Chapter IV

Discussion and conclusion

The use of animal seizure models is essential in the discovery and development of new drugs for the treatment of epileptic seizures. Discovery of a new therapeutic agent begins with the hypothesis that there is a relationship between the experimental seizure model and the initiation and propagation of the seizure, and that the experimental seizure approximates the pathophysiology underlying the human condition. Phenytoin (PHT), carbamazepine (CBZ), and valproate (VPA) were identified by relatively simple screening procedure that do not provide insight into a drug's mechanism of action. Two concepts were assumed: either seizure spread or seizure threshold was affected. When animal models that used electrical stimulation or chemoconvulsant were developed, they were systematically validated using the then-known clinically effective compounds. The pharmacologic activity of the potential anticonvulsants was then profiled to predict their utility in the various types of human epilepsy. The therapeutic activity as well as the toxicity of these new agents was then demonstrated in various animal models and species. The utilization of different animal seizure models has been critical for identifying many of the newly marketed antiepileptic drugs (AEDs)(Kupferberg, 2001).

Löscher et al. studied several important test parameters. In a series of articles, they meticulously presented the role of technical, biologic, and pharmacologic factors in the laboratory evaluation of AEDs. They included the influence of vehicle, the maximal electroshock seizure models, the pentylenetetrazole seizure models, protective indices, and seasonal influence in pharmacodynamic responses (Kupferberg, 2001).

The MES and PTZ models represent the highly reproducible *in vivo* systems that are the most commonly used for antiepileptic drugs screening models. Results in these two models are employed in the search for effective anticonvulsant drugs and sometimes as a test of possible mechanisms of action as well. The MES test is an excellent animal model for the identification of new AEDs that block seizures

spread and are likely to be effective for the management of generalized tonic-clonic seizure in human. On the other hand, the PTZ test is an effective model that identifies the AEDs that raise seizure threshold and are likely to couple with AEDs that are effective in the treatment of absence seizure (Rogawski and Porter, 1990; White, 1997; Lösher, 1998). In addition, the PTZ-induced clonic seizure are blocked by drugs acting at the $GABA_A$ receptor e.g. benzodiazepine and phenobarbital whereas the hind limb extension observed in MES test is effectively blocked by AEDs such as phenytoin and carbamazepine which are known to inhibit voltage-sensitive sodium channels (Rogawski and Porter, 1990; White, 1997).

The fact that ameltolide is very potent, though with narrow spectrum, attracted many investigators to further modify its structure in search for ameltolide analogs with improving pharmacological profiles (Clark, 1988;Potts et al, 1989; and Robertson et al, 1991). Many of them have been found to be ineffective while some of them became broad-spectrum anticonvulsants exhibiting protection in both MES and PTZ models (Diouf et al, 1997; and Vamecq et al, 1998). CU-17-06 is one of them. The result of the present studies demonstrated that intraperitoneally given CU-17-06 exhibited a selective anticonvulsant activity in only the MES test (Figure5; Table7). In accordance with previous work (Clark, 1988), ameltolide exclusively exerts its anticonvulsant activity in MES but not PTZ model (Figure6; Table 7). Taking into consideration that lamotrigine, the new AED which exerts it anticonvulsant activity by selective blockade of sodium channels of glutamatergic neurons, posses the same profile of anticonvulsant activity in these two models, similar to those exhibited by phenytoin and carbamazepine (White, 1997 ; Rho and Sankar, 1999). It is suggestive that CU-17-06 may exert its anticonvulsant activity in the same manner, as did its precedents.

Apparently, CU-17-06 was less effective than ameltolide, which exhibited its ED_{50} of 1.08 mg/kg B.W. at pretreated time of 30 min in MES test. However in terms of safety, CU-17-06 seems to safer than ameltolide as it did not produce any lethality in the dose up to 1,000 mg/kg (Table 7) implying that CU-17-06 may clinically less potent but safer than ameltolide.

Regarding the duration of anticonvulsant activity, it was apparent that intraperitoneal administration of either CU-17-06 or ameltolide exhibited protection against MES at least within 6 hour after dosing, however, with an increment of the ED_{50} values (Figure 8). The ED_{50} values of CU-17-06 were always higher than that of ameltolide at any given time, suggesting the lower potency of CU-17-06 than that of ameltolide throughout the observation period.

