CHAPTER I

GENERAL INTRODUCTION

Introduction to polyphenols

Over the past decades, researchers searching for new drugs to use in oncology have refocused on natural polyphenolic compounds. Phenolic compounds, or polyphenols, constitute one of the most numerous and widely distributed groups of substances in the plant kingdom, with more than 8,000 phenolic structures currently known. "Phenolic compound" is standed for encompass molecules that possess an aromatic ring bearing one or more hydroxyl substituents. Natural polyphenols can range from simple molecules such as phenolic acid, flavonoids and large highly polymerized compounds such as tannins. Conjugated forms of polyphenols are the most common, where various sugar molecules, organic acids and lipids are linked with the phenolic ring structure (Harborne, 1980).

Health benefits of polyphenols

Flavonoids exert a wide range of biological activities including antioxidant, anticarcinogenic, antiproliferative, and antiviral actions (Aherne and O'Brien, 1999; Formica and Regelson, 1995; Csokay *et al.*, 1997). Moreover, flavonoids such as genistein, apigenin, and quercetin, are also known as apoptotic inducers (Wang *et al.*, 1999; Yin *et al.*, 1999; Choi *et al.*, 2001) and MDR modulators (Conseil *et al.*, 1998; De Wet *et al.*, 2001). Among bioflavonoids, quercetin frequently uses for testing the pharmacological properties. The potential beneficial use of quercetin in preventing ischemia/reperfusion-induced myocardial damage by reactive oxygen species has

been reported. By using a normal cell such as H9c2 cardiomyoblast cell, quercetin could protect hydrogen peroxide from inducing H9c2 cardiomyoblast cells from undergoing apoptosis (Park *et al.*, 2003). It was also reported that quercetin showed a higher value of antioxidant activity than Vitamin C, Vitamin E and β-carotene on a molar basis (Rice-Evans *et al.*, 1995) and probably due to the antioxidant action, it prevented the generation of reactive oxygen species by cyclosporine and thereby suppressed the cyclosporine-induced nephrotoxicity (Satyanarayana *et al.*, 2001). This is strong evidence suggesting that quercetin is safe and has potential for exploring their *in vivo* toxicity.

Potential use of polyphenolic compounds in cancer prevention

Polyphenols are demonstrated to be beneficial against degenerative conditions such as cardiovascular disease and carcinogenesis (Manach *et al.*, 2005; Chung *et al.*, 2003). The cancer prevention effects of dietary polyphenols are also the subjects studied by many research groups (Gurjeet *et al.* 2006, Dolara *et al.*, 2005; Kris-Etherton *et al.*, 2002). Dolara P *et al.* reported that polyphenols from red wine inhibit the process of chemical colon carcinogenesis in rodents, modify colon microbial ecology, reduce colonic mucosa DNA oxidation and have complicated effects on gene regulation, possibly affecting the mucosal response to inflammatory and carcinogenic agents. It is not clear at present how the observed variations in gene regulation are specifically connected to protection from oxidative damage and/or inhibition of carcinogenesis.

Potential use of polyphenolic compounds in cancer intervention

According to the recent studies, polyphenol is considered as an effective general inhibitor of cancer cell growth, and induces apoptosis against various cancer cell lines including human colon carcinoma cells (Wenzel *et al.*, 2000), human prostate cancer cell lines (Romero *et al.*, 2002; Hsieh *et al.* 1999). The relationship of chemical structures of polyphenols and anticancer has been widely studied with *in vitro* and *in vivo* system, and polyphenol concentrations required for anticancer effects vary depending on the types of cancer cell lines (Kothan, 2004).

Recently it was reported that quercetin (10 μM, at 1 h after exposure) induced apoptosis in a concentration- and time-dependent manner against in drug-sensitive K562, drug-resistant K562/*adr*, drug-sensitive GLC4 and drug-resistant GLC4/*adr* cells. At such a low quercetin concentration as 10 μM, an increase followed by a decrease in $|\Delta\Psi_m|$ value associated with an induction of apoptosis was detected at 1h (Kothan *et al.,* 2004). In addition, it was also reported that quercetin (60 μM) induced apoptosis in HL-60 cells via decrease in $\Delta\Psi_m$ due to cytochrome c release and induction of caspase-9 processing (Wang *et al.*, 1999; Murphy *et al.*, 1999). In fact quercetin provoked its cytotoxicity at mitochondria level, impairing mitochondrial energetic state followed by an induction of apoptosis and inhibition of cancer cell growth (Kothan *et al.*, 2004).

Cellular drug resistance in cancer chemotherapy

Multidrug resistance (MDR) or intrinsic resistance of tumor cells to anticancer agents remains a major cause of treatment failure in cancer therapy. MDR phenomenon is always associated with a decreased intracellular accumulation of anticancer drugs, consequently there is a decline in the therapeutic effect due to an

over-expression of MDR protein transporters including P-glycoprotein (Pgp) (Gottesman *et al.*, 2002), multidrug resistance-associated protein (MRP) (Young *et al.*, 2001) and lung resistance-related protein (LRP) (Rybarova *et al.*, 2004). MDR is always multifactorial. Many research groups reported that a modification of cellular anticancer drug distribution might be perturbed by an altered pH gradient across different cell compartments. Particularly, acidic organelles such lysosomal sequestration following enhanced exocytosis, which favors a reduced intracellular accumulation of antineoplastic drugs, reducing efficiency, was characterized in various MDR cell types (Miraglia *et al.*, 2005; Altan *et al.*, 1998). Although the combination of Pgp-mediated flux and the intracellular drug sequestration governed the MDR phenotype was not considered by those researchers.

