

5. DISCUSSION AND CONCLUSION

In this study, pharmacokinetics of ivermectin was analyzed using both compartment and non-compartment models. That is because pharmacokinetics of this drug in several animals, reported by several investigators had been described based on either compartment, or non-compartment model. Regarding to compartment model analyses, some investigators reported that pharmacokinetics of ivermectin was fitted with 1-compartment model, while the others reported with 2-compartment model. To be able to compare non-statistically with other studies or animals, both compartmental and non-compartmental approaches were used to describe kinetics of ivermectin in normal cats.

In this study, 1-compartment model was chosen. That is because no distribution phase appeared separately in the serum concentration-time profiles of ivermectin and values of coefficient of variation were acceptable. In addition, AIC values obtained from pharmacokinetic analyses with aid of the WinNonlin software in each experimental cat showed minimum AIC values. Therefore, the compartment model with minimum AIC is chosen as the best representation for the serum concentration-time profiles of ivermectin (Yamaoka *et al.*, 1978). Since blood sampling interval after administration of ivermectin in normal cats during distribution phase was slightly far, so in the infected cat, a few blood sampling times were added to confirm correct selection of the model. The results showed that the serum concentration-time profiles of ivermectin were not changed. This confirmed that 1-compartment model would be suitable to describe kinetics of ivermectin in cats.

As a result of 1-compartment and non-compartment analyses, the

pharmacokinetic parameters representing absorption, distribution, and elimination were not significantly different, excepting C_{max} and k_{ab} in absorption phase. For absorption phase, the mean values derived from 1-compartment and non-compartment models of t_{max} 1.13 v.s. 1.09 day and $AUC_{0 \rightarrow \infty}$ 94.23 v.s. 94.84 ng.day/ml were not significantly different ($p > 0.05$) but were significantly different in ($p < 0.05$) C_{max} 16.17 v.s. 17.53 ng/ml and k_{ab} 1.72 v.s. 1.16 day^{-1} . This result may be related with sampling interval which was slightly far in normal cats. It may be affected to missing value of the C_{max} and k_{ab} parameters. Because of the C_{max} and k_{ab} values derived from 1-compartment model were estimated from predicted curve, while non-compartment model were estimated from observed curve. The distribution and elimination parameters resulted from 1-compartment and non-compartment analysis were not significantly different ($p > 0.05$), i.e. V_d/F 8.18 v.s. V_z/F 7.23 L/kg; k_e 0.20 v.s. λ_z 0.24 day^{-1} ; $t_{1/2 \text{ el}}$ 2.53 v.s. $t_{1/2 \lambda_z}$ 2.30 days; and Cl/F 5.68 v.s. Cl/F 5.68 L/day.

Considering the serum concentration-time profile of each individual, there were 2 cats (animal code 05 and 06) whose serum concentrations of ivermectin lasted until day 25, while those of the rest were undetectable after day 15. These two animals seemed to eliminate ivermectin slowly. The elimination half-life was longer (approximately 6 days) than that of the other 6 animals (ranging from 1-3 days). That is due to the lower elimination rate constant, i.e. approximately 0.1 day^{-1} , compared with the rest, i.e. ranging from $0.20\text{-}0.45 \text{ day}^{-1}$. The slow elimination process in these two animals may be resulted from the delayed transfer rate of the drug from plasma to the gastrointestinal tract during biliary excretion, which leads to a lower rate of faecal excretion of drug (Perez *et al.*, 2001). Ivermectin is normally excreted into the bile before eliminating via the faeces with 90% of excreted drug as parent compound (Bogan & McKellar, 1988; Chiu *et al.*, 1999; Zulalian *et al.*,

1994a, 1994b). Therefore, hepatic metabolism has a minor role for drug elimination.

When considering their pharmacokinetic data, both animals seemed to have higher volume of distribution (ranging from 13.01-21.84 L/kg), compared to the other 6 animals (ranging from 5.28-9.61 L/kg). This may be involved with greater proportion of fat in body composition, which may facilitate longer $t_{1/2\text{ el}}$ of the drug. However, values of Cl/F (approximately 5.00 L/day) did not differ from the other 6 animals, i.e. ranging from 4.47-9.67 L/day.