Most AEDs suffer from unwanted effect such as ataxia, sedative and impairment of motor function (Deckers et al, 1997). Rotarod test of Dunham and Miya (1957) is the most commonly used screening test to estimate the neurological deficit in experimental animals, which show the motor impairment such as muscle incoordination or relaxation (Löscher, Nolting and Fassnender, 1990). Mice receiving either NSS or PEG400 were able to maintain their equilibrium on the rotating rod. However, neurologlcal deficit in a dose dependent manner was noted in experimental animal being intraperitoneally injected by either CU-17-06 or ameltolide. As shown in Table 7, the median neurotoxic dose (TD_{50}) of CU-17-06 and ameltolide were 323.59 and 9.09 mg/kg B.W.,respectively resulting in protective index ($PI=TD_{50}/ ED_{50}$) of 4.16 and 9 for CU-17-06 and ameltolide, respectively. Therefore, it can be anticipated that therapeutic dose of both compounds should produce acceptable neurological deficit in terms of motor impairment. Since it has been previously proposed that compounds with an estimated PI of at least 2 in the MES model should be further evaluated (Löscher and Nolting, 1990), the present finding renders such as opportunity for both compounds.

Most AEDs exerts their antiepileptic properties through a few neurochemical mechanisms that are meanwhile basic pathophysiological mechanisms thought to cause seizures. The activity of numerous drugs is associated with an increase in GABAergic activity. Another group of drugs decreases excitatory mechanism, through the inhibition of ionic channels, or through a decrease in the activity of excitatory neurotransmitters. This understanding may make it possible to develop a group of respective agonists or antagonists with intended antiepileptic actions (Engelnorghs et al, 2000; Moshe, 2000; Graeme and Martin, 2001; and Saidon, 2003).

In the present studies, CU-17-06 and ameltolide in the dose of 2 times of their respective ED (155.24 and 2 mg/kg B.W., respectively) did not significantly decrease the total amount of glutamate and aspartate (Figure14 and 15). Furthermore, no significant change in the levels of glycine and GABA (Figure 16 and 17) was exhibited by both compounds. Based on the result of the ability of ameltolide to displace[^{3}H]flunitrazepam from the BZD receptor produced only 15.3% inhibition at concentration up to 500 μM and ameltolide had no significant effect on [^{3}H]GABA binding in concentrations up to 250μM. It is unlikely that modulation at postsynaptic of GABA plays an important role in anticonvulsant activity of ameltolide and probably CU-17-06 as well.

Regarding to postsynaptic modulation at NMDA receptor, previous studied of Bhuthabthim(2002) demonstrated that ameltolide and NTQ (N-(*p*-aminobenzoly)-1,2,3,4-tetrahydroquinoline), a newly synthesized ameltolide analog significantly inhibited NMDA-induced current demonstrating IC_{50} of 0.10 and 0.12 μM, respectively. While both of them did induce neither current nor a shift in membrane potential of *Xenopus* oocyte. Therefore it is suggestive that inhibition of excitation of NMDA receptor may, at least in part, accounted for anticonvulsant activity in animal models of ameltolide, though, precise mechanism of action remain unknown. In addition to inhibitory effect of ameltolide on NMDA receptor, it is tempting to study further its effect on Na^+ channel.

In conclusion, the present studies demonstrated a rather selective anticonvulsant activity of a new ameltolide analog, CU-17-06. Similar to its parent compound, CU-17-06 was ineffective in PTZ model. Considering from rather high ratio between LD_{50} and ED_{50} ,CU-17-06 should be rather safe in therapeutic dose. Furthermore, CU-17-06 seemed to possess the same mode of anticonvulsant mechanism as those of ameltolide as reflected by ability of CU-17-06 to produce anticonvulsant activity in MES but not PTZ model as well as the finding that CU-17-06 and ameltolide did not exhibit any effect on the levels of cortical amino acid neurotransmitters.

Further structural modification of CU-17-06 may lead to a discovery of new ameltolide analogs with favorable pharmacological and toxicological properties.