 71 ng/ml)were found in the cerebrospinal fluid (CSF) after dosing with 20, 40, and 80 mg/kg respectively. AM levels in the CSF were < 10% of the AM observed in the plasma concentration (Classen et al., 1999).

These limited and incomparable data indicates that the oil-soluble derivatives, AE (0.89% of total dose in brain) and AM (10% of plasma level in CSF) appear to possess the ability to cross the BBB more readily than water-soluble ART compounds, like AL (0.1% of total dose in brain). If this is true, then the higher degree of neurotoxicity of AE and AM compared to the neurotoxicity observed after treatment with water soluble forms of artemisinin is easily understood. However, this deduction is not supported by the observation that AS has the ability to easily cross the BBB. The largest fraction of AS has been found in rat brains after intravenous administration by radioimmunoassay (Zhao & Song, 1989). Recent studies found that the levels of ^{14}C-AS in rat brains were more than 2 fold higher in the brain than in plasma, and the AUCs of radioactivity observed in the brain and in plasma were 70.7 μg·h/g and 29.4 μg·h/ml, respectively, by both of quantitative whole-body autoradiography (QWBA) and tissue dissection techniques (LSC) methods (Li et al., 2008). During a 192 hour period, 1.27% of the total radioactivity dose was detected in the rat brain. The half-life of ^{14}C-AS in the brain tissue was 94.2 hours, which is significantly longer than the 63.7 hour half-life observed in the plasma (Li et al., 2006a; Li et al., 2008). These results showed that the resident time of ^{14}C-AS was longer in the brain than in plasma. This may reflect a "sink effect" of DHA uptake transfer by the lipid-rich structures of the brain (Kearney & Aweeka, 1999).

After intravenous AS injection, samples were found in CSF that contained no parent drug which is consistent with the low lipid solubility of the metabolites of AS (Davis et al., 2001). The types of AS metabolites present in the CNS are completely unknown (Li et al., 2005a), and whether these metabolites in the brain are associated with neurotoxicity is also unknown. AS is converted stoichiometrically to DHA and the DHA concentrations observed peak at 5-10 min after dosing in plasma (Batty et al., 1998; Davis et al., 2001). DHA is highly lipid soluble and has a low molecular mass (284 Da), and both of these factors favor penetration of DHA into the CSF (Kearney & Aweeka, 1999). Since DHA is poorly soluble in

water, it should be able to cross cell membranes and the BBB. After treatment with AS in patients, however, the parent drug (AS) cannot be detected in the CSF. DHA levels in the CSF after AS treatment increase with time while DHA levels in plasma decrease. These finding suggest a continuing influx but a slower efflux of DHA in which DHA might accumulate in the CSF during frequent AS dosing (Davis et al., 2003; Kearney & Aweeka, 1999).

AS and/or its metabolites have been shown to easily cross the BBB and have higher accumulation in brain tissues when compared to the other ARTs, as described above (Kearney & Aweeka, 1999; Jiang et al., 1989; Li et al., 2005a; Zhao & Song, 1989). After intravenous injection of 120 mg AS in malaria patients, DHA levels in the CSF were shown to increase ranging from 1,100-1,450 ng/ml, which is much higher than the DHA levels observed in plasma which ranged from 104-120 ng/ml (Davis et al., 2003). No neurotoxicity, however, was observed in these studies. Therefore, while the ART agents distribute in brain tissue and CSF, they do not appear to be an important factor in induction of neurotoxicity.

2.3 The role played by the drug exposure level of artemisinins in neurotoxicity

Recognizing the fact that if there is no drug exposure, there is no toxicity leads us to the conclusion that if there is exposure, toxicity can ensue when exposure exceeds a certain level and/or time and that will be dependent on pharmacokinetics and pharmacodynamics.

2.3.1 PK profiles of ARTs in rats

After daily intramuscular injections of AE at a dose of 25 mg/kg for 7 days, accumulation and prolonged exposure of AE in plasma was noted in rats with severe neurotoxicity. This accumulation was shown to be due to slow and prolonged absorption of AE in the muscle injection site (Li et al., 1999b). The absorption of AE from muscle at the injection site was incomplete in the first 48 hours after a single injection. At 24 and 48 hours after dosing, 38% and 22% of the total dose of AE still remained in the injection site, respectively. The amount of AE in muscle rapidly decreased for 1-2 hours and then exhibited a much slower rate of decrease. Half-lives for the fast and slow absorption phases in the muscle were 1.0 hour and 26.3 hours, respectively. It was expected that the remaining dose amount would be absorbed later because an intramuscular total dose of AE with sesame oil should be 100% absorbed in the absence of decomposition or metabolism at the injection site.

The analysis of toxicokinetic (TK) parameters on day 1 of this study was similar to that of the single intramuscular dose. After day 1, however, the kinetic data estimates from days 2-7 showed a notable change in the TK parameter estimated for day 1 (Figure 1, top). The C_{max} of AE (410 ng/ml) on day 7 was found to be three times higher than on day 1 (130 ng/ml). The AUC of AE observed after the intramuscular dose was found to be 4.5-fold higher on day 7 at steady state (4367 ng·h/ml) than on day 1 (905 ng·h/ml) (Table 2). This indicates that the exposure concentration of AE was significantly increased, and the system of excretion and biotransformation in the rats in this study may not have been normal (Rowland & Tozer, 1995). Only a pathophysiological factor could change the slopes of distribution and elimination associated with biotransformation, excretion, and protein or tissue binding (Mayer, 1995; Shargel & Yu, 1993).

Neurotoxic Severity / Animal & PK Parameters	Severe & Death	Moderate	Minimal	Not affected
Sprague-Dawley Rats	Arteether, IM (Sesame oil) 25 mg/kg x 7	Arteether, IM (1:2 CRM/ saline) 25 mg/kg x 7	Arteether, IM** (Sesame oil) 12.5 mg/kg x 7	Artesunate, IM (5% NaHCO$_3$) 25 mg/kg x 7
AUC$_{1-7D}$ ($\mu g \cdot h/ml$)	16.92 ± 4.04	46.29 ± 2.06	12.31 ± 1.57	8.57 ± 3.34
C$_{max\ Day\ 1}$ (ng/ml)	130 ± 39	1227 ± 171	65 ± 19	3981 ± 992
C$_{max\ Day\ 7}$ (ng/ml)	410 ± 91	1826 ± 118	205 ± 45	2078 ± 416
t$_{1/2\ Day\ 1}$ (hr)	13.74 ± 1.71	6.96 ± 0.93	13.23 ± 1.32	0.43 ± 0.05
t$_{1/2\ Day\ 7}$ (hr)	31.24 ± 4.31	9.06 ± 1.69	22.03 ± 4.83	0.39 ± 0.04
LONEL (ng/ml)	41.32	41.32	41.32	NA
Neuro-exposure time (hr)*	164.3 ± 7.9	103.0 ± 5.3	67.1 ± 5.6	NA
Sprague-Dawley Rats		Artelinic acid Oral (Suspension) 160 mg/kg x 9	Artelinic acid Oral (Suspension) 288 mg/kg x 5	Artelinate IM (5% NaHCO$_3$) 25 mg/kg x 7
AUC$_{1-7\ or\ 9D}$ ($\mu g \cdot h/ml$)		1420 ± 454	1302 ± 905	86.84 ± 5.59
C$_{max\ Day\ 1}$ (ng/ml)		11293 ± 8291	14069 ± 10905	20044 ± 3459
C$_{max\ Day\ 7\ or\ 9}$ (ng/ml)		12230 ± 7139	14341 ± 9339	15585 ± 1476
t$_{1/2\ Day\ 1}$ (hr)		2.84 ± 0.52	3.33 ± 0.25	1.89 ± 0.22
t$_{1/2\ Day\ 7\ or\ 9}$ (hr)		10.68 ± 1.84	3.60 ± 0.25	1.73 ± 0.17
LONEL (ng/ml)		346.26	346.26	NA
Neuro-exposure time (hr)*		186.0 ± 28.0	75.0 ± 12.0	NA
Beagle Dogs	Arteether, IM (Sesame oil) 15 mg/kg x 14	Artemether, IM (Peanut oil) 40 mg/kg x 7	Artemether, IM (Peanut oil) 20 mg/kg x 7	Artelinic acid Oral (Capsules) 25 mg/kg x 14
AUC$_{1-7,\ 14,\ or\ 28D}$ ($\mu g \cdot h/ml$)	24.39 ± 18.04	86.27	32.13	191.14 ± 90.80
C$_{max\ Day\ 1}$ (ng/ml)	90 ± 15	580	190	6279 ± 2995
C$_{max\ Day\ 7,\ 14,\ or\ 28}$ (ng/ml)	352 ± 203	720	346	7177 ± 6248
t$_{1/2\ Day\ 1}$ (hr)	9.23 ± 1.32	17.24	10.93	1.51 ± 0.16
t$_{1/2\ Day\ 7,\ 14,\ or\ 28}$ (hr)	21.53 ± 2.08	22.43	20.75	1.94 ± 0.56
LONEL (ng/ml)	40.92	75.69	75.69	346.26
Neuro-exposure time (hr)*	277.6 ± 10.7	147.8	113.2	14.6 ± 1.8
Rhesus monkeys***		Arteether, IM (Sesame oil) 16 mg/kg x 14	Arteether IM** (Sesame oil) 8 mg/kg x 14	
AUC$_{1-14D}$ ($\mu g \cdot h/ml$)		70.96 ± 4.59	31.25 ± 2.61	
C$_{max\ Day\ 1}$ (ng/ml)		63 ± 9	38 ± 5	
C$_{max\ Day\ 14}$ (ng/ml)		1038 ± 622	527 ± 306	
t$_{1/2\ Day\ 1}$ (hr)		22.59 ± 1.55	16.56 ± 1.20	
t$_{1/2\ Day\ 14}$ (hr)		82.09 ± 41.98	50.58 ± 16.77	
LONEL (ng/ml)		193.80	193.80	
Neuro-exposure time (hr)*		307.4	179.5	

