CHAPTER 4
DISCUSSION AND CONCLUSION

4.1 Discussion

This is the first report on the quantitative analysis of ganoderic acids A and F in various Ling Zhi preparations available in Thailand and their pharmacokinetic study after a single oral administration of MG2FB-WE in healthy Thai male volunteers. Results of quantitative study of ganoderic acids A and F demonstrated that the contents of both ganoderic acids varied considerably among Ling Zhi preparations. It ranged from below the LOQ to a remarkably high content. On the basis of the extraction method, the content of active ingredients in extracts is generally significantly greater than that in raw materials. Therefore, it was not surprising that highest contents of total ganoderic acids were those of 100% Ling Zhi extract namely NPN, MG2FB-WE, and DXN-r, respectively. However, this is not always true, since some Ling Zhi extract preparations in this study, e.g., GEC (100% Ling Zhi mycelium extract), DXN-g (100% Ling Zhi mycelium and sprout extract) and BNR (60% Ling Zhi water extract) demonstrated only a negligible content of ganoderic acids. These findings might result from at least two possibilities. Firstly, Ling Zhi mycelium and/or sprout might contain a low level of ganoderic acids compared to Ling Zhi fruiting bodies. Therefore, study to determine the contents of ganoderic acids in the different parts of Ling Zhi should be further investigated. Secondly, variation in contents of ganoderic acids might depend on Ling Zhi strains, cultivating condition and areas, extraction procedure, or manufacturing processes [76]. Nonetheless, our data mandate that the pharmaceutical manufacturer should pay more attention to quality control of biologically active compounds present in raw materials. Indeed, raw materials should be screened not only for total ganoderic acids or triterpenes, but also for total polysaccharides if the immunomodulating effect of Ling Zhi is the main marketing issue. Furthermore, the content of biologically active compounds should be labeled on the packaging of commercial Ling Zhi products in order to help consumers to make decision based on cost-effective context.

The pharmacological activities of ganoderic acid A are different from those of ganoderic acid F. Ganoderic acid A has been reported to exert anti-nociceptive [8], anti-oxidative [9], FPT inhibitory [10], hepatoprotective [11, 12], and anti-cancer activities [14, 15], whereas ganoderic acid F demonstrates anti-hypertensive [7] and anti-cancer activities [13, 14, 16]. The present study revealed that ganoderic acid A was the major compound in most Ling Zhi preparations, except NPN in which ganoderic acid F was the major compound. The discrepancy in major biologically active compounds might contribute to the difference in pharmacological effects as well as clinical applications of each Ling Zhi preparation.

Dosage forms of Ling Zhi are also an important factor affecting orally administered doses of ganoderic acids. Based on the equivalent dose of ganoderic acids present in different Ling Zhi preparations, the dosage forms of capsule, granule, and solution prepared from instant tea powder theoretically seem to provide a greater oral dose of ganoderic acids compared to a solution prepared from a tea bag or decoction prepared from fruiting bodies since preparation of tea (using tea bag) or decoction usually causes incomplete dissolution of ganoderic acids into the solutions. In our preliminary experiments, immersion of 1 sachet of DHP-t tea bag (2 g) containing 3,477 μg of ganoderic acids (A and F) in 100 mL hot water for 4 min yielded a tea solution containing only 1,600 μg of ganoderic acids per serving. Similarly, decoction of 10 g of sliced MG2FB containing 10,045 μg of ganoderic acids in 500 mL hot water for 30 min provided a decoction containing 5,227 μg of total ganoderic acids per 500 mL serving size. This finding indicates that only approximately 50% of ganoderic acids in the tea bag or sliced fruiting bodies could be dissolved into the solutions during the preparation process. Therefore, in some clinical setting in which a high dose of ganoderic acids is warranted (such as in the treatment of cancers), oral administration of Ling Zhi extract in the dosage form of capsule, granule or instant tea, containing sufficiently high contents of ganoderic acids, is preferred.