Pharmacokinetics of a single dose of 200 $\mu\text{g/kg}$ of ivermectin subcutaneously injected to normal cats appeared to differ from those in other animals. The mean value of the maximum concentration (C_{max}) of 16.17 ± 1.43 ng/ml was lower than those obtained in sheep (24.09 ± 6.57 ng/ml: Echeverria *et al.*, 2002, cattle (31.70 ± 2.75 ng/ml: Toutain *et al.*, 1997), and pigs (33.30 ± 4.10 ng/ml: Lifschitz *et al.*, 1999a). The mean value of the time to maximum serum concentration (t_{max}) of 1.13 ± 0.18 day was shorter than that registered in sheep (2.67 ± 0.52 days: Echeverria *et al.*, 2002), pigs (2.80 ± 0.40 days: Lifschitz *et al.*, 1999a), and cattle (3.98 ± 0.28 days: Toutain *et al.*, 1997). Absorption rate constant (k_a) in cats was 1.72 ± 0.66 day^{-1} . No value of k_a in other animals was reported except in sheep that was 1.30 ± 0.5 day^{-1} (Cerkvenik *et al.*, 2002). Therefore, ivermectin was more rapidly absorbed in cats than sheep. However, the mean value of the area under the serum concentration-time curves ($\text{AUC}_{0 \rightarrow \infty}$) that refers to the extent of absorption was 94.23 ± 10.79 ng.day/ml, which was lower than those obtained in pigs (165.00 ± 21.60 ng.day/ml: Lifschitz *et al.*, 1999a), sheep (207.50 ± 46.5 ng.day/ml: Echeverria *et al.*, 2002), and cattle (361.00 ± 17.00 ng.day/ml: Toutain *et al.*, 1997). Therefore, extent of absorption of ivermectin in cats was the least among other animals.

The mean value of the elimination half-life ($t_{1/2\text{ el}}$) obtained in cats was 2.53 ± 0.79 days, which was lower than those reported in pigs (3.50 ± 0.50 days: Lifschitz *et al.*, 1999a), cattle (4.32 ± 0.25 days: Toutain *et al.*, 1997; 6.30 ± 0.80 days: Lifschitz *et al.*, 1999a; 5.90 ± 3.36 days: Lifschitz *et al.*, 1999b) and sheep (5.60 ± 1.20 days: Echeverria *et al.*, 2002). Therefore, ivermectin was more slowly eliminated in cats than pigs, cattle and sheep. Elimination rate constant (k_e) in cats was $0.20 \pm 0.05 \text{ day}^{-1}$. No value of k_e in other animals was reported except in sheep that was $0.30 \pm 0.20 \text{ day}^{-1}$ (Cerkvenik *et al.*, 2002). Thus, ivermectin was more rapidly cleared in cats than sheep. This led to a low mean residence time of ivermectin ($\text{MRT}_{0 \rightarrow \infty}$) (4.58 ± 0.90 days), which was shorter than that reported in pigs (6.30 ± 0.60 days: Lifschitz *et al.*, 1999a) and cattle (6.10 ± 0.50 days: Lifschitz *et al.*, 1999a; 9.49 ± 3.86 days: Lifschitz *et al.*, 1999b). This suggests the short residence of the drug in the bloodstream in normal cats. However, elimination half life and mean residence time of ivermectin in these animals may be depended on the transfer rate of the drug during biliary excretion, the metabolic rate, the rate of faecal excretion, proportion of fat in body composition and normal variation in individual cats as same as those two animals.

The pharmacokinetic parameters of ivermectin in a *B. malayi*-naturally infected cat were placed within the range of normal cats. At this step, it is unlikely to state that pharmacokinetic behaviors, i.e. absorption, distribution and elimination of this drug in the infected cat would be similar to that of the normal ones. In the preliminary study, it was aimed to evaluate microfilaricidal efficacy and pharmacodynamic of ivermectin in naturally infected cat. However, only one animal was used because these must be led from endemic area of lymphatic filariasis (Narathiwat province), which is a risky area. This restriction leads to trouble for searching the naturally infected cat.

Comparison of pharmacokinetics of ivermectin in normal and infected cats can be done when a larger amount of the infected animals is included, possibly in further study.

The number of *B. malayi* microfilariae in an infected cat was found the highest at night. The nocturnally subperiodic type also exhibits their highest levels of parasites in blood circulation at night, but 40 to 60% of peak levels persist during the day (Dondero *et al.*, 1971). From this study, approximately 20 to 50% of highest levels of microfilariae presented at night and persisted through out the day. Thus, the *B. malayi* in the infected cat taken from Narathiwat, the endemic area of lymphatic filariasis in this study was likely to be a nocturnally subperiodic type.

During the experiment, process of identification of the parasite species was cautiously done by using Giemsa-stained method and observing under light microscope (40x). Since microfilariae of *B. pahangi* and *Dirofilaria* species, common natural parasites in cats, are similar to *B. malayi*, it may be confused in differentiation if not performed by the specialist. However, this problem was solved by consulting with the specialist at the Filariasis Project Pigultong Development Center in Narathiwat Province. Using Giemsa-stained method, differentiation between *B. malayi* and *Dirofilaria* species were based on cephalic nuclei, sheath, terminal nuclei and cephalic space. *Dirofilaria* species showed two cephalic nuclei, unsheathed, no terminal nuclei and large cephalic space, while the *B. malayi* showed one cephalic nucleus, sheathed, two terminal nuclei and small cephalic space (WHO, 1987). Differentiation between *B. malayi* and *B. pahangi* cannot be distinguished using this method. According to the study in *B. malayi*-naturally infected cats in Narathiwat province by Kob-asa *et al.* (2004) using PCR-based methods, the Brugia type found in this endemic area was only *B. malayi*.