Polyphenols modulate the cellular energetic state

Several studies revealed that MDR cells need more cellular ATP than that of their corresponding sensitive cells (Miccadei *et al.*, 1996; Dorward *et al.*, 1997; Mieminen *et al.*, 1994; Jia *et al.*, 1996). A strong evidence is that ATP depletion in MDR cells, even partial, will block P-glycoprotein and MRP1 pump activity leading to an increase in cellular drug accumulation (Versantvoort *et al.*, 1992; Mankhetkorn *et al.*, 1996). Indeed, to understand the source of cellular ATP production and cellular energetic state of MDR cells is crucial data to overcome MDR phenomena. In other words, we must get insight how the important role of mitochondria play in the MDR phenotype since they supply the cellular ATP pool.

The mitochondrial membrane potential ($\Delta\Psi_m$) is a sensitive indicator which indicates the energetic state of mitochondria and cells. An increase followed by a decrease in $|\Delta\Psi_m|$ value was associated to an induction of apoptosis (Kothan, 2004).

In fact, some flavonoids induced apoptosis in HL-60, K562 and K562/*adr* cells via decrease in $\Delta\Psi_m$ due to cytochrome c release and induction of caspase-9 processing (Wang *et al.*, 1999; Murphy, 1999). It can be noted that with increasing concentrations of quercetin, the $|\Delta\Psi_m|$ decreases in very narrow range (from 160 ± 1.0 mV to 150 ± 0.6 mV for K562 and from 145 ± 1.2 mV to 135 ± 1.1 mV in K562/*adr*), whereas the percentage of early apoptotic cells increases in greater degree of range (from 1.5 ± 0.4% to 45 ± 3.2%)(Kothan, 2004).

Polyphenols as inhibitors of MDR transporters

Experimental approaches aiming to determine the direct interaction between flavonoids and P-glycoprotein were studied by many research groups. On one hand, Conseil *et al.* (1998) studied the fixation of flavonoids at vicinal ATP-binding site. They measured the resonance-energy transfer of tryptophan-intrinsic fluorescence of H6-NBD2, a highly soluble recombinant protein, from mouse P-glycoprotein and flavonoids (Conseil *et al.,* 1998). Similar results were obtained from the series of experiments dealing with the inhibition the photolabelling of ATP analogues on the ATP-binding site within the C-terminal nucleotide-binding domain of mouse P-glycoprotein using 30 flavonoids (De Wet *et al.* 2001). De Wet *et al.* (2001) showed the structure-activity relationships of 30 flavonoids on their ability to bind to the vicinal ATP- and steroid-binding site. On the other hand, Phang *et al.* (1993) reported that flavonols (quercetin, kaempferol and galangin) were potent stimulators of the Pgp-mediated efflux of 7, 12-dimethylbenz (a)-anthracence in multidrug-resistant breast cancer cells (Phang *et al.*, 1993). Consistently with previously cited data, Critchfield *et al.* (1994) found that galangin, kaempferol and quercetin reduced [^{14}C]

ADR accumulation and that phenomenon was blocked by verapamil, vinblastine and quinidine in HCT-15 colon cells (Critchfield *et al.*, 1994).

As previously mentioned, almost reports were obtained from the series of experiments performed using both cancer cell lines and purified P-glycoprotein. In order to investigate the potential use of polyphenols as anticancer agents, their interaction with normal cells as well as cancer cells at cell culture laboratory and in animal models are essential.

OBJECTIVES

1. The aim of a first set of experiments is to study the anticancer and apoptosis-inducing activities of the Siamois® red wine polyphenols. Several experiments were performed including:
    - Investigating the antiproliferation and resistance reversion of compounds (by cytotoxic assay in drug-sensitive and drug-resistant cells) compared with normal myocytes
    - Investigating the effects of quercetin, the most abundant plant flavonoids on the mitochondrial function.
    - Investigating the apoptosis-inducing activities of these polyphenols.
2. The second set of experiments is to investigate the aptitude of the polyphenols to induce apoptosis of cancer cells *in vitro* and *in vivo* situations by using molecular imaging and histochemical techniques.

The organization of the thesis dissertation

This study provides an original of results and new methodology which allows earning more insight the molecular and cellular interaction of polyphenols, particularly in drug-sensitive and drug-resistant cells. The outputs of this thesis dissertation are:

1. Red wine polyphenols promote normal myocyte growth but exhibit anticancer and apoptosis-inducing activities against cancer multidrug-resistant cells. *Submitted to Journal of Nutrition and Cancer (2006).*
2. Quercetin, extracts of mamoa wood and Siamois® red wine induce apoptosis in human breast cancer MDA-MB-435 cells xenografts *in vivo*. *Submitted to Journal of Cancer Biology and Therapy (2006).*
3. P-glycoprotein-mediated efflux and lysosomal sequestration of drugs confer advantages of K562 MDR sublines to survive prolonged exposure to cytotoxic agents. *Submitted to Indian Journal of Biomedical Sciences (2006).*
4. Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2', 7'-dichlorofluorescein diacetate assay. *Published in Journal of Radiation Physics and Chemistry, (2005) 72: 323–331.*
5. Spontaneous mitochondrial membrane potential change during apoptotic induction by quercetin in K562 and K562/*adr* cells. *Published in Canadian Journal of Pharmacology and Physiology, (2004) 82: 1084–1090.*

The thesis was edited and presented in article research formats, chronologically demonstrated from the determination of potential intracellular target of polyphenols, their interaction with normal myocytes and cancer cells, the extension of anticancer action from *in vitro* to *in vivo* studies, and two article presented as appendix, including the determination of intracellular active oxygen species and role of P-glycoprotein-mediated efflux and lysosomal sequestration in MDR cells, respectively.

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