Owing to the pharmacokinetic investigation of ganoderic acids in human has not yet been available, we performed the pharmacokinetic analysis of ganoderic acids A and F after a single oral administration of MG2FB-WE in healthy volunteers. Since the study design of this pharmacokinetic study was similar to that of bioequivalence

testing, the minimum number of 12 subjects were enrolled in the study according to the guideline on investigation of bioequivalence [105]. Additionally, a two-phase crossover study was conducted in order to minimize subject variability between fasted and fed conditions.

The MG2FB-WE used in our pharmacokinetic study is currently under intensive investigation at the Faculty of Medicine, CMU, for its efficacy in treatment of advanced gynecologic and other advanced-stage cancers using a dosage of 3,000 mg twice daily (6,000 mg/day) for 3 months. This dosage was selected in accordance to the study previously reported by Gao *et al* [91] exhibiting that oral administration of 5,400 mg/day of Ling Zhi extract for 12 wk significantly enhances the immune responses in patients with advanced-stage cancers. Indeed, MG2FB-WE used in the ongoing clinical trials was prepared as granular formulation dissolved in 200 mL of warm water before oral administration. This formulation has proved to be easier and more acceptable for the cancer patients to consume than other dosage forms, such as a single dose of 6 capsules (500 mg/capsule) each time. Therefore, a single dosage of 3,000 mg of MG2FB-WE in granular formulation was investigated in the present study based on the dosage and formulation used in the ongoing clinical trials mentioned above. In addition, the granular formulation was considered to be superior to other formulations (capsules and tablets) in this pharmacokinetic study because the granules are readily dissolved and absorbed without necessity to evaluate for its dissolution and disintegration profiles, which are the major confounding factors during an absorptive phase.

The measurement of plasma ganoderic acids A and F was performed by using the LC-MS method due to its rapid (runtime of 20 min) and high sensitivity (LLOQ of 0.50 ng/mL) in comparison to longer runtime (runtime of 60 min) and lower sensitivity (LLOQ of 2.50 μg/mL) by HPLC technique in our preliminary experiments. The results of validation of LC-MS assay demonstrated a wide linear range (0.50-20.00 ng/mL), validity in precision, accuracy, recovery as well as stability following the U.S. FDA guidance, thus showing the suitability of this method for analysis of ganoderic acids A and F in plasma samples.

According to plasma concentration-time curves under the fasted condition, ganoderic acids A and F could be detected in the plasma as early as 5-10 min after an

oral administration and reached their T_{max} at approximately 30 min. Both ganoderic acids A and F had a very short elimination $t_{1/2}$ of 37.20 min and 28.80 min, respectively. These findings are in agreement with the previously reported pharmacokinetic parameters in animals that revealed rapid absorption and elimination of *G. lucidum* triterpenes as evidenced by a T_{max} value range from 18-110 min and elimination $t_{1/2}$ of 35-143 min [77, 79, 106]. AUC of ganoderic acids A and F were low in spite of large dose of Ling Zhi preparation containing relatively high contents of ganoderic acids A (4253.40 μg) and F (642.75 μg) was administered. This data suggests low oral bioavailability of ganoderic acids A and F, which was consistent to the bioavailability of approximately 10% of ganoderic acid A reported in previous studies [77]. We postulated that the relatively low oral bioavailability of ganoderic acids A and F could probably not be resulted from the poor absorption from gastrointestinal tract because their absorption appeared to be very rapid. However, this poor oral bioavailability might be due to their extensive hepatic first-pass metabolism coupled with partial conversion of some triterpenes to their metabolites by intestinal bacteria as reported in rat feces, but not in plasma and urine [106]. Further studies should be investigated to identify the exact mechanisms involving in this poor oral bioavailability.