*The neuro-exposure times above LONEL concentration were calculated with neurotoxic outcome.
** PK data were simulated from our previous studies.
***The monkey results are from our unpublished data. Neurotoxic severity were graded as minimal = 1-5 neurons affected, moderate = 10-30 neurons affected, and severe = >40 neurons affected.
DHA = Dihydroartemisinin; CRM = cremophore; AUC = area under the curve; IM = intramuscular

Table 2. Drug accumulation, exposure level and neurotoxic exposure time of arteether (AE), Artemether (AM), artesunate (AS) and artelinic acid (AL) at above the lowest observed neurotoxic effect level (LONEL) in rats, dogs, and monkeys concerning with histopathological neurotoxicity after various dose regimens (Li et al., 2007a; Si et al., 2007).
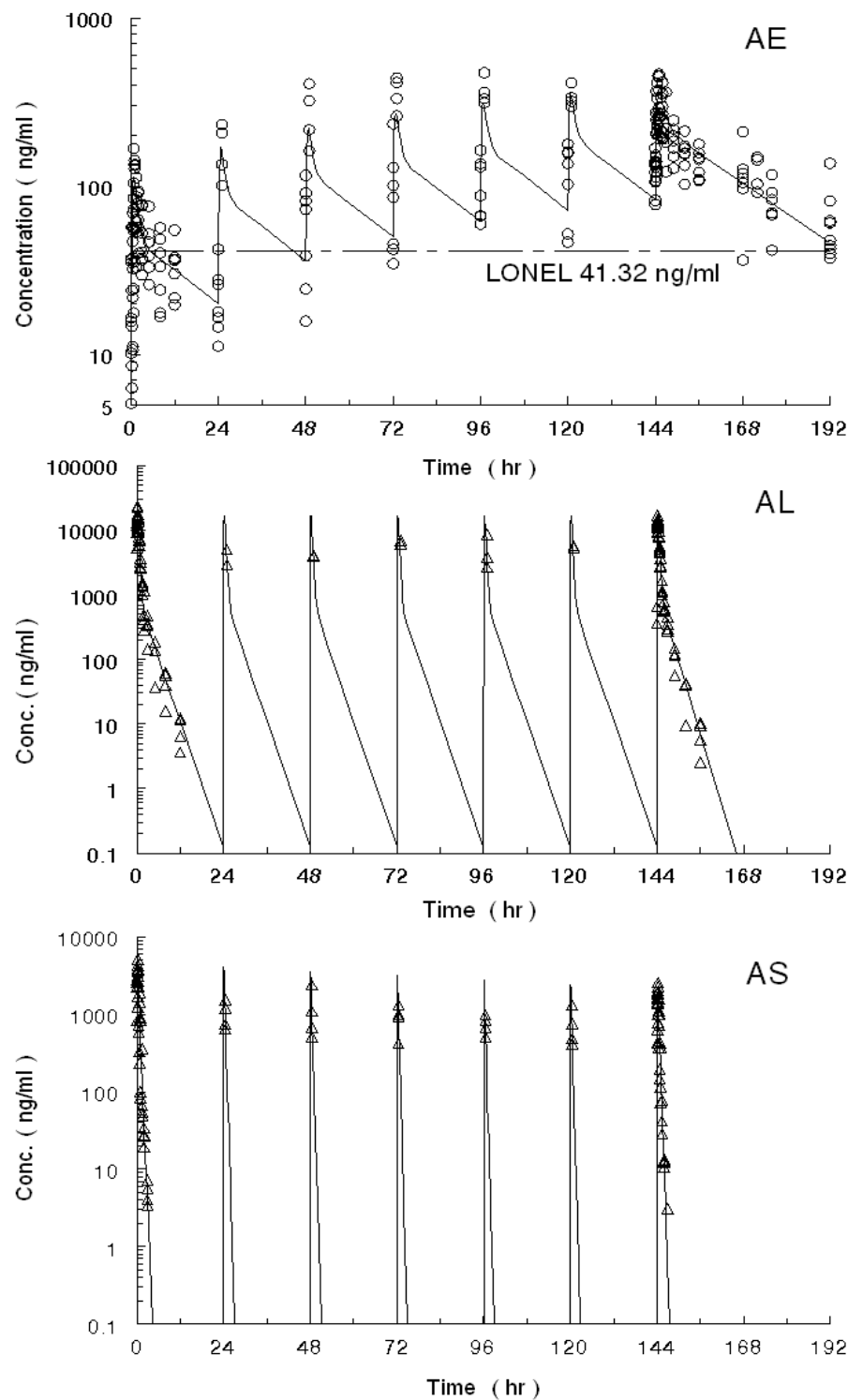
Fig. 1. Pharmacokinetic profiles measured by HPLC-ECD (markers) and computer fitted curves (solid line) of 25 mg/kg of arteether with sesame oil (top, n = 4, Li et al., 2007a). Artelinate (middle) and artesunate (bottom) with 5% $NaHCO_3$/0.9% saline following daily intramuscular injection 25 mg/kg daily for 7 days in male rats. (n = 3, Li et al., 2005b). The lowest observed neurotoxic effect level (LONEL) from AE measurement was estimated at 41.32 ng/ml (dashed line) in male rats.

The drug exposure level shown by the total AUC data observed after a 7 day intramuscular treatment dosing of 25 mg/kg AE was 16.92 μg·h/ml, and this dosing regimen induced severe neurotoxicity and animal death (Li et al., 1999b). Following intramuscular injection, the exposure concentration level of AE in this study was just one-fifth of the total AUC of AL (86.84 μg·h/ml) with the same dosing regimen (Table 2) (Li et al., 2005b). Clinical observation of rats in this experiment treated with AL did not show any indications of neurotoxicity (Figure 1, middle), even after multiple intramuscular injections of high AL doses of 100 mg/kg (our unpublished data). Computer simulation indicated that the total AUC after 7 day treatment with 100 mg/kg AL was 341.88 μg·h/ml, 21 times higher than that of AE treated rats. This result suggests that the 5-21 fold higher exposure concentrations of AL did not seem to be a principal independent factor in causing neurotoxicity (Table 2).

In order to demonstrate that the neurotoxicity of AE is related to drug accumulation in blood as a consequence of slow and prolonged absorption from the intramuscular injection sites, a study was conducted designed to decrease the accumulation and toxicity of AE through the replacement of traditional sesame oil with a cremophore vehicle (Li et al., 2002). When administered at a daily dose of 25 mg/kg for 7 days, the AUC of AE formulated in sesame oil (AESO) was 7.5-fold higher on day 7 (last day of dosing) than on day 1 (first day of dosing), while treatment with AE formulated with cremophore (AECM) resulted in only a 1.8-fold higher AUC. The C_{max} of AECM (1826 ng/ml) on day 7 was found to be only slightly higher than on day 1 (1227 ng/ml).

Although the accumulation of AECM was greatly reduced, its total exposure level (total AUC_{1-7D} of 46.29 μg·h/ml) was still 2.7-fold higher than AESO (total AUC_{1-7D} of 16.92 μg·h/ml) due to the higher bioavailability of AECM (74.5%) compared with AESO (20.3%), and the data for this study are shown in Table 2. While the histopathological examinations of the brain demonstrated neurotoxic changes in both groups, the animals in the AESO group showed significantly more severe neurotoxicity than in the AECM group. Brain injury scores in animals treated with AECM were mild to moderate (severity index 2.3 to 3.0), but in animals treated with AESO they were moderate to severe (3.0 to 4.7) on day 7 and day 10, respectively. This study further demonstrates that the toxicity of AE is not dependent on its exposure level in animals.

2.3.2 PK profiles of ARTs in dogs

Daily intramuscular administration of 15 mg/kg AE for 14 days resulted in drug accumulation in the plasma of beagle dogs who also presented clinically with severe neurotoxicity and death (Li et al., 2000). The peak concentration (C_{max}) of AE (352 ng/ml) was found to be four times higher on the last dosing day (day 14) compared to the C_{max} observed on the first dosing day (90 ng/ml). The AUC (7.09 μg·h/ml) of AE observed on the last dosing day was 8.7-fold higher than on the first dosing day (0.81 μg·h/ml), indicating that the exposure concentration of AE had been greatly increased (Figure 2, middle). The total AUC observed during the 14 days of daily intramuscular AE dosing at 15 mg/kg was 24.39 μg·h/ml (Table 2).

Similar accumulation results for AM were reported in dog plasma after daily intramuscular administration of 20, 40, and 80 mg/kg for 7 days (Classen et al., 1999). The PK profiles of AM dosed IM at 20 and 40 mg/kg in beagles is shown in Table 2. The analysis of the toxicokinetic parameters on day 2-7 was changed, with the parameter estimated on day 1. In the dogs treated with 20 mg/kg dosed IM minimal neurotoxicity was observed, and the

C_{max} of AM (346 ng/ml) doubled on day 7 from the C_{max} observed on day 1 (190 ng/ml). The AUC (6.16 µg·h/ml) of AM observed on day 7 was 2.7-fold higher than the AUC of AM observed on day 1 (2.25 µg·h/ml) after IM dosing. Similar results were found in animals treated IM with AM at 40 mg/kg daily for 7 days. The C_{max} of AM (720 ng/ml) on day 7 was much higher than on day 1 (580 ng/ml), and the AUC (12.52 µg·h/ml) doubled on day 7 from the AUC observed on day 1 (6.70 µg·h/ml), indicating that the exposure concentration of AM had been increased (Classen et al., 1999).

In comparison to the dogs treated with intramuscular AE, the total AUCs observed in animals treated with AM dosed IM at 20 mg/kg (32.13 µg·h/ml) and 40 mg/kg (86.27 µg·h/ml) were significantly higher than the AUC observed after dosing with AE at 15 mg/kg (24.39 µg·h/ml) (Classen et al., 1999; Li et al., 1999a). The severity of neurotoxicity observed after dosing with intramuscular AM in dogs, however, was minimal in the 20 mg/kg group and moderate in the 40 mg/kg group. The observed neurotoxicity was much less in AM treated animals than in AE treated dogs who presented with severe neurotoxicity and death. These comparison studies demonstrate that the neurotoxicity of AE and AM is not dependent on plasma concentrations in dogs.

