Title: USE OF GINSENSOIDS Rh2 & Rg3, AND AGLYCON GINSENSOIDS FOR THE PREVENTION OF CANCER

Effects of carenseng and its major components on CYP3A4

- Careseng2131
- Rh2
- PPD
- PPT
- Rg3

Abstract: This invention pertains to a method of preventing cancer in humans or other animals by administering to the human or other animals ginsenosides Rh2, Rh3, aglycon ginsenosides, or any combination thereof. These ginsenosides and aglycon ginsenosides prevent or reduce the chances of developing cancer or precancerous malignancies by inhibiting the activities of cytochrome p450 enzymes. This invention also pertains to pharmaceutical and nonpharmaceutical compositions for the prevention of cancer in humans or other animals.
Use of Ginsenosides Rh2 & Rg3, and Aglycon Ginsenosides for the Prevention of Cancer

FIELD OF THE INVENTION

The present invention is directed to ginsenosides Rh2 and Rg3, as well as aglycon ginsenosides including aglycon protopanaxadiol (aPPD) and aglycon protopanaxatriol (aPPT), or any combinations of the above for the prevention of cancers. More specifically, the present invention relates to the use of ginsenosides Rh2 and Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of the above to reduce the production of carcinogens in the body by inhibiting certain members of the cytochrome P450 superfamily.

BACKGROUND

Carcinogens

A carcinogen is a substance that causes cancer, such as aflatoxin B1, PAHs, NNK, and 6-aminochrysene. (10th Report on Carcinogens, http://ehp.niehs.nih.gov/roc/toc10.html)

Aflatoxin B1

Aflatoxins are toxic and carcinogenic. Aflatoxin B1 is one of the most toxic compounds known to cause cancer. It is formed commonly in crop plant materials held at relatively high moisture and temperature for long periods. At lower levels and following prolonged exposure in humans, aflatoxins can cause liver cancer. Toxicsis only takes place after consumption of the contaminated plant materials. Animals tend to avoid contaminated feed, but as B1 is so highly toxic, even large animals can be killed by small, almost undetectable quantities.

PAHs

Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 different chemicals that are formed during the incomplete burning of coal, oil and gas, garbage, or other organic substances like tobacco or charbroiled meat. PAHs are usually found as a mixture containing two or more of these compounds, such as soot. PAHs can cause harmful effects on the skin
and in body fluids, and can affect the body’s ability to fight disease after both short- and long-term exposure.

The Department of Health and Human Services of USA (DHHS) has determined that some PAHs may reasonably be expected to be carcinogens. Some people who have breathed or touched mixtures of PAHs and other chemicals for long periods of time have developed cancer. Some PAHs have caused cancer in laboratory animals when they breathed air containing PAHs (lung cancer), ingested PAHs in food (stomach cancer), or had PAHs applied to their skin (skin cancer).

NNK, 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE

4-(Methylamino)-1-(3-pyridyl)-1-butane (NNK) has been found in a variety of tobacco products (chewing tobacco, snuff, cigarettes and cigars), in mainstream and sidestream smoke from cigars and cigarettes, in the saliva of chewers of betel quid with tobacco, and in the saliva of oral-snuff users. Some of the NNK in saliva appears to be formed endogenously from salivary nitrate and nicotine. Thus, there is widespread exposure to NNK among users of tobacco products and those exposed to sidestream smoke.

NNK was tested for carcinogenicity in several studies by subcutaneous injection in rats and hamsters and by intraperitoneal injection in mice. In rats, it induced carcinomas of the nasal cavity, lung and liver, with a clear dose-response relationship. In hamsters, it induced benign and malignant tumours of the nasal cavity, trachea and lung, even after only a single administration. In mice, NNK and its metabolites 4-(methylamino)-1-(3-pyridyl-N-oxide)-1-butane and 4-(methylamino)-1-(3-pyridyl)butan-1-ol induced benign and malignant tumours of the lung. NNK and its metabolites can cross the placental barrier in mice. NNK can be metabolically activated by mouse foetal tissues. Administration of NNK to rats results in abnormal DNA methylation in the liver and the lungs. NNK is mutagenic to Salmonella typhimurium in the presence of an exogenous metabolic system. It induces unscheduled DNA synthesis in primary cultures of rat hepatocytes.

