CHAPTER I

INTRODUCTION

Temozolomide (TMZ) is an imidazotetrazine derivative of the alkylating agent dacarbazine, which can degrade in physiological fluid to generate the cytotoxic methylating agent, MTIC (5(3-methyl-1-triazeno) imidazole-4-carboxamide), which subsequently fragments to yield the DNA-methylating agent, methyldiazonium (Tsang *et al.,* 1991). TMZ exhibits schedule-dependent antineoplastic activity by inhibiting DNA replication through methylating nucleotide bases to generate a number of methyl adducts such as N-methylpurines, N^3-methyladenine and O^6-methylguanine. Among them, O^6-methylguanine is primarily responsible for the antitumour activity, since tumour cell sensitivity inversely correlates with the levels of O^6-alkylguanine DNA alkyltransferase, and requires an intact mismatch repair system (Dean *et al.*, 1992). In preclinical testing, TMZ has shown a broad spectrum of antineoplastic activity, (Steven *et al.*, 1987; Raymond *et al.*, 1997) and has demonstrated similar clinical responses to procarbazine and dacarbazine in malignant glioma and melanoma (Darkes, 2002). It is currently indicated for the treatment of malignant glioma and is available as a capsule for oral administration. Phase II trials of TMZ have confirmed that it has significant activity in patients with metastatic malignant melanoma *via* oral administration (Bleehen *et al.*, 1995). Recently, a phase III trial indicated that TMZ is as effective as dacarbazine in patients with advanced metastatic melanoma (Middleton *et al.*, 2000) with favorable side effects. Furthermore a recent study revealed that the combination regimen of TMZ with thalidomide offers an effective therapy for intra-cranial metastases as well as long-term control of metastatic melanoma (Hwu *et al.*, 2001).

Generally TMZ is well tolerated and common adverse effects are manageable. However, in addition to nausea and vomiting, TMZ has been shown to have dose-limiting myelotoxicity following oral administration (Su *et al.*, 2004) and haematological toxicity often causes concern and occasionally disrupts treatment (Bent *et al.*, 2003). Therefore, it is useful to examine other potential routes of delivery. However, poor solubility in both aqueous and organic media has resulted in serious difficulties for pharmaceutical studies and exploration of alternative delivery routes. As a result, only a limited number of studies have been carried out with attempts to reduce the toxicity *via* intrathecal delivery of a solubilised formulation (Sampson *et al.*, 1999) and intracerebral microinfusion (Heimberger *et al.*, 2000) of TMZ.

Skin delivery approach using TMZ ester derivatives was recently proposed as a potential route especially for the treatment of metastasis melanoma. It was found that among the esters, temozolomide hexyl ester (TMZ-HE) demonstrated an adequate balance of skin permeation and retention by means of the promising permeability coefficient (K_p) and flux (J_{ss}) values (Suppasansatorn *et al.,* 2006). During the permeation studies on rat and human skin, this agent was extensive hydrolysed and generated biological active TMZA. With only one publication shown that TMZA is a biological active compound exerted an equi-cytotoxicity as TMZ against TLX5 lymphoma cells *in vitro* (Tsang *et al.,* 1990). Furthermore, bioactivity of TMZ-HE has not yet been investigated. It is therefore of great interest to evaluate the cytotoxicity of TMZ-HE and TMZA against a variety of cancer cell lines, particularly melanoma and glioma cell lines in comparison with TMZ.

In this project, the cytotoxicity testing of TMZ, TMZA and TMZ-HE was performed on a variety of cancer cell lines. Topical application of 5 % (w/v) TMZ-HE in DMSO solution was also tested on solid tumor of BALB/c nude mice, which were inoculated with MV3 human melanoma. Finally, topical formulation development of TMZ-HE was studies. With one exception of dimethylsulfoxide (DMSO) as a solvent, TMZ and TMZ-HE has a little solubility in both aqueous and organic solvent. Microemulsions (ME) were proposed as they are promising vehicles for skin delivery of drugs, demonstrating high drug loading capacity and a penetration enhancer effect (Kreilgaard 2002). As a starting point, Vitamin E-TPGS (VE-TGPS) was chosen as a surfactant, oleic acid (OA) or isopropyl myristate (IPM) as an oil phase and isopropyl alcohol (IPA) as co-surfactant where appropriate. A gel VE-TPGS ME system was developed and a number of formulations were prepared. The ME system and formulations were characterized using polarization microscopy and freeze fracture electron microscopy (FFEM). *In vitro* permeation of TMZ-HE from the ME formulations was studied using silicone membrane for 8 h and full-thickness hairless mice skin for 24 h with Franz diffusion cells.

The objectives of this study were; 1) to evaluate whether TMZ-HE could be a candidate for development into an anti-skin cancer drug through skin delivery by assessing its cytotoxicity against a panel of cancer cell lines *in vitro*, and *in vivo* by using its DMSO solution on an animal model (nude mice); 2) to choose available pharmaceutical excipients to form microemulsion for assessing whether microemulsion could be a potential vehicle for delivering TMZ-HE topically.