In another comparison study, dogs treated with oral AL showed much higher plasma concentrations. The AUC observed after daily oral dosing with AL for 14 days at 20 mg/kg in a suspension formulation was 231.6 µg·h/ml. The AUC value dropped to 191.1 µg·h/ml in animals dosed with oral AL in a 25 mg/kg capsule formulation given daily for 14 days (Table 2). The dogs in both groups did not show any clinical toxicity (Li et al., 1994). In dogs treated with AL at two different dose regimens the exposure concentrations were 2-9 times higher than in dogs treated with AE and AM. No neurotoxicity was detected in those animals, however, dosed with 20 or 25 mg/kg oral AL daily for 14 days (Noker & Lin, 2000).

The drug exposure levels of these treatments suggest that a much higher total exposure concentration of AL in dogs (AUC_{1-14D} = 191.14 µg·h/ml) does not correlate with clinical observations of neurotoxicity while the drug exposure levels of AE (AUC_{1-14D} = 24.39 µg·h/ml) and AM (AUC_{1-7D} = 32.13 µg·h/ml), correlated well with neurotoxicity at much lower exposure concentrations in dogs (Li et al., 2007a). These results also showed the exposure level of AM at daily dose of 20 and 40 mg/kg (AUC_{1-7D} = 32.13 and 86.27 µg·h/ml) is higher than the exposure level of AE in dogs (AUC_{1-14D} = 24.39 µg·h/ml), but the clinical observations of neurotoxicity were less severe in dogs treated with AM than with AE (Table 2). These studies further demonstrate that the toxicity of AE, AM or AL is not dependent on exposure levels in dogs.

2.3.3 PK studies in monkeys

Significant accumulations of AE were shown in the plasma of rhesus monkeys after daily intramuscular administration of AE at 16 mg/kg for 14 days (Figure 2, bottom). The concentration-time profile of AE dosed at 16 mg/kg in monkeys showed that the C_{max} of AE (1038 ng/ml) on day 14 was 19-fold higher than the C_{max} of AE observed on day 1 (63 ± 9 ng/ml). The AUC (53.10 µg·h/ml) of AE observed on the last dosing day was shown to be 60-fold higher than the AUC of AE observed after intramuscular dosing on the first day (0.88 µg·h/ml), indicating that the exposure concentration of AE was greatly increased. The total AUC of AE observed during the entire 14 days of treatment was 70.96 µg·h/ml (Li & Hickman, 2011).
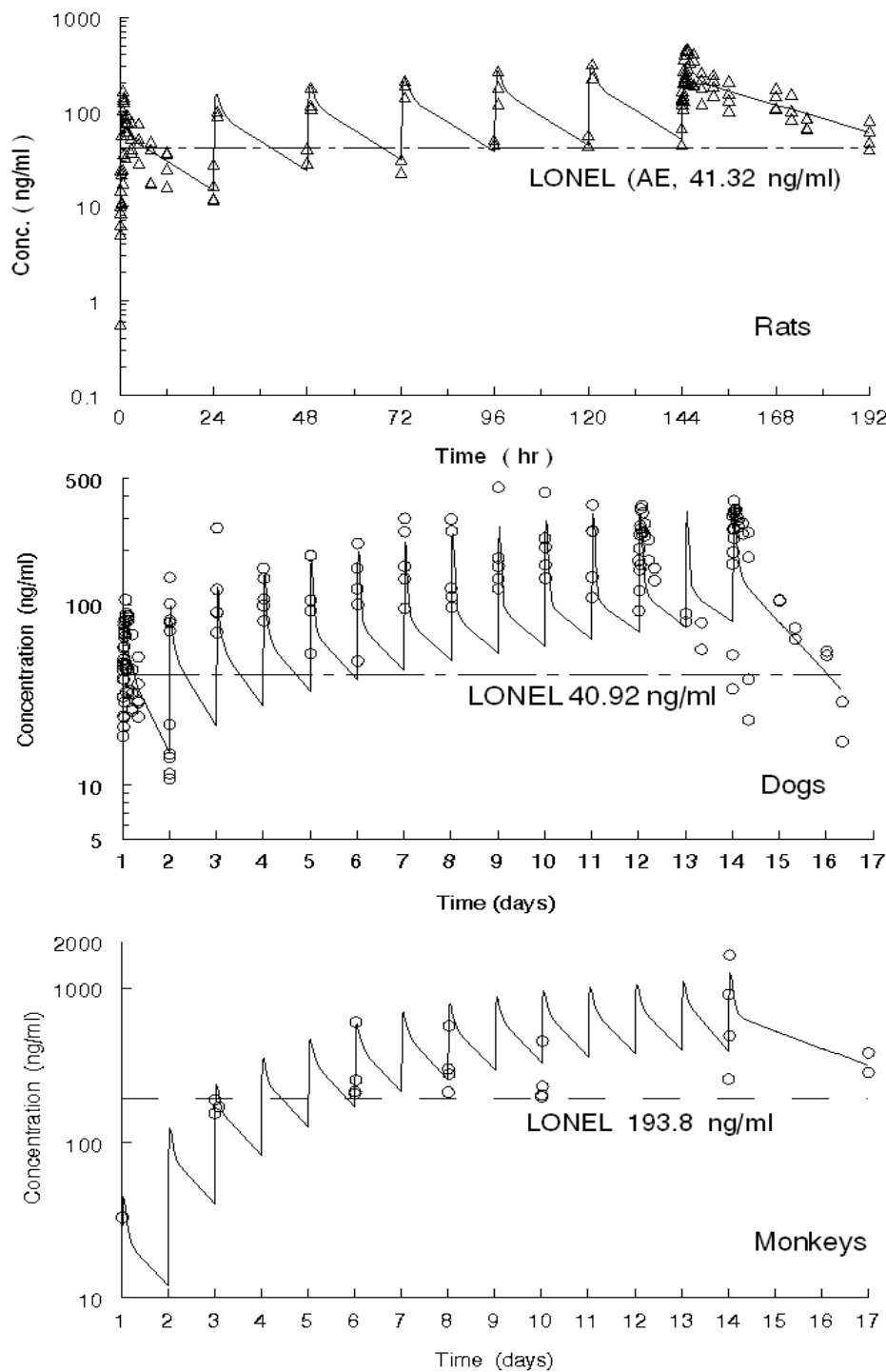
Fig. 2. Pharmacokinetic profiles measured by HPLC-ECD (markers) and computer fitted curves (solid line) of arteether (AE) in sesame oil in rats at daily dose of 12.5 mg/kg for 7 days (top, Li et al., 1999b), in beagle dogs at daily dose of 15 mg/kg for 14 days (middle, Li et al., 2000), and in rhesus monkeys at daily dose of 16 mg/kg for 14 days (bottom, Li & Hickman, 2011). The lowest observed neurotoxic effect level (LONEL, dashed line) from AE measurement was estimated 41.32 ng/ml in rats, 40.92 ng/ml in dogs, and 193.8 ng/ml in rhesus monkeys, respectively.

2.4 The importance of artemisinin drug half-life in neurotoxicity

While the exposure level of ARTs has not been shown to be a major factor involved in the induction of neurotoxicity, the exposure time may play a role in causing neurotoxicity. There are a number of similar reports of exposure investigations in the literature that have obtained the same observations that continued exposure of artemisinins over time (drug exposure time) rather than a high concentration (drug exposure level) over a short time (interval time) substantially contribute to neurotoxicity (Jorgensen, 1980; Rangan et al., 1997; Rozman & Doull 2000).

2.4.1 Half lives of ARTs in rats

The biological half-life of test drugs in blood controls the time exposure of drugs in animals and humans. The biological half-life is the amount of time it takes the body to eliminate one half of the drug initially present. In the animal PK studies with ARTs, the half lives of drugs administered were noted. Rats treated with daily intramuscular injections of AE at 25 mg/kg for 7 days presented with clinical observations of severe neurotoxicity (Li et al., 1999b). The absorption of AE from the muscle at the injection site was incomplete in the first 48 hours after a single injection. The elimination half life of AE in plasma after 7 daily doses was prolonged from 13.74 hours on day 1 to 31.24 hours on day 7, suggesting that the elimination half life of AE had been greatly extended.

To examine the question of whether AE accumulation in blood is due to the slow and prolonged absorption of drug from the intramuscular injection sites, a comparison study was conducted designed to decrease the accumulation of AE through the replacement of the sesame oil formulation with a cremophore vehicle (Li et al., 2002). When administered at a daily dose of AE at 25 mg/kg for 7 days, the half life (13.74 hr) of AE with sesame oil (AESO) on the first dosing day (day 1) showed a 2.5-fold higher half life than AE formulated with cremophore (AECM, 6.96 hr). Similar results were observed on the last dosing day (day 7), and the half life of AE in animals treated with AESO was 31.24 hr, which was 3.3-fold higher than AECM treated rats (9.06 hr) on the same day. Although the new formulation of AE with cremophore greatly increased the absorption rate from the muscles of rats, the half life observed in AECM treated animals was slightly lengthened from 6.96 hr on day 1 to 9.06 hr on day 7, suggesting that the exposure time of AECM was greatly reduced compared to animals treated with AESO. In this comparison, the neurotoxic severity observed was also significantly reduced from severe with death in animals treated with AESO to moderate in animals treated with AECM, and the drug half-lives appear to strongly correlate to clinical observations of neurotoxicity observed in these animals (Li et al., 2002).

No prolonged half lives and neurotoxicity were found in animals treated with intramuscular AL and AS at a dose of 25 mg/kg per day for 7 days, which closely follows the conditions of the experiments conducted in the previously mentioned AE study (Li et al., 1999b; Li et al., 2005b). The half-lives of AL and AS, both of which are water soluble, were very short ranging from 0.39 to 1.89 hr (Table 2), which is significantly shorter than the half life observed after dosing with AE intramuscularly. In addition, the half lives of AL and AS were not prolonged when comparing the half life observed on day 7 to the half life observed on day 1 post-injection (Figure 1, middle & bottom), suggesting that drug accumulation was not observed in those animals treated with repeated intramuscular doses of AL and AS. This

result suggests that the significantly shorter half-lives of AL and AS compared to the half-life of AE seem to be an important element in avoiding clinical neurotoxicity. In other words, treatment of animals with intramuscular AL and AS is one means by which these animals avoided neurotoxic risk.