6-aminochrysene

6-aminochrysene is one of the amino-substituted PAHs proven to be potential liver and lung carcinogens. Studies have shown that 6-aminochrysene can cause DNA single strand cleavage to form a carcinogen-DNA adduct.
P450 Superfamily and Activation of Carcinogens

P450 Superfamily

Cytochrome P450s are now known to comprise an ancient superfamily of mostly microsomal and mitochondrial heme-thiolate proteins. Having been found in every major domain of living organisms, Bacteria, Archaea and Eukarya, modern cytochrome P450s are believed to originate from an ancestral gene that existed approximately three and a half billion years ago. More than 2000 identified cytochrome P450 genomic and cDNA sequences have been divided into a total of 265 different families. Multiple cytochrome P450 genes can be expressed simultaneously and the number of genes per species is highly variable with a tendency for higher eukaryotes to possess large numbers of paralogously-related sequences. The completion of several genome sequencing projects has revealed that yeasts have up to three cytochrome P450 genes while Caenorhabditis elegans has 80 cytochrome P450 genes (6 of which appear to be pseudogenes). Drosophila melanogaster has 90 cytochrome P450 genes, 6 of which appear to be pseudogenes, and Homo sapiens have 75 cytochrome P450 genes, including 19 predicted pseudogenes. It is among plants, however, that the multiplicity of the cytochrome P450 gene family is most evident. To date, a minimum of 224 cytochrome P450 genes, including 52 predicted pseudogenes, have been identified in the genome of rice (Oryza sativa) and in Arabidopsis thaliana, for which 100% of the genome has been sequenced, a record 273 cytochrome P450 genes, including 26 predicted pseudogenes, have been identified.

Bioactivities of Different P450s

The cytochrome P450 superfamily is a group of enzymes that are responsible for metabolizing many endogenous and exogenous substances.

Prokaryotic cytochrome P450s participate in the biosynthesis of antiboiotics. Fungal cytochrome P450s are required to synthesize ergosterol and facilitate phatogenesis by detoxifying the chemical defenses of target host cells. In plants, cytochrome P450s serve a wide range of functions including phytohormone, petal pigment and phytoalexin biosynthesis. In arthropods, cytochrome P450s play important roles in metabolic resistance to pesticides and in development and reproduction. Cytochrome P450s are key players in mammalian drug
metabolism, steroidogenesis, the metabolism of fatty acids and the conversion of procarcinogens and promutagens to deleterious genotoxic compounds.

The CYP enzymes are the principle cytochrome P450 enzymes which metabolize and inactivate drugs, and in some cases activate them to active, toxic or carcinogenic products. It has been reported that certain cytochrome P450s can activate carcinogens which induce cancers. For example, carcinogens which induce bladder cancer are converted by hepatic drug metabolizing enzymes to products that are excreted and concentrated in urine. A ten-fold variation in expression was observed for cytochrome P450 CYP3A4, with similar ranges in normal mucosa and cancer tissue. P450 CYP2C9 and CYP2C19 were intermittently expressed. Those that are expressed have a potential for contributing to carcinogenesis of bladder cancer in man. (M. Romkes-Sparks, Mrna expression of individual cytochrome P450s in bladder mucosal tissue in man: potential for local production of carcinogene, Published in Cancer Detection and Prevention 1993; 17(1)).

Certain Cytochrome P450s (CYP3A4, CYP2D6, CYP2C9, CYP2C19 And CYP2B6) Can Cause Precancerous Malignancies

Many cytochrome P450s, including CYP3A4, CYP2D6, CYP2C9, CYP2C19 and CYP2B6, activate procarcinogens to carcinogens, such as aflatoxin B1, PAHs, NNK, and 6-aminochrysene, that cause carcinogenesis.