After receiving a single subcutaneous dose of 200 µg/kg of ivermectin, the maximum reduction of microfilariae was achieved within 4 days. Percentages of reduction were more than 60% and lasted up to 25 days. Whether the effect will last longer, that is beyond the study results. The blood microfilariae were suppressed due to the action of ivermectin, which affects only the blood microfilariae but not the adult worms (Dreyer *et al.*, 1996). Although the serum concentrations of ivermectin were gradually decreasing, the number of microfilariae was still low. That may be because the microfilariae in blood circulation come from adult worms residing in the lymphatic system. Plaisier *et al.* (2000) has been suggested that the lasting suppression of microfilaraemia generally observed after ivermectin treatment in human is due to a reduced microfilariae output from the adult females.

This study results correspond to that of Phantana *et al.* (2002) who studying the efficacy of ivermectin (200, 400 and 1,000 µg/kg) on zoonotic *B. malayi* in naturally infected cats. At the dose of 200 µg/kg of ivermectin, the maximum microfilariae clearance of 84% was achieved within 30 days. The authors have shown that the microfilaricidal effect of ivermectin is independent of drug dose. However, repeating the same dose at 30 days after the first treatment was able to further reduce blood microfilariae, i.e. 90.20% at maximum. Therefore, using high dose of ivermectin may not be useful for lymphatic filariasis in cats. Repeating dose may be more reasonable. However, result of the maximum microfilariae clearance differed from our result that maximum decreasing at 65% approximately. It may be related with number of microfilariae before post-dose, which were higher than that studied by Phantana *et al.* (2002).

In this study, administration of a single dose of 200 µg/kg of ivermectin in cats did not cause any side effects. This finding corresponds with those

reported by Phantana *et al.* (2002). In their study, the cats naturally infected with *B. malayi* were used. The animals were subcutaneously injected using a single dose of 200 µg/kg of ivermectin. The common side effects, such as abnormal behavior, ataxia, lethargy, weakness, tremors and recumbence, were not observed in treated cats.

Regarding to demographic data in some animals (animal code 01) would be anemia. This is due to the lower hemoglobin (7.30 g %) and hematocrit (22.00 %), compared with normal range of hemoglobin (8.00-15.00 g %) and hematocrit (24.00-45.00 %), respectively. It was received the ferrous sulphate (FeSO_4) at 1 month approximately before administration of ivermectin. However, following the demographic data in this animal after receiving FeSO_4 was not proceeded.

In this study, HPLC technique with fluorescence detection was used to determine the levels of ivermectin in cat serum. Although ivermectin can be detected by using ultraviolet (UV) detection at 245 nm, but such method is not quite sensitive due to matrix interference of ivermectin in a biological sample. Method of extraction of ivermectin from serum samples was based on that described by Lifschitz *et al.* (1999a), which was modified from De Montigny *et al.* (1990). Some procedures were adapted in this study. Solvent and sample were mixed using vortex for 30 sec instead of rotation for 20 min because an instrument was not supported. Drying of samples was not done under a stream of nitrogen but under speed vacuum at 45-50 °C. The advantage is that the sample was rapidly dried.

Method for analysis selected to use in this study can analyze the minor component of ivermectin. As stated earlier, ivermectin and abamectin compose of the major and the minor components. The major component of ivermectin (H_2B_{1a}) and abamectin (B_{1a}) were seen in the chromatogram at

12.50 min and 7.00 min, respectively. It was noticed that there were small peaks presented next to the peak of the major components of both ivermectin and abamectin (t_R of 9.80 min and 4.50 min, respectively). Those peaks were suspected to represent the minor components of ivermectin (H_2B_{1b}) and abamectin (B_{1b}). This was confirmed by varying concentrations of both ivermectin and abamectin spiked in serum samples. It was found that when the concentrations of ivermectin and abamectin increased, the peak responses represented to both major and minor components increased.

In conclusion, a single dose of 200 $\mu\text{g}/\text{kg}$ of ivermectin after subcutaneous injection is rapidly absorbed and slowly eliminated in healthy cats. Pharmacokinetic analyses reveal simple behavior of drug disposition. With a single low dose, the drug effectively and rapidly reduces blood microfilariae in a *B. malayi*-naturally infected cat despite of incomplete clearance of microfilariae. Effect of either a larger dose or repeated dose on eliminating blood microfilariae as well as concurrent blood drug level determination should be further investigated to find out the optimal dose and interval of application in the endemic area.