After changing the administration route from intravenous and intramuscular to intragastric, AL showed moderate neurotoxicity in rats treated with 160 mg/kg daily in 9 multiple doses for 9 days but not in animals dosed with 288 mg/kg every other day in 5 multiple doses for 9 days (Si et al., 2007). The elimination t½ of AL in plasma after oral dosing at 160 mg/kg daily for 9 days doses was prolonged from 2.84 hrs on day 1 to 10.68 hrs on day 9, suggesting that the elimination half life of AL had been greatly extended (Figure 4, middle). A slight increase of the half life, however, from 3.33 hrs on day 1 to 3.60 hrs on day 9 was noted in the rats treated with 288 mg/kg every other day in 5 multiple doses for 9 days (Figure 4, bottom). The neurotoxic severity of AL changed from moderate in the group treated with an oral daily dose of 160 mg/kg for 9 days to minimal in the group treated orally with AL every other day at a dose of 288 mg/kg for 9 days. These results indicate a correlation between observed clinical symptoms of neuropathology in rats and prolonged AL half lives in the animals dosed daily. The data further suggest that if the drug half life were shortened it might be possible to reduce the neurotoxic risk in animals treated with ARTs (Li et al., 2006b).

2.4.2 Half lives of ARTs in dogs

Beagle dogs treated with multiple daily intramuscular doses of AE at 15 mg/kg for 14 days developed severe neurotoxicity with death (Li et al., 2000). Similar to what was observed in the rat AE PK studies; the dogs demonstrated prolonged AE half-lives and drug accumulation. (Figure 2, middle). In this study, the most striking data observed was the elimination t½ of AE during the two weeks of daily dosing. The half life was prolonged from 9.23 hrs on the first dosing day (day 1) to 21.53 hrs on the last dosing day (day 14), suggesting that the exposure time of AE had been extended by two-fold (Table 2). In addition, half life extensions s were also reported in dog plasma after daily AM intramuscular administration of 20, 40, and 80 mg/kg dissolved in peanut oil for 7 days (Classen et al., 1999). The concentration-time profile of AM dosed at 20 and 40 mg/kg in beagles is shown in Table 2 and Figure 4. The elimination t½ of AM dosed at 20 mg/kg after 7 days daily dosing was prolonged from 10.93 hours on day 1 to 20.75 hours on day 7, suggesting that the exposure time of AM had been significantly increased. Similar results were found in animals treated with AM dosed intramuscularly at 40 mg/kg. Daily AM dosing for 7 days did not result in severe neurotoxicity, unlike the 14 day treatment with AE where severe neurotoxicity and death were noted. The difference in dosing time from 7 days to 14 days is the most likely factor related to this difference in clinical outcomes (Classen et al., 1999).

The minimal AE dose of multiple intramuscular injections which induced neuropathological findings was 6 mg/kg for 28 days in dogs (Brewer et al., 1994b; Davidson, 1994; Dayan, 1998). Through PK simulation of data from our dogs treated with intramuscular AE, the elimination half-lives of AE were found to be slightly prolonged from 8.22 hrs on day 1 to 10.45 hrs on day 28 with no significant difference. The neurotoxicity findings were also significantly reduced when comparing different compound dosing groups and treatment

durations. Severe neurotoxicity was observed in dogs treated with AE at 15 mg/kg daily for 14 days, and minimal toxicity in animals treated with AE at 6 mg/kg daily dose for 28 days (Table 2). When compared to the drug half-lives of AE and AM, much shorter half lives were found in dogs treated with AL orally at 25 mg/kg daily for 14 days. The half lives of AL in dogs were 1.51 hrs on day 1 and 1.94 hrs on day 14 after oral capsule administration. The half life of AL observed was much shorter and no prolonged elimination half lives were found after oral daily dosing for 14 days. No neurotoxicity was observed in any of the AL treated animals (Noker & Lin, 2000). These studies further demonstrate that the neurotoxicity in beagles following AE, AM or AL administration is likely dependent on the half life of drug.

These studies strongly support the findings that neurotoxicity is half life (exposure time) dependent in the animals regardless of drug formulations and administration routes. As a result, the half lives of ARTs in animals are critical elements in the induction of neurotoxicity (Li et al., 2006b). Other pharmacokinetic parameters, such as drug distribution in the brain, toxicity of DHA, the active metabolite of ARTs or drug exposure level, tend to be of minor importance.

2.4.3 Half lives of ARTs in monkeys

AE dissolved in sesame oil formulation showed high plasma concentrations in rhesus monkeys after daily intramuscular administrations of AE at 16 mg/kg for 14 days (Li & Hickman, 2011). The concentration-time profile of monkeys dosed with AE at 16 mg/kg showed the C_{max} of AE (1038 ng/ml) on day 14 was 19 fold higher than on day 1 (63 ng/ml). The half lives of intramuscular AE were 22.59 hrs on day 1 and 82.09 hrs on day 14 after daily multiple dose regimens. Accordingly, the half life of AE was significantly prolonged from the first dosing day to the last dosing day after intramuscular AE administrations. The monkey showed moderate neurotoxicity after this intramuscular AE dosing regimen (Petras et al., 1997, 2000).

Since AE-induced brainstem neuropathology in monkeys has been shown to occur at a minimal dose of 8 mg/kg dosed daily for 14 days, we chose to simulate the main AE PK parameters on day1 and day 14 from our own rhesus PK data (Table 2). We noted a drug half life of 50.28 hrs on day 14 which was extended from 16.56 hrs on day 1 in monkeys treated with multiple doses of 8 mg/kg. This half life value is still shorter than the 82.09 hrs half life observed in animals treated with multiple intramuscular doses of AE at 16 mg/kg. The former dose induced minimal neurotoxicity, and the latter doses produced moderate toxicity in monkeys. Therefore, the AE half lives in monkeys appear to also have an important impact on the induction of neurotoxicity.

In conclusion, the data on ART half lives in animals supports the hypothesis that induction of neurotoxicity is much more dependent on the exposure time than other PK parameters. The summary data illustrates that the water-soluble ARTs, AL and AS, have very short half lives of 0.39-1.94 hrs in animals without any neurotoxic observations with the exception of oral AL dosed in rats at a very high dose of 160 mg/kg daily for 9 days. High dose AL treatment resulted in a significantly prolonged drug half life on day 9, the animals showed moderate neurotoxicity, and this treatment was also associated with GI toxicity. In rats or dogs treated with the oil-soluble ARTs, AE and AM, the drug half-lives were significantly extended due to slow absorption from the site of the intramuscular injection. Those animals

presented with a moderate to severe neurotoxicity and some died. It is notable that the drug half-lives (9.23 – 82.09 hrs) in animals treated with AE and AM were extremely extended compared to the half lives observed after AL and AS treatment. Although we lacked a full set of PK data after AE dosing in monkeys, we noted several PK results in different animals that showed the half lives on day 14 after intramuscular AE was dosed at 16 mg/kg daily were much longer than those observed in rats and dogs.

In addition, the monkeys dosed with AE for 14 days showed moderate neurotoxicity, suggesting that monkeys appear less sensitive to AE-induced neurotoxicity than rats and dogs which showed severe neurotoxicity and death when dosed with AE for 14 days. We conclude, from the dose amounts of ARTs used in various experiments to induce neurotoxicity, that the rank order of ARTs in terms of toxic potency is AE first followed by AM, AL, and lastly by AS. There is a strong correlation between the drug half-lives and the severity of neurotoxicity observed after treatment which supports the hypothesis that the drug exposure time during treatment plays a very important role in the induction of neurotoxicity.

3. Blood accumulation of ARTs leads to prolonged half-lives

The studies described thus far support the hypothesis that neurotoxicity induced by ARTs is highly dependent on the half-lives of these drugs. These studies also show that the ART drug half-life is due to the accumulation of ART drugs in the plasma following multiple doses of AE and AM administered intramuscularly and AL administered orally. In accordance with the published literatures and our research experience, ARTs (QHS, AS, AM, and DHA) elicit auto-induction of a drug metabolism pathway during multiple oral treatments in malaria patients and healthy subjects (van Agtmael et al., 1999; Ashton et al., 1996 and 1998; Khanh et al., 1999: Li et al., 2004; Park et al., 1998). The C_{max} and AUC values of these patients were markedly reduced from one-third to one-seventh on the last dose day compared with the first dosing day. The decrease in drug exposure levels during treatment is not disease-related, since the PK parameters of ART drugs in treated patients is similar to that reported in healthy subjects. Similar time-dependent declines were also found in animals treated with intravenous AS (Figure 3, top, Li et al., 2005a), intramuscular AS (Figure 3, middle, Li et al., 2007a), and oral AM (Figure 3, bottom, Classen et al., 1999). This data leads to pertinent questions: if all ART drugs have a pathway of metabolism auto-induction, why do drugs accumulate in animals treated with AE and AM after multiple intramuscular doses and with AL after repeated oral dosing?