CYP3A4

The CYP3A4 enzyme is the most important drug-metabolizing CYP in human livers. About 30-40% of the total hepatic CYP content consists of CYP3A4 (Shimada et al. 1994, Imaoka et al. 1996) and it is also present in small intestines (Kolars et al. 1992). It has been estimated that about 50% of the drugs metabolized by CYPs are metabolized by CYP3A4 (Bertz & Granneman 1997). The substrates for this enzyme include drugs, such as quinidine, nifedipine, diltiazem, lidocaine, lovastatin, erythromycin, cyclosporin, triazolam, and midazolam, and endogenous substances, including testosterone, progesterone, and androstenedione (Pelkonen et al. 1998, Guengerich 1999). Midazolam and erythromycin have been used as in vivo probes for CYP3A4 activity (Thummel & Wilkinson 1998). CYP3A4 also activates procarcinogens, including aflatoxin B1 (Aoyama et al. 1990), PAHs, NNK (Hecht 1999), and 6-aminochrysene (Yamazaki et al. 1995). Three variant alleles have been
detected for the CYP3A4 gene. A 5′-flanking allelic variant has been associated with prostate cancer (Rebbeck et al. 1998) and leukemia (Felix et al. 1998).

**CYP2 Family**

The human CYP2 family is a heterogeneous group of enzymes. It contains the subfamilies CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, CYP2F, and CYP2J (Nelson et al. 1996). CYP2B6, CYP2D6, CYP2E1, CYP2F1, and CYP2J2 are the only functional members in their respective subfamilies, whereas the CYP2A subfamily contains two functional enzymes and CYP2C contains four functional enzymes. Unlike the CYP1 family, the members of the CYP2 family do not share features of regulation. The substrate and tissue specificities of these enzymes also differ markedly.

**CYP2B6**

CYP2B6 is a minor CYP formed in human livers, accounting for only 1-2% of total hepatic CYP (Mimura et al. 1993, Shimada et al. 1994, Imaoka et al. 1996). Its expression appears to be regulated tissue-specifically, since in the lungs and kidneys it is expressed as a splicing variant (Czerwinski et al. 1994, Nelson et al. 1996, Gervot et al. 1999). This splicing variant was previously called CYP2B7. The substrates for CYP2B6 include 6-aminochrysene (Mimura et al. 1993), methoxychlor (Dehal & Kupfer 1994), NNK (Code et al. 1997), and cyclophosphamide (Chang et al. 1993).

**CYP2C**

The human CYP2C subfamily contains four highly homologous genes: 2C8, 2C9, 2C18 and 2C19, which are located in a cluster on chromosome 10 (Gray et al. 1995, Nelson et al. 1996). These CYP enzymes are the principle enzymes which metabolize and inactivate drugs, and in some cases activate them to active, toxic or carcinogenic products. Polymorphisms in CYP2C19 affect the efficacy of the common anti-ulcer agent omeprazole, one of the ten most prescribed drugs worldwide. Individuals carrying the wild-type allele have lower blood levels which results in lower cure rates. Presence of the allele is considered a risk factor for gastric cancer. (Joyce A. Goldstein, University of Texas Southwestern Medical School, Pharmacology, 1968. http://dir.niehs.nih.gov/dirlpc/human.htm.)
CYP2C9 is the major CYP2C in human liver. It metabolizes drugs such as the anticonvulsant phenytoin, the common anticoagulant warfarin, antidiabetic agents such as glipizide and tolbutamide, and nonsteroidal anti-inflammatory drugs such as celecoxib, rofecoxib, and ibuprofen. Several drug-metabolizing enzymes such as CYP2D6, CYP2C19, CYP2C9, CYP2A6, CYP1A2, CYP1B1, CYP2A6, glutathione S-transferase M1 (GSTM1), N-acetyl transferase 2 (NAT2) are involved in the metabolism of many drugs and metabolic activation of pro-carcinogens.