It is well known that food may positively or negatively affect the rate and/or extent of bioavailability of various drugs [107-110]. This study revealed that food caused a significant decrease in C_{max} and rate of absorption (T_{max}) of ganoderic acid A, but not the extent (AUC) of absorption. Since it is established that most drugs are ordinarily absorbed from the small intestine and delayed gastric emptying will delay absorption of those drugs that are absorbed predominantly from the small intestine [107, 109], we postulated that food affected rate but not extent of ganoderic acid A absorption through slowing of gastric emptying. Indeed, many dietary factors, such as solid food, high-fat content and high osmolarity, have been found to delay gastric emptying [107-109]. Nonetheless, concomitant food administration also significantly prolonged the $t_{1/2}$ of ganoderic acid A. This finding was presumably resulted from the delayed ganoderic acid A absorption due to prolonged gastric emptying by food, yielding sustained plasma levels and distorted the terminal $t_{1/2}$ under fed condition.

The absorption of ganoderic acid F was probably affected by food in the same manner as that of ganoderic acid A. Since the concentrations of ganoderic acid F were already low under fasted condition, the effect of food would then impede the absorption to the point that its plasma concentrations were lower than the LLOQ. Its pharmacokinetic parameters, likewise, could not be assessed. Owing to food intake generally impairs the rate and/or extent of ganoderic acids and perhaps other triterpenes, we recommend that Ling Zhi preparations should be taken on an empty stomach whenever possible.

Several *in vitro* studies have demonstrated that cytotoxicity against various human cancer cell lines expressed as IC_{50} values are in the range of 9.47-26.50 μM (approximately 4,900-13,700 ng/mL) for ganoderic acid A [14] and 9.62-19.50 μM (approximately 5,500-11,000 ng/mL) for ganoderic acid F [14, 16]. These targeted concentrations are much higher than the mean C_{max} of 10.99 ± 4.02 ng/mL for ganoderic acid A and 2.57 ± 0.91 ng/mL for ganoderic acid F found in this study. Therefore, it is unlikely to achieve cytotoxic effects *in vivo* although a relatively high dose or multiple dosage regimen of Ling Zhi extract is used. However, Ling Zhi is well documented to contain over 150 types of triterpenes [3-6], and many of them have been demonstrated to possess direct anti-cancer activity through different mechanisms of action e.g., induction of cell cycle arrest and apoptosis [46, 50, 51, 56], inhibition of proliferation, migration, invasion, metastasis and angiogenesis of carcinoma cell lines [13, 15, 16, 47, 52, 53, 55]. Therefore, *in vivo* anti-cancer activity might be exerted via synergistic effects among these triterpenes and with other biologically active compounds such as immunomodulatory protein, Ling Zhi-8 [42-44]. Additionally, polysaccharide fractions might also play some additional benefits through activation of an immune response against cancer [17, 24-27].

The major limitation of present study was the limited ability of LC-MS technique to measure very low levels of ganoderic acid F in plasma samples, especially under fed condition, because its plasma concentrations at any time points were lower than the LLOQ value of an analytical method, being unable to establish individual plasma concentration-time data and hence calculation for pharmacokinetic parameters. Therefore, more sensitive analytical method such as LC-MS/MS or study using Ling Zhi preparation containing high content of ganoderic acid F are suggested for the

determination of human plasma ganoderic acid F and other triterpenes concentrations in future studies.

4.2 Conclusion

The contents of ganoderic acids A and F varied considerably among 19 Ling Zhi preparations and the total contents of ganoderic acids did not correlate with their prices. Ganoderic acid A was the major compound in most Ling Zhi preparations, except one preparation of which ganoderic acid F was the major compound. In pharmacokinetic study investigating a single oral administration of MG2FB-WE under fasted condition, both ganoderic acids reached their T_{max} at approximately 30 min. Ganoderic acids A and F had a very short elimination $t_{1/2}$ of 37.20 min and 28.80 min, respectively. The single oral administration of MG2FB-WE under fed condition resulted in significantly delayed rate but no influence on the extent of ganoderic acid A absorption, whereas concomitant food intake markedly impeded both rate and extent of ganoderic acid F absorption.