3.1 Cause of AM and AE accumulation after intramuscular injection

PK data from various animal studies have shown that AE and AM will accumulate in the plasma of rats (Li et al., 1999b), beagle dogs (Classen et al., 1999), rhesus monkeys (Li et al., 2007a), and humans (Kager et al., 1994) following multiple intramuscular injections. Data collected from rat studies showed that the accumulation of AE in the plasma is due to a slow and prolonged absorption from the injection sites. The elimination t½ of AE after 7 daily doses at 25 mg/kg was prolonged from 13.7 hr on the first dosing day to 31.2 hr on the last dosing day (Li et al., 1999b) (Fig. 1, top). AE accumulation was also observed in beagle dogs with severe neurotoxicity and death after daily intramuscular administration of AE at 15 mg/kg for 14 days (Li et al., 2006b). The elimination t½ of AE after dosing the dogs for 14 days was prolonged from 9.23 hr on day 1 to 21.53 hr on day 14 (Fig. 2, middle).
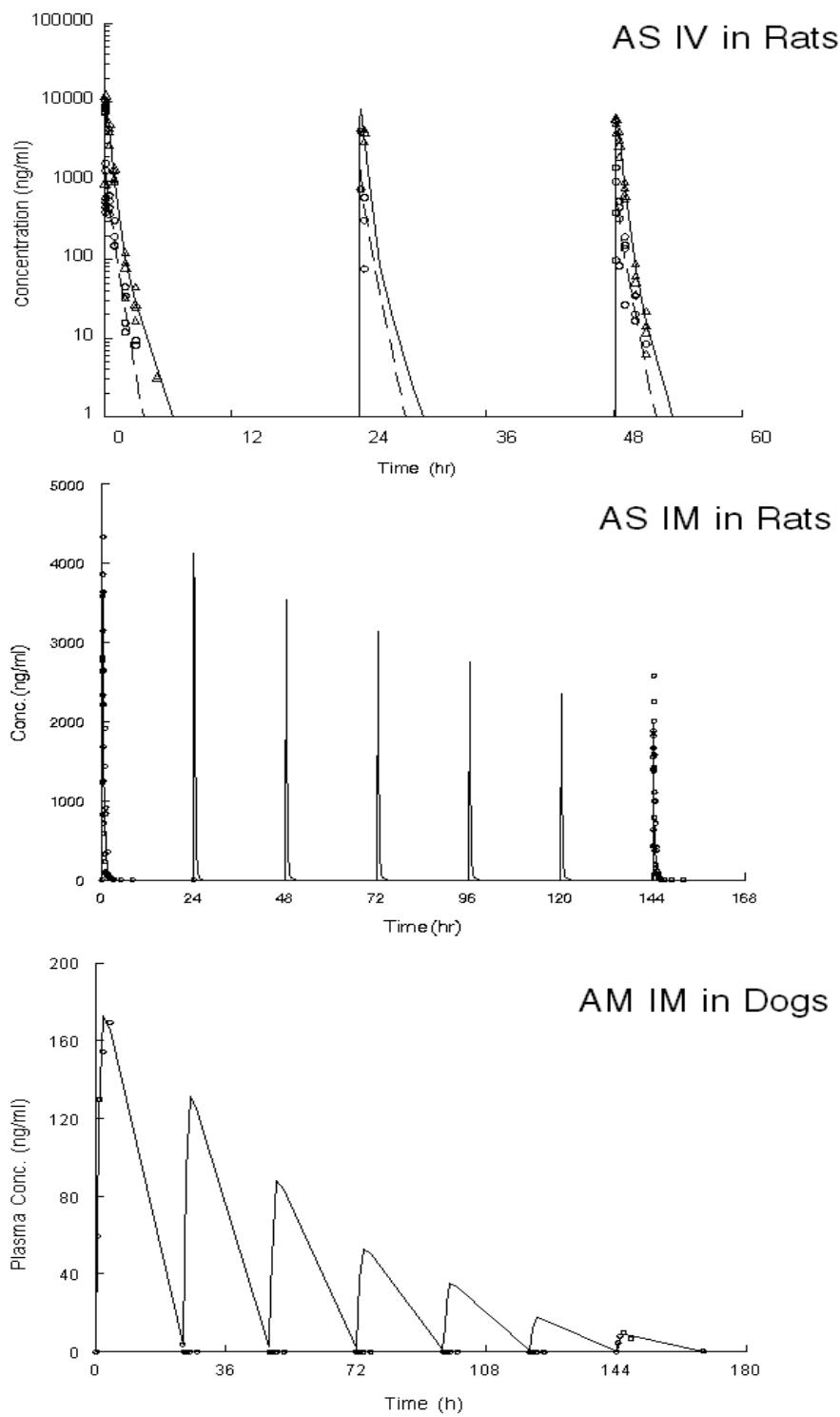
Fig. 3. Auto-induction metabolic profiles measured by HPLC-ECD (markers) and computer fitted curves of 36.7 mg/kg of intravenous artesunate (AS, top, dashed line), and its active metabolite DHA (top, solid line) once daily for 3 days (Li et al., 2005a). Study of 25 mg/kg of intramuscular AS (middle) was dosed daily for 7 days in rats (Li et al., 2007a). Another PK profiles after oral dose at 600 mg/kg of AM (bottom) daily for 7 days in beagle dogs (Classen et al., 1999).
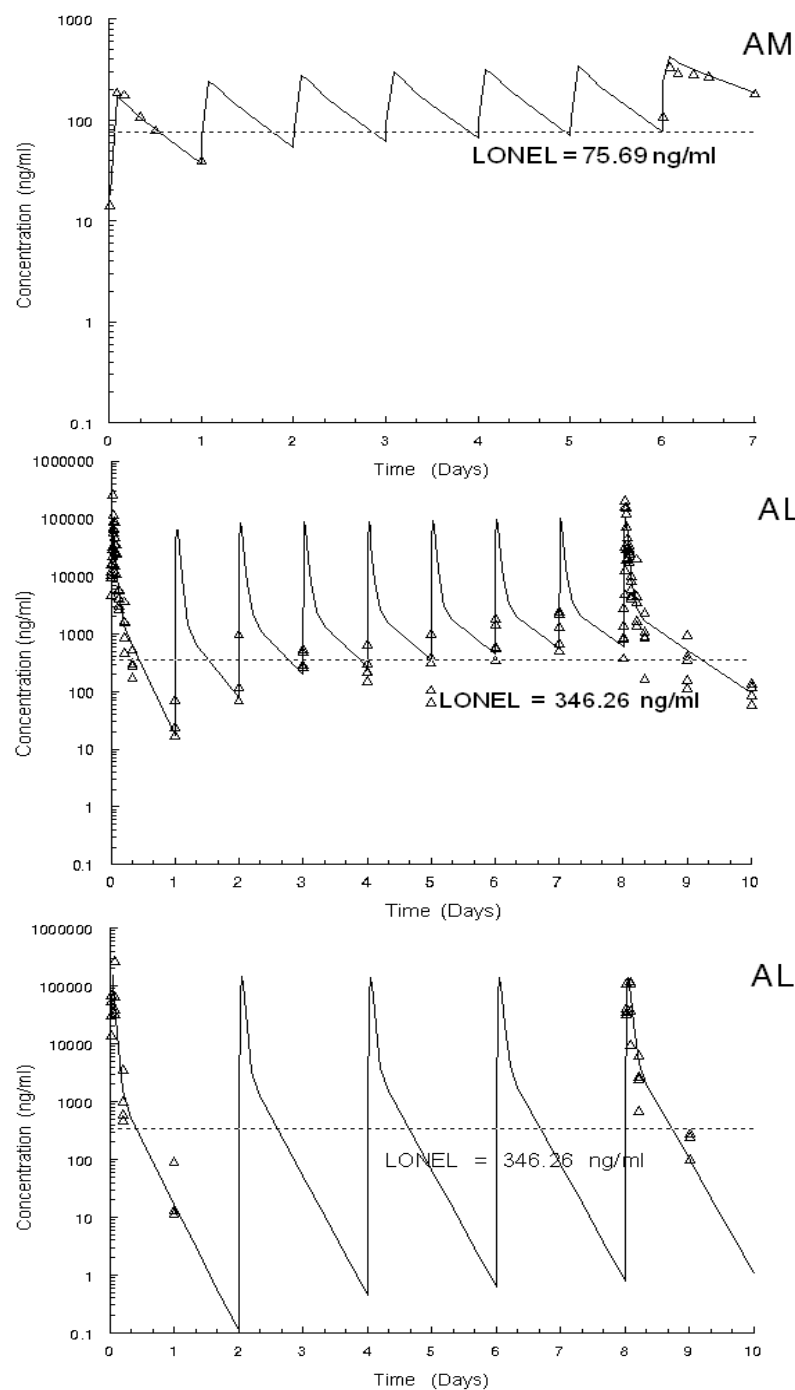
Fig. 4. Pharmacokinetic profiles measured by HPLC-ECD (markers) and computer fitted curves (solid line) of intramuscular artemether (AM) in peanut oil at 20 mg/kg daily for 7 days in beagle dogs (top, Classen et al., 1999). Oral artelinic acid (AL) in suspension at 160 mg/kg daily for 9 dosages (middle, n = 5) and oral AL at 288 mg/kg every other daily for 5 dosages in rats (bottom, n = 4, Si et al., 2007). The two regimens of AL have the same total dose with 1440 mg/kg and same treatment period for 9 days. The lowest observed neurotoxic effect level (LONEL) was defined as the plasma level of 75.69 ng/ml of AM in dogs and 346.26 ng/ml of AL in rats, at which anorectic and neuropathological toxicities were noted in daily dosing cohort.

Similar accumulation results for AM were reported in dog plasma after daily intramuscular administration of AM at 20, 40, and 80 mg/kg for 7 days (Classen et al., 1999). The analysis of TK parameters on day 2-7 was significantly different when compared to the parameters estimated on day 1. The elimination t½ of AM at 20 mg/kg after 7 daily doses increased from 10.93 hr on the first day of dosing day to 20.75 hr on the last day of dosing (Table 2; Figure 4, top), suggesting that the exposure time of AM had greatly increased. In addition, accumulation of AE was shown in the plasma of rhesus monkeys after daily intramuscular administration of AE at 16 mg/kg for 14 doses (Fig. 2, bottom). The elimination t½ of AE after 14 daily doses increased from 22.59 hr on day 1 to 82.09 hr on day 14 (Li et al., 2006b).

The intramuscular administration of AM and AE was associated with slow absorption because the drugs were dissolved in sesame oil or peanut oil that, when injected, formed a depot from which the drug was slowly released (Kager et al., 1994: Li et al., 2004). The slow elimination of AE was recently demonstrated in a rat study, which also found significant accumulation of AE in the plasma from injection sites (Li et al., 1999b). The results of a rat study conducted by daily intramuscular injections of AE at 25 mg/kg in sesame oil for 7 days confirmed and extended the results of earlier studies (Li et al., 1998b) by demonstrating that the absorption of AE from the injection site of the muscle was incomplete. This study also indicated that up to 38% of the total single dose of AE remained in the injection site for 24 hr after dosing, and 22% of the total single dose still remained in the muscle 48 hr after dosing. Following 7 days of daily intramuscular injections of AE (25 mg/kg), 91.4% of a single dose (25 mg/kg/day) was still left in the muscles from the injection sites 24 hr after the last dose (Li et al., 1999b).