*CYP2D6*

The CYP2D subfamily has one gene and four pseudogenes (Nelson et al. 1996). The CYP2D6 polymorphism was the first defect in drug metabolism to be specifically associated with altered expression of a CYP enzyme (Gonzalez et al. 1988). The CYP2D6 poor metabolizer (PM) phenotype is detected in about 6% of Caucasians (Ingelman-Sundberg et al. 1999), and it has profound effects on the metabolism of several commonly used pharmaceuticals, including several tricyclic antidepressants, haloperidol, metoprolol, propranolol, codeine, and dextromethorphan (Pelkonen et al. 1998). There are at least 30 different defective CYP2D6 alleles, six of which contribute to 95-99% of PM phenotypes (Ingelman-Sundberg et al. 1999). Interestingly, duplications of the CYP2D6 gene, up to 13 gene copies, have been reported (Johansson et al. 1993), giving rise to the ultra-rapid metabolizer phenotype. Ultra-rapid metabolizers show increased metabolism and decreased drug effects of CYP2D6 substrates, such as tricyclic antidepressants (Dalen et al. 1998).

About 4% of Caucasians have multiple CYP2D6 genes (Ingelman-Sundberg et al. 1999). It has been speculated that since CYP2D6 is not inducible, the duplications are a way to adapt to environmental chemical pressures, most likely to alkaloids in the diet (Ingelman-Sundberg et al. 1999). CYP2D6 polymorphisms have been linked to altered susceptibility to Parkinson’s disease and lung cancer. Although meta-analysis has not revealed a link to Parkinson’s disease, PM individuals seem to be somewhat protected (OR = 0.69) against lung cancer (Rostami-Hodjegan et al. 1998). CYP2D6 has a minor, but not crucial, role in the activation of tobacco-derived nitrosamine NNK (Crespi et al. 1991, Hecht 1998). It has been speculated that the CYP2D6 polymorphism might affect the risk of lung cancer through modulating smoking behavior, since CYP2D6 might be involved in the signal transduction of the dopaminergic pathway in brain (Saarikoski et al. 2000). CYP2D6 constitutes about 2% of
total hepatic CYP (Shimada et al. 1994, Imaoka et al. 1996), and the protein is also expressed in the duodenum and brain (Pelkonen & Raunio 1997).

**Ginsenosides and Ginseng Extracts**

A great deal of research has proven that ginsenosides are the main active components of ginseng, and that they have various medicinal effects. Thus, the use of ginseng has various pharmacological benefits.

Individual, isolated ginsenosides are named according to the orders of their Rf values on thin-layer chromatograms, such as Ra, Rb1, Rb2, Re, Rd, Re, and Rf. Based on their aglycons, i.e. the skeletons of their molecules, ginsenosides are classified into three types: 1) Dammarane, 2) Ocotillol, and 3) Oleanane. The tetracyclic-terpene-structured dammarane type can be further divided into two subtypes: protopanaxatriol and protopanaxadiol (Chan, But, 2000; Chen, Zhang, 1990; Cheng, Su, 1987; Corthout, Naessens, 1999; Cui, Garle, 1996; Cui, Song, 2000; Elkin, Makhankov, 1993; Kaku and Kawashima, 1980; Karikura, Miyase, 1990; Liu, Luo, 1989; Ong and Yong, 2000; Oura, Hiiai, 1975; Shao1984; Takino, Odani, 1982; Wang, Sakurna, 1999; Yang and Xu, 1987; Yip, Lau, 1985; Yoshikawa, Murakami, 1998; Zhang, Chen, 1989; Zhao and Yuan, 1993). The structures of Rh1, Rh2, and Rg3, the structural types and classification of common ginsenosides, are as follows:

It was reported that Rh1 and Rh2 could induce differentiation in B16 melanoma and F9 teratocarcinoma stem cells (Lee, Lee, 1996; Odashima, Ohta, 1985; Xia and Han, 1996). Rh2 can inhibit the proliferation of murine melanoma, human MCF-7 breast cancer cells, ovarian cancer cells, and liver cancer SK-HEP-1 cells in vitro (Kikuchi, et al. 1991 □Lee, et al. 1996 □Ota, Maeda, Odashima, Ninomiya, and Tatsuka, 1997 □Oh, et al. 1999 □Popovich and Kitts, 2002). Recent studies about the mechanism of Rh2's tumor-inhibitory actions have led to a speculation: the cancer-inhibitory effects of Rh2 may involve expression of p27kip1 (Lee, et al. 1996), p21WAF1/CIP1 (Oh, et al. 1999), and/or suppression of cyclin-dependent kinase-2 (Cdk2) (Ota, Maeda, Odashima, Ninomiya, and Tatsuka, 1997).
Ginsenoside Rg3 is very similar to Rh2 in its chemical structure (one more glucose at C-3 than Rh2), and can inhibit invasion by rat ascites hepatoma, mouse melanoma, human small-cell lung carcinoma, and human pancreatic adenocarcinoma cells \textit{in vitro} (Shinkai, Akedo, Mukai, Imamura, Isai, Kobayashi, and Kitagawa, 1996). In vivo studies have found that Rg3 can inhibit lung metastasis by highly metastatic melanoma and colon carcinoma in mice (Iishi, Tatsuta, Baba, Uehara, Nakaizumi, Shinkai, Akedo, Funai, Ishiguro, and Kitagawa, 1997). However, because of its multi-glucose chemical structure, Rg3’s cytotoxicity is much lower than that of Rh2 and sapogenins.

Having no glucose in its chemical structure, the aglycon of protopanaxadiol ginsenosides ("aPPD") has been reported to have the strong inhibitory effects on B16 melanoma cell proliferation (Ota, Maeda, Odashima, 1991) THP-1 leukemia cells (Popovich and Kitts2002), and multi-drug resistant P388/ACM leukemia cells (Hasegawa, Sung, Matsumiya, Uchiyama, Inouye, Kasai, and Yamasaki, 1995). The aglycon of protopanaxatriol ginsenosides ("aPPT") which has no glucose on its chemical structure, has also been reported to have the similar anticancer effects as PPD (Shibata, 2001 Popovich and Kitts2002 Hasegawa, Sung, Matsumiya, Uchiyama, Inouye, Kasai, and Yamasaki, 1995). The only structural difference between aPPD and aPPT is at C-6, with an "-H" for aPPD, but an "-OH" for aPPT. The two known sapogenins, aglycon protopanaxadiol and aglycon protopanaxadiol, have further been demonstrated to be effective in chemosensitizing multi-drug resistant (MDR) cancer cells when used with other chemotherapy drugs \textit{in vitro} (U.S. Patent Application No. 09/957,082).

Ginsenoside Rh2, Rg3, aPPD and aPPT are found in the plants of the ginseng family in extremely scarce amounts. For example, in Korean red ginseng, the content of Rh2 is only 0.001% (1 g of Rh2 in every 100 kg of red ginseng), and the contents of 20(S)-Rg3 and 20(R)-Rg3 in red ginseng is 0.015% and 0.014% respectively (Shibata, 2001).

**Protopanaxadiol (PPD)/Protopanaxatriol (PPT), Aglycon Ginsenosides, and Aglycon Protopanaxadiol (aPPD)/ Aglycon Protopanaxatriol (aPPT)**

There is inconsistent use of the terminology for PPD/ PPT and aPPD/ aPPT in this field.

PPD/PPT is broadly used to indicate a group of compounds with the following chemical structures, wherein the $R_1$ and $R_2$ have different components: (CHEMTECH 1998, 28(4), 26-32.)
Table 1. The ginseng saponins of protopanaxadiol.