Date*	Findings	Sesame oil control	AE	AM	Student** T-Test
Day 7	fascial inflammation, pseudocysts, muscle necrosis, & hemorrhage	1.75 ± 0.43	3.17 ± 0.37	2.50 ± 0.50	P = 0.0019
Day 10	fascial inflammation, pseudocysts, & hemorrhage	1.50 ± 0.50	3.00 ± 0.58	2.00 ± 0.0	P = 0.0045

Finding severity were grading as 0 = no significant lesions, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, and 5 = severe. *Day 7 and 10 are one day after last treatment of daily dose for 7 and 10 days. The date is given the dosing day as day 0. **The t-test was conducted between animals treated both with AE and AM in sesame oil.

Table 3. Muscle injury severity in the injection site areas following repeated arteether (AE) and artemether (AM) injection with vehicle sesame oil by multiple daily intramuscular dosing at 25 mg/kg for 7 days (n = 3) in rats (Li & Hickman, 2011).

Our histopathological data demonstrated that the cytotoxicity induced by AE in muscle cells occurred at the injection site. The severity of the damage observed after dosing was graded where no significant lesion detected was scored as 0; subacute to minimal damage and mild inflammation was scored as 1-2; damage along the connective tissue and between major skeletal muscle bundles showing subacute to chronic inflammation was scored as 2-3;

inflammation observed rarely extending into or between muscle fibers was scored as 3-4; prominent muscle damage with coagulative necrosis surrounded by a reparative response of fibroplasia, fibrosis, skeletal muscle regeneration, and muscle atrophy was scored as 4-5. The distribution of damage observed involves major muscle bundles and occurred in linear tracts, suggesting the damage may have been the result of a direct effect of the injected drug and/or vehicle on the muscle. Overall, the rats in the 7-day group (24 hr after last treatment) had more severe lesions than those in the 10-day group (96 hr after last dosing), and animals treated with AE had significantly more severe lesions than those treated with AM. The results showed that the chronic inflammation of the muscle located at the site of AE injection is more severe (moderate severity 3.00-3.17) than the inflammation noted at the AM injection site (mild severity 2.00-2.50)(Table 3). The inflammation of muscles induced by AE and AM injection may, therefore, be a factor prolonging the absorption of drugs from the muscles (Li & Hickman, 2011).

3.2 Cause of AL accumulation after intragastric administration

Oral administration of AL has also been shown to result in gastrointestinal toxicity that is associated with delayed gastric emptying. Similar findings of gastrointestinal toxicity were observed in rats treated intramuscularly with other ARTs (Li et al., 1998a). Rats treated with AL at a daily oral dose of 160 mg/kg for 9 days showed moderate neurotoxicity during treatment which was associated with prolonged absorption of AL in the stomach. Comparison of the day 1 and day 9 results revealed that the PK parameters were very different. A significantly longer (3.82-fold) elimination half-life was noted on day 9 (10.68 hr) in comparison to that observed on day 1 (2.84 hr), and this prolonged elimination results in drug accumulation (Figure 4, middle). The mean AUC of AL was higher on day 9, the last dosing day, (168.01 µg·h/ml) than the AUC observed on day 1, the first dosing day (128.38 µg·h/ml). Furthermore, progressive delays in gastric emptying and drug accretion were only found in rats treated with oral AL at 160 mg/kg. These results imply that the observations of delayed gastric emptying in turn results in AL accumulation, and the mean half-life of AL was correspondingly extended on the last dosing day compared to day 1 (Si et al., 2007).

This report of drug accumulation is illustrated in rats dosed daily with 160 mg/kg of AL orally for 9 days. These rats showed moderate neurotoxicity due to prolonged oral absorption in the stomach. This reservoir is similar to the depot effect seen with the oil-soluble ARTs (AM and AE) at intramuscular injection sites. The stomach contents of rats dosed orally with AL at 160 mg/kg daily for 9 days were examined at 8 and 24 hr after dosing at different time points during the study. On days 3, 5, 7, and 9, we detected 0, 46.52, 178.59, and 486.21 µg of AL/g in the stomach contents at 8 hr after the last dose. The increasing amount of AL observed remaining in the stomach over the course of dosing showed that gastric emptying was inhibited. The inhibition of gastric emptying progressed from mild inhibition seen on day 5 to severe inhibition observed on day 9. The inhibition of gastric emptying persisted even at 24 hr post dosing. At twenty-four hr post dosing on days 5, 7, and 9, we observed 5.74, 25.50, and 29.11 µg of AL/g, respectively, in the stomach contents. Similar anorectic toxicity was also reported in animals treated with AM, AE and DHA (Li et al., 1998a). It is postulated that this decrease in GI motility could result from a decrease in vagal tone as a consequence of a decline in sympathetic outflow (Hull & Maher 1990; Si et al., 2007).

4. Lowest observed neurotoxic effect level (LONEL) as a neurotoxic indicator

The body of literature cited supports the hypothesis that drug accumulation extends the drug exposure time, which we believe is a major causative factor in ART-induced neurotoxicity (Li et al., 2006b). The current methodology for assessing the no observed adverse effect level (NOAEL) involves identifying the highest concentration or dose administered that does not cause a statistically significant or biologically significant response to treatment in comparison to the control group. The lowest observed neurotoxic effect level (LONEL) represents the minimal plasma concentration associate with the lowest dose that is found to cause a neurologically and/or statistically significant response to treatment in comparison to the control group. Therefore, determining the neurotoxic exposure time (drug exposure time spent above the LONEL) is critical, and the LONEL should also be determined before evaluating the drug neurotoxic exposure time for each drug in various animals.

4.1 Neurotoxicity induced by intramuscular AE and oral AL in rats

A minimal daily dose of AE at 6.25 mg/kg for 7 days was estimated as a no observed neurotoxic effect dose (NONED), which was defined by AE dosing that did not result in histopathological findings in rats (Genovese et al., 1998). Significant changes of neuropathology in brain stem nuclei, however, were observed in a group of rats treated with AE at 12.5 mg/kg for 7 days. These results demonstrate that AE-induced brainstem neuropathology in rats can occur at the relatively high dose of 12.5 mg/kg for 7 days. The lowest plasma concentration of AE in rats was shown to be 41.32 ng/ml following intramuscular dosing of AE at 12.5 mg/kg in previous studies (Li et al., 1999b and 2002). Based on the NONED value of 6.25 mg/kg obtained in previous studies, it was possible to correctly identify the minimal neurotoxic effect level at 41.32 ng per ml, which has thus been defined as a LONEL in rat plasma (Figure 2, top). Administration above this concentration with a certain exposure time should result in neuropathological effects (Dayan 1998; van Agtmael et al., 1999; Brewer et al., 1994b).

In a PK simulation, 7 multiple AE treatments at a dose of 12.5 mg/kg were administered intramuscularly in rats (Li et al., 2002). The exposure time of 67.1 hr in plasma was estimated as the minimum time spent above the LONEL concentration of 41.32 ng/ml that was sufficient to induce positive neurotoxicity (Figure 2, top). After histological examination this estimate of 67.1 hours of AE dosing above LONEL to induce brainstem injury was confirmed by histopathological examination (Genovese et al., 1998) (Table 2). This finding confirmed the estimated LONEL (41.32 ng/ml) for AE in rats after dosing at 12.5 mg/kg for 7 days to induce neurotoxicity based on 100% positive findings of neuropathology (Fig. 2, top) (Li et al., 1999b & 2002).

Recently, AL was shown to induce moderate neurotoxicity after treatment with 160 mg/kg daily for 9 days confirmed by histopathology, and a minimal neuronal degeneration was observed following 5 doses of AL at 288 mg/kg every other day in rats. Although the total dose (1440 mg/kg) and duration (9 days) were identical (Si et al., 2007), different neurotoxic results were observed. This observation could be due to a prolonged exposure time in the 160 mg/kg group in comparison to the shorter time spent above the LONEL in the

288 mg/kg group (Table 2). Based on the minimal inhibition of gastric emptying on day 5 and histopathological data obtained from animals treated with oral AL daily for 9 days, the LONEL was calculated as 346.26 ng/ml in this study (Figure 4, middle). This data demonstrates that AL induced moderate brainstem injury in rats at a severity of 3.25 during a neurotoxic exposure time of 186.0 hr at daily doses of 160 mg/kg, and AL induced minimal brainstem injury in rats at a severity of 1.17 during a neurotoxic exposure time of 75.0 hr at a daily dose of 288 mg/kg dosed every other day. The reduction of the neurotoxic exposure time from 186.0 hrs to 75.0 hrs correlates well with the reduction of neurotoxicity observed between the two doses regimens.

This study also showed that the LONEL value (346.26 ng/ml) following oral AL administration in rats is approximately 8-fold higher than the LONEL observed after dosing with intramuscular AE (41.32 ng/ml). A tissue distribution study was also conducted comparing the intramuscular injection of ^{14}C-AE to intravenous administration of ^{14}C-AL in rats. The results of this study showed 0.89% total radioactivity in the brain after ^{14}C-AE administration while the administration of ^{14}C-AL in rats showed 0.1% of total radioactivity in the brain (Li et al., 2005a). This result suggests that ^{14}C-labelled AL seems to be less capable of penetrating through the blood-brain barrier than ^{14}C-AE.

4.2 Neurotoxicity induced by intramuscular AE and AM in dogs

Davidson (1994) showed that a daily dose of AE at 3 mg/kg for 28 days is a NONED that does not cause clinical neurotoxicity or pathology in beagles. AE-induced brainstem neuropathology in dogs has been detected after administration of a dose as low as 5 mg/kg/day as shown by Brewer (1994a, 1994b, 1998), 6.25 mg/kg observed by Dayan (1998), and 6.75 mg/kg dosed daily for 28 days observed by Davidson (1994). Based on these three findings, the calculated average of the minimal dose necessary to produce neurotoxicity by histopathology in dogs is 6 mg/kg daily for 28 days. The minimal plasma concentration of AE with a dose of 6.0 mg/kg daily for 28 days has been estimated at 40.92 ng/ml, and this value could, therefore, be defined as a LONEL in plasma. The LONEL of 40.92 ng/ml should be the first "at risk" level for causing neurotoxicity in dogs at a daily dose of 6 mg/kg for 28 days (Li et al., 2006b). As a result, the LONEL for toxicity of AE in dogs was estimated based on all positive findings of neuropathology in these animal studies.

A TK simulation of 28 repeated AE treatments at 6 mg/kg dosed intramuscularly in beagles was conducted, and an exposure time of 103.7 hr in plasma was estimated as a minimum time above the LONEL (40.92 ng/ml) to induce positive neurotoxicity, which was confirmed by histopathological examination (Brewer et al., 1994b; Davidson 1994; Dayan 1998) (Table 2). The result demonstrated that AE-induced brainstem injury in dogs occurred during a minimal period of 103.7 hr with plasma exposure of AE above this LONEL. Therefore, the LONEL for neurotoxicity of AE was simulated based on 100% positive findings of neuropathology at a daily dose of 6 mg/kg for 28 days in beagles (Li et al., 2006b and 2007a).

Classen (1999) showed that a minimal daily dose of AM at 20 mg/kg given for 7 days caused a minimal clinical neurotoxicity or pathology in beagles. The minimal plasma

concentration of AM achieved after a dose of 20 mg/kg given daily for 7 days has been estimated at 75.69 ng/ml, and this value could, therefore, be defined as a LONEL in dog plasma. The LONEL of 75.69 ng/ml should be the first "at risk" level for causing neurotoxicity in dogs at a daily dose of 20 mg/kg for 7 days (Figure 4, top). As a result, the LONEL for toxicity of AM in dogs was estimated based on all positive findings of neuropathology in these animals (5 out of 8 dogs). PK analyses of 7 repeated AM treatments of beagles at 20 and 40 mg/kg dosed intramuscularly were conducted, and exposure times of 153 and 188 hrs, respectively, were estimated as a minimum time above the LONEL (75.69 ng/ml) to induce neurotoxicity, which was confirmed by histopathological examination (Table 2). The results of this study showed that AM-induced brainstem injury occurred during a minimal period over 153 hrs at drug plasma level above this LONEL. It is to be noted that the LONEL of AM (75.69 ng/ml) has been shown to be much higher than that of AE in beagles (40.92 ng/ml), suggesting that AE may have a higher potential for neurotoxicity than AM.

4.3 Neurotoxicity induced by intramuscular AE in monkeys

Since AE-induced brainstem neuropathology in rhesus monkeys occurred at a minimal dose of 8 mg/kg dosed daily for 14 days (Petras et al., 1997, 2000), the LONEL of AE was estimated to be 193.8 ng/ml. This estimate is based on our TK analysis conducted by dosing monkeys with AE at 16 mg/kg dosed daily for 14 days which should, based on a number of repeat dosing studies, result in evidence of AE-induced neurotoxicity (Petras et al., 1997; Li et al., 2006b, 2007a). By conducting TK simulations of 14 repeated AE treatments of monkeys dosed intramuscularly at 8 mg/kg, a neurotoxic exposure time of 179.5 hr in plasma was calculated as the minimum time spent above the LONEL (193.8 ng/ml) required to induce pathological neurotoxicity (Figure 2, bottom). The results of this study showed that AE-induced brainstem injury occurred during a minimal duration of 179.5 hr spent above the LONEL of AE (Li et al., 2006b, 2007a). The data also showed that the LONEL value of 193.8 ng/ml in rhesus monkeys is 4-fold higher than that of rats (41.32 ng/ml) and dogs (40.92 ng/ml), indicating that rats and dogs appear to be more vulnerable than rhesus monkeys to neurotoxic ART-induced toxicity.

5. TK/TD analysis of artemisinin-induced neurotoxicity

To determine the relationship between drug exposure time (toxicokinetics, TK) and neurotoxic effects (toxicodynamics, TD) after administration of ARTs in varying dose and time regimens, we calculated the time required for the drug plasma concentration to reach a neurotoxic level above the LONEL. Although the LONEL is a minimum observed neurotoxic effect level, a certain exposure time was required to be necessary to determine the neurotoxic effects in our previous studies where ARTs were shown to produce neurotoxicity. This process gave us an initial estimate of drug LONEL and exposure times and allowed us to correlate these data with observed histopathology or neurotoxic effects from ARTs tested in various regimens in different animal species. For the evaluation of toxicity, if the recovery (consisting of adaptation, repair and reversibility) half-life of an organism is longer than the half-life of the causative agent in the organism, then TD would become the rate-determining or rate-limiting step, and the organism would survive. If the

TK half-life of the compound is longer than the recovery half-life, then TK will be a rate-determining or rate limiting step. In this scenario, the TK exposure time would be identical to a TD exposure time, and the organism might die. Therefore, neurotoxicity induced by ARTs in animals may be determined through either TD or TK/TD processes.

5.1 Exposure time with neurotoxic effects of AE and AL in SD rats

A neurotoxicity study was conducted to compare AE in two vehicles, sesame oil and cremophore (Li et al., 2002). The authors calculated the neurotoxic exposure time in rats of AE in sesame oil to be 164.3 hrs after dosing over the LONEL (41.32 ng/ml) during treatment with 25 mg/kg AE dosed intramuscularly daily for 7 days (Figure 1, top). The total neurotoxic exposure time of AE in a formulation of 1:2 cremophore/saline at the same dose regimen was 103.0 hrs. The exposure time for AE in a cremophore formulation was over one-third less than the exposure time observed with AE in sesame oil. Neurotoxicity outcomes in these rats were reduced from severe to moderate. After simulating the TK data on daily dosing at 12.5 mg/kg for 7 days (Li et al., 2007a), the drug exposure time spent over the LONEL was only 67.1 h, and the severity of neuropathological toxicity was further reduced to a minimum (Genovese et al., 1998), demonstrating that drug exposure time plays a key role that correlates with the development of neurotoxicity (Figure 2, top).

In a further study, the neurotoxic exposure time (drug exposure time spent over the LONEL at 346.26 ng/ml) for oral AL was shown to be 186 hr which defines the exposure period threshold to achieve neurotoxicity (Fig. 4, middle). These results indicated a correlation between the neuropathology observed in rats and a prolonged AL exposure time. In this study, the AL exposure time was related to an accumulation of drug in plasma likely resulting from delayed gastric emptying, which, in turn, was hypothesized to induce prolonged absorption of the drug from the stomach (Si et al., 2007). When rats were treated intermittently with 5 doses of AL at 288 mg/kg every other day for 9 days, neuronal degeneration was minimal until day 7 after the last treatment (Table 2). The minimal exposure time required to induce neurotoxicity in these animals was calculated to be 75 hr (Fig. 4, bottom). The data, therefore, supports the hypothesis that shortening the drug exposure time above LONEL level may reduce the risk of neurotoxicity.

The LONEL cannot be estimated for intramuscular AL and AS dosed in rats daily at 25 mg/kg for 7 days because no neurotoxicity (pathological or/and behavioral neurotoxicity) was detected in those animals. PK data showed that drug accumulation in the plasma was not observed in these animals. The lack of AL and AS drug accumulation is likely due to their rapid elimination and short half-lives with clear and long time intermissions between each dosing without drug exposure (Figure 1, middle and bottom). Confirmation of the hypothesis that the short drug exposure times of AL and AS after intramuscular injection do not induce neurotoxicity (Table 2) is a very relevant finding as animals treated with AL and AS intramuscularly would likely avoid the risk of neurotoxic outcome due to a reduction of drug exposure times. This result suggests that the shorter exposure times of AL and AS without drug exposure at LONEL appears to be a major contributing factor for avoiding clinical neurotoxicity.

5.2 Exposure time with neurotoxic effects of AE, AM and AL in dogs

Drug accumulation was also observed in the plasma of beagles after daily intramuscular administration of AE at 15 mg/kg for 14 days (Li et al., 2006). The mean concentration-time profile of AE in beagles obtained from this experiment is shown in Fig. 2 (middle). The most significant data observed were found to involve different parameters of the AUC and the drug half-lives. The elimination t½ of AE was prolonged from 9.23 hr on the first dosing day to 21.53 hr on the last dosing day, suggesting that the exposure time of AE had nearly doubled. Also, the LONEL of AE (40.92 ng/ml) in beagles was first reached in that study on day 6-7 (Li et al., 2006b). At this time, the food intake of these animals decreased by 70%, and the QT interval also increased by more than 25%. We believe, based on the TK data analysis, that day 6-7 is the earliest time possible to induce minimal neurotoxicity in beagles, which also showed GI toxicity, following high level AE dosing. The exposure time of AE in this 14 day study which induced severe neurotoxicity and death involved daily intramuscular injections of 15 mg/kg AE in dogs was estimated to be at concentrations above the LONEL (40.92 ng/ml), and the exposure period was calculated to be 277.6 (Table 2).

Although AM and AE are both oil-soluble ARTs, there are differences in the TK and TD profiles of these two drugs: 1) AM injection has been shown to result in less local inflammatory toxicity at the muscle injection site than AE formulated with the same sesame oil vehicle; 2) AM has been shown to have a significantly higher absorption rate (C_{max}) from intramuscular injection sites than AE; 3) AM has been shown to have much higher drug exposure levels (total AUC) than AE which likely results in much less drug accumulation; and 4) AM has been shown to have a LONEL that is almost two fold higher (75.69 ng/ml) than AE (41.32 ng/ml) in beagles. Although the multiple dose levels of AM employed in this study were higher than those used for AE (Table 2), the AM dosing period of 7 days was half that of the 14 day AE dosing period. Therefore, the LONEL data for AM and AE would suggest that the predicted neurotoxic exposure time for AM (113.2 – 147.8 hrs) required to induce neurotoxicity should be significantly shorter than the neurotoxic exposure time for AE (277.6 hrs) required to induce neurotoxicity. Correspondingly, the clinical observations of animals dosed with AM showed a lower degree of neurotoxicity than dogs treated with AE intramuscularly (Table 2). This finding supports the hypothesis that intramuscular AM treatment is less prone to induce neurotoxicity in animals than intramuscular AE.

The neurotoxic exposure time spent above the LONEL after oral AL dosing (346.26 ng/ml) in dogs was calculated to be 17.9 hr for a suspension formulation of AL (Li et al., 2005b) and 14.6 hr for a capsule formulation of AL both dosed at 25 mg/kg daily for 14 days (Table 2). Similar to intramuscular administration, animals treated with oral AL did not show any evidence of induction of neurotoxicity due to the shorter drug exposure times above the LONEL. The drug exposure times observed for AL were much shorter than the neurotoxic exposure time above the LONEL of 75.0 h, which is required to induce a minimal pathological neurotoxicity in rats (Table 2).

5.3 Exposure time with neurotoxic effects of AE in rhesus monkeys

Substantial plasma accumulation of AE was shown in the plasma of rhesus monkeys after daily intramuscular administration of AE at 16 mg/kg for 14 days (Fig. 2, bottom). Based on

the minimal injury observed to the neuronal tissues in the monkeys treated with 8 mg/kg daily for 14 days, it was possible to correctly identify 193.8 ng/ml as the LONEL (Li & Hickman, 2011). The neurotoxic exposure period of AE was calculated as 307.4 hr in this study following daily intramuscular injection of 16 mg/kg for 14 days in monkeys with moderate neurotoxicity (Petras et al., 1997 and 2000) (Table 2). This calculation was based on the estimated exposure time of AE above the LONEL concentration (193.8 ng/ml). The rhesus monkeys treated with intramuscular AE at 8 mg/kg daily for 14 days showed that the drug exposure time spent above LONEL with minimal pathological toxicity was estimated at 179.5 hr, which was shorter than the exposure time (307.4 hr) from a previous study following daily intramuscular administration of AE at 16 mg/kg for 14 days (Li et al., 2006b). The longer neurotoxic exposure time is likely the key factor involved in the induction of neurotoxicity in rhesus monkeys (Li et al., 2007a).

6. Neurotoxic consideration of ARTs in antimalarial and anticancer treatments

Though no animal model exists capable of completely mimicking ARTs-induced neurotoxicity, the comparison of monkeys to humans is the closest that can be achieved. With animal experiments, only certain aspects of the whole complex TK/TD environment can be analyzed. In order to achieve the best prediction of neurotoxicity based on TK/TD parameters, the choice of animals and experimental design requires careful consideration to represent the conditions existing in humans in as suitable a model as possible. The more the model deviates from human TK/TD conditions, the less likely the prediction will be relevant. Today more information is available on the TK/TD properties of ARTs in animals. This body of literature will provide data on the neurotoxic doses of ARTs and on their non–neurotoxic doses relevant to man.

Studies with laboratory animals have demonstrated fatal neurotoxicity associated with intramuscular administration of AM and AE or oral administration of AL. These effects suggest that the exposure time of ARTs was extended in these studies due to the accumulation of drug in the bloodstream, and this accumulation, in turn, resulted in neurotoxicity. In our previous studies, the drug exposure time with a neurotoxic outcome (neurotoxic exposure time) was evaluated as a predictor of neurotoxicity *in vivo* (Li & Hickman, 2011). The neurotoxic exposure time represents a total time spent above the lowest observed neurotoxic effect levels (LONEL) in plasma. The effects of ARTs do vary in different animal species. For example, the dose of AE required to induce minimal neurotoxicity requires a 2-3 fold longer exposure time in rhesus monkeys (179.5 hr) than in rats (67.1 hr) and dogs (103.7 hr) when using a daily dose of 6-12.5 mg/kg for 7-28 days. This finding suggests that the safe drug dosing of LONEL duration in monkeys should be longer than 7.0 days (> 168 hrs) under this exposure.

In addition, the effects of ARTs vary from compound to compound. For example the LONEL for intramuscular AM required to induce neurotoxicity (75.69 ng/ml) is twice as high as the LONEL for intramuscular AE required to induce neurotoxicity (40.92 ng/ml). Oral AL treatment required a LONEL to induce neurotoxicity (346.26 ng/ml) that is 4-fold higher than the LONEL of AM required to induce neurotoxicity (75.69 ng/ml) and 8-fold higher than the LONEL of AE (40.92 ng/ml), required to induce neurotoxicity. A

determination of LONEL for oral and injectable AS was not possible because no neurotoxicity was observed in animals treated with various AS dose regimens, suggesting that water-soluble ARTs (AS and AL) appear to be much safer than oil-soluble ARTs.

The various animal species treated with different ARTs clearly show the different neurotoxic effects associated with corresponding exposure times. The current marketed drugs for ART therapy are based on oral administration of drug and combination therapies to malaria patients. Oral administration results in lower peak concentrations and shorter exposure times which is less likely to induce neurotoxicity than intramuscular ARTs. Since more than 99% of malaria patients have been treated with oral ARTs or intravenous AS, this may be the reason for the lack of neurotoxicity observed in malaria patients. When relating the animal and human neurotoxicity of ARTs, the different neurotoxic exposure times may possibly provide a greater margin of safety in humans. The current clinical dose regimens of three-day ART combined therapies (ACTs) for uncomplicated cases of malaria, and the dose regimens recommended for intravenous AS treatments for severe malaria, which include a few days of a loading dose, may be too short of a drug exposure time to induce neurotoxicity in humans. Also, with regard to acute toxicity, humans appear to be less sensitive than animals (Geyer et al., 1990; Kimbrough 1990), and humans have much better repair capabilities than animals to respond to such toxicity (Culotta & Koshland 1994).

Although the water-soluble ARTs, like AS, appear to be much safer, further study is needed when employing ARTs as anticancer agents (Li & Hickman, 2011). At high concentrations, ARTs appear to be active against cancer *in vivo*. The use of ARTs at high concentrations or for long drug exposure times, however, has substantial risk of inducing severe toxicities, such as the neurotoxic effects we have described here. Animal studies have shown that high concentrations of AS and DHA can induce embryotoxicity, and longer exposure times have been shown to be associated with fatal neurotoxicity (Li et al., 2007a). To provide maximum benefit and minimal risk of toxicity, ARTs should be combined with other anticancer agents to increase the efficacy of cancer drugs, enhance the survival rate of patients with cancer, and prolong the time to progression (Zhang et al., 2008). The diversity in targets of ARTs supports the possibility that these compounds could be used in combination with other agents, which mimics the current strategy promoted by the WHO as policy recommendations on the use of ARTs for malaria therapy (WHO 2006).

7. Conclusion

Studies with laboratory animals have demonstrated neurotoxicity following administration of some intramuscular doses of oil-soluble AM and AE, or intragastric treatment of water-soluble AL. The various PK studies of ARTs conducted using numerous animal models show that the drug exposure time appears to be a more important factor than other PK parameters measured for inducing neurotoxicity. There are significant differences in neurotoxicity observed when comparing the effects of ARTs on rats, dogs and monkeys suggesting that the exposure time required inducing neurotoxicity after dosing with ARTs is likely to be longer in humans. Extensive TK/TD analyses of neurotoxicity after ART treatment of rats, dogs, and monkeys have provided a wealth of data which can be used to

predict the neurotoxic exposure time of ARTs in humans. Based on the dose amounts of ARTs used in various experiments to induce neurotoxicity we would rank the ARTs in terms of toxic potential with AE being the most toxic followed in order by AM, AL, and lastly by AS. There is a strong correlation between the drug half-lives of ARTs and the severity of neurotoxicity observed after treatment which supports the hypothesis that the drug exposure time during treatment plays a very important role in the induction of neurotoxicity.

In the this chapter, the lowest observed neurotoxic effect level (LONEL) represents the minimal plasma concentration associated with the lowest dose that is found to cause a neurologically and/or statistically significant response to treatment in comparison to the control group. Based on the determination of the LONEL in various animal species for ARTs, the neurotoxic exposure time (drug exposure time spent above the LONEL) was evaluated. Our analysis of this data leads us to the prediction that the safe dosing duration of AE or AM in humans should be longer than 7 days (> 168 hr), and the safe dosing duration of AS therapy is likely much longer than that. Accordingly, the 3-5 days dosing duration currently used in antimalarial therapy of ARTs should be quite safe. Neurotoxicity may be caused in humans, however, treated with inappropriate dose regimens, and therefore, evaluation of the sustained drug exposure times appears to be the critical factor to assess and prevent neurotoxicity. Advances in our knowledge of ART-induced neurotoxicity can help refine the treatment regimens used as therapies for malaria and cancer with ART-based combination therapy and injectable AS products to avoid drug accumulation and reduce the risk of toxicity.

8. Acknowledgments

9. References

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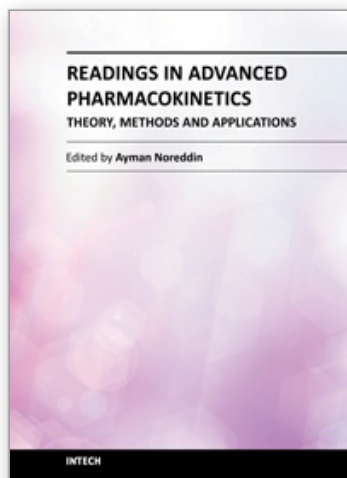
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This book, "Readings in Advanced Pharmacokinetics - Theory, Methods and Applications", covers up to date information and practical topics related to the study of drug pharmacokinetics in humans and in animals. The book is designed to offer scientists, clinicians and researchers a choice to logically build their knowledge in pharmacokinetics from basic concepts to advanced applications. This book is organized into two sections. The first section discusses advanced theories that include a wide range of topics; from bioequivalence studies, pharmacogenomics in relation to pharmacokinetics, computer based simulation concepts to drug interactions of herbal medicines and veterinary pharmacokinetics. The second section advances theory to practice offering several examples of methods and applications in advanced pharmacokinetics.

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