Other diols include Rα₁, Rα₂, Rα₃, Rβ₃, Rg₅, Rh₂, Rg₁, Rg₂, Q-R₄, and mRd. All compounds are in 20S configuration except Rg₅, which is a mixture of S and R configurations. Rh₂, Rg₁, and NG-R₄ are found only in red ginseng. Glc = glucose; Ma = malonyl; Ara(p) = arabinose in pyranose form, Ara(f) = arabinose in furanose form.
Table 2. The ginseng saponins of protopanaxatriol.
All compounds have a 20S configuration except Rg₂ and Rh₁. The R forms of Rg₂ and Rh₁ may be produced during the production process and are characteristic ginsenosides of red ginseng. Rha = rhamnose; Xyl = xylose.

In this disclosure, the term “aglycon ginsenosides” is used to describe a group of ginseng sapogenins, which are totally free of glucose in their chemical structures, including, but not limited to, compounds such as aPPD, aPPT, PAM-120, PBM-110, PBM-100.

In this disclosure, the terms “aPPD” and “aPPT” are used to describe the following two specific aglycon sapogenins:

aPPD:

aPPT:

(Note: the hydroxyl group of aPPT above in position 6 may be R or S epimers.)

The terms PAM-120, PBM-110, and PBM-100 are used to describe the following specific aglycon sapogenins:

PAM-120

PBM-110
In this disclosure, the term “Combination” is used to describe any combinations of a group of two or more ginseng saponins or sapogenins, including, but not limited to, compounds such as ginsenoside Rh2, Rg3, aPPD, aPPT, PAM-120, PBM-110, and PBM-100. In this disclosure, one example of these combinations, named Careseng 2131 which is a combination of ginsenoside Rh2 (1%-10%), aPPD(20%-50%), aPPT(15%-45%) and small portion of other ginsenosides(<5%), is used.

The interaction of ginseng extracts with cytochrome P450s has been studied previously. Cytochrome P-450 metabolism can be affected by traditional Chinese medicines (including Ginseng Dao, Panax Ginseng Extractum, and Ginseng-Royal Jelly) in vitro (FOSTER et al.). Certain traditional Chinese medicines have been associated with adverse reactions in which cytochrome P-450 was thought to be involved. In vitro assays of cytochrome P450 enzymes CYP2C9, 2C19, 2D6, and 3A4 in the presence of 12 different traditional Chinese medicines, directly or as extracts. Most extracts of traditional Chinese medicines inhibited between 25 to 100% of the activity of the enzymes tested.

However, those studies were conducted using either crude ginseng extracts or ginsenosides-Rb1, -Rb2, -Rc, -Rd, -Re, Rf or -Rg1. Although the ginseng extracts had some inhibitory effect on cytochrome P450s, the individual ginsenosides did not demonstrate significant effect on cytochrome P450s (Chang, et al., Dorinovan, et al., Gurley, et al., Henderson, et al., Ioannides, Lee, et al.).

The inhibitory effects of ginsenoside Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, and combinations of these ginsenosides, on cytochrome P450s have not been reported.
SUMMARY OF THE INVENTION

An object of the present invention is to provide a use of Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, and any combinations of the above for reducing the production of carcinogens in the body by inhibiting certain cytochrome P450s.

In accordance with a first aspect of the present invention, there is provided a method of preventing cancers in humans comprising administering to humans an effective amount of Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of these compounds.

In accordance with a second aspect of the invention, there is provided a pharmaceutical composition comprising Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of these compounds useful for preventing a human subject from developing cancer.

In accordance with a third aspect of the invention, there is provided a non-pharmaceutical composition comprising Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of these compounds useful for preventing a human subject from developing cancer.

In accordance with a fourth aspect of the invention, there is provided a kit comprising one or more pharmaceutical compositions comprising Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of these compounds, useful for preventing a human subject from developing cancer.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a graphical representation of the inhibitory effects of Rh2, Rg3, aglycon ginsenosides aPPD and aPPT, and one combination of the above on the human cytochrome P450 CYP3A4.

FIG. 2 provides a graphical representation of the inhibitory effects of Rh2, aglycon ginsenosides including aPPD and aPPT, and one combination of the above on the human cytochrome P450 CYP2D6.
FIG. 3 provides a graphical representation of the inhibitory effects of Rh2, aglycon ginsenosides including aPPD and aPPT, and one combination of the above on the human cytochrome P450 CYP2C9;

FIG. 4 provides a graphical representation of the inhibitory effects of Rh2, aglycon ginsenosides including aPPD and aPPT, and one combination of the above on the human cytochrome P450 CYP2C19;

FIG. 5 provides a graphical representation of the inhibitory effects of Rh2, aglycon ginsenosides including aPPD and aPPT, and one combination of the above on the human cytochrome P450 CYP2B6.

DETAILED DESCRIPTION OF THE INVENTION

Throughout the following description, specific details are set forth in order to provide a more thorough understanding of the invention. However, the invention may be practiced without these particulars. In other instances, well known elements have not been shown or described in detail to avoid unnecessarily obscuring the invention. Accordingly, the specification and drawings are to be regarded in an illustrative, rather than a restrictive, sense.

Definition of Cancer Prevention

The fundamental difference between “cancer prevention” and “cancer treatment” is whether or not the person who receives the product has cancer when the product is administered. For the purpose of this application, “cancer prevention” is defined as the following: preventing any type of cancer or precancer malignancy from developing, or reducing the chances of any type of cancer or precancer malignancy from developing.

Methods of Cancer Prevention

Effectively inhibiting the level of carcinogen-related P450s in human tissues is one of the key issues in preventing cancers. The inventors have unexpectedly found that Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, and any combinations of these compounds, can inhibit certain members of the cytochrome P450 superfamily. More specifically, Rh2, Rg3, aglycon ginsenosides aPPD and aPPT, and combinations of these ginsenosides, have strong dose-dependent inhibitory effects on the following P450 enzymes: CYP3A4, CYP2D6,
CYP2C9, CYP2C19 and CYP2B6. These P450 iso-enzymes are known to produce carcinogens. Therefore, inhibition of these enzymes reduces the production of carcinogens in the human body. The end result is a decrease in carcinogenesis. Therefore, when P450s are inhibited, they prevent or reduce the chances of developing cancer.

The production of carcinogens in the body is widespread and occurs in almost all of the organs. Therefore, the invention disclosed herein will reduce carcinogenesis in many organs. Many types of cancer are therefore prevented.

In one embodiment, the invention pertains to a method of preventing cancer in humans or other animals. The method involves the administration of an effective amount Rh2, Rg3, aglycon ginsenosides, including aPPD, aPPT, PAM-120, PBM-100, and PBM-110, or any combination of these compounds, to inhibit the activity of cytochrome P450 enzymes, including CYP3A4, CYP2D6, CYP2C9, CYP2C19, and CYP2B6.

The method can be administered with compounds Rh2, Rg3, and aglycon ginsenosides including aPPD and aPPT, in a non-purified form, in which the purity of each compound is lower than 50%. The method can also be administered with compounds Rh2, Rg3, and aglycon ginsenosides including aPPD and aPPT, in a purified form in which the purity of each compound is above 50%.

When the method is administered using a combination of any of the compounds Rh2, Rg3, and aglycon ginsenosides including aPPD and aPPT, the concentration of each compound in the combination formula may range between 0.2% and 99% of each component in the formula.

The method can be administered to a healthy person, or a person who belongs to one of following groups of people who are at high-risk of developing cancer: smokers, ex-smokers, workers in certain industries such as mining, persons with a family history of cancer, persons who have had diseases that are known to cause cancer, such as hepatitis and AIDS, persons who are exposed to radiation and certain chemicals that are known to cause cancer. Furthermore, Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of the above can be used as cancer prevention agents administered to a cancer survivor whose cancer is cured to prevent cancer from recurring.
The method can be used to prevent cancers such as lung, breast, prostate, colon, rectal, pancreatic, bladder, kidney, uterine, mouth, stomach, ovarian, brain, thyroid, cervical, esophageal, laryngeal, and testical cancers, as well as non-Hodgkin’s lymphoma, melanoma, leukemia, multiple myeloma, and Hodgkin’s Disease.

In the method, Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of the above can be administered as pharmaceutical or non-pharmaceutical compositions.

**Pharmaceutical Compositions**

The isolation and purification of Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of the above from natural sources has been described in the art. The pharmaceutically active compound may be administered as pharmaceutical compositions with an appropriate pharmaceutically acceptable carrier, diluent, recipient or vehicle. The pharmaceutical compositions may also be formulated to contain Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of the above compounds for administration to a human subject.

The pharmaceutical compositions of the present invention may be administered orally, topically, by injection, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The terms parenteral as used herein include subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques.

The pharmaceutical compositions may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to methods known to the art for the manufacture of pharmaceutical compositions and may contain one or more agents selected from the group of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with suitable non-toxic pharmaceutically acceptable excipients including, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, such as corn starch, or alginic acid; binding agents, such as starch, gelatine or acacia, and lubricating agents, such as
magnesium stearate, stearic acid or talc. The tablets can be uncoated, or they may be coated by known techniques in order to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed.

5 Pharmaceutical compositions for oral use may also be presented as hard gelatine capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatine capsules wherein the active ingredient is mixed with water or an oil medium such as peanut oil, liquid paraffin or olive oil.

10 Aqueous suspensions contain the active compound in admixture with suitable excipients including, for example, suspending agents, such as sodium carboxymethylcellulose, methyl cellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents such as a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example, polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example, hepta-decaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol for example, polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example, polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxy-benzoate, one or more colouring agents, one or more flavouring agents or one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example, arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example, beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and/or flavouring agents may be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active compound in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents
and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavouring and colouring agents, may also be present.

Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oil phase may be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil, for example, liquid paraffin, or it may be a mixture of these oils. Suitable emulsifying agents may be naturally-occurring gums, for example, gum acacia or gum tragacanth; naturally-occurring phosphatides, for example, soy bean, lecithin; or esters or partial esters derived from fatty acids and hexitol, anhydrides, for example, sorbitan monoleate, and condensation products of the said partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monoleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example, glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, and/or flavouring and colouring agents.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to known art using suitable dispersing or wetting agents and suspending agents such as those mentioned above. The sterile injectable preparation may also be sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Acceptable vehicles and solvents that may be employed include, but are not limited to, water, Ringer's solution, lactated Ringer's solution and isotonic sodium chloride solution. Other examples are, sterile, fixed oils which are conventionally employed as a solvent or suspending medium, and a variety of bland fixed oils including, for example, synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Other pharmaceutical compositions and methods of preparing pharmaceutical compositions are known in the art and are described, for example, in "Remington: The Science and Practice of Pharmacy," Gennaro, A., Lippincott, Williams & Wilkins, Philidelphia, PA (2000) (formerly "Remington's Pharmaceutical Sciences").
Non-Pharmaceutical Compositions

An important aspect of the invention provides for ginsenoside Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of the above to be administered as a non-pharmaceutical composition in an appropriate pharmacologically acceptable medium such as a buffer, a solvent, a diluent, an inert carrier, an oil, a creme, or an edible material. The non-pharmaceutical formulation may be in the form of, for example, a nutraceutical composition, a food, a health food, a natural health product, a functional food, a nutritional supplement, a dietary supplement, an herbal supplement, an herb, an alternative medicine, or a naturopathic product. In one embodiment of the invention, the composition of the present invention is provided as a non-pharmaceutical formulation in a pharmacologically acceptable medium. The non-pharmaceutical formulations disclosed herein may typically be orally administrable in the form of a tablet, a powder, a suspension, an emulsion, a capsule, a granule, a troche, a lozenge, a pill, a liquid, a spirit, a syrup, an elixir, and a drink. The non-pharmaceutical composition may also be administered topically in the form of a drop, a paste, an ointment, a liquid, a powder, a plaster, a suppository, an aerosol, a liniment, a lotion, an enema and an emulsion.

Administration and Dosage Protocols

In one embodiment of the invention, the pharmaceutical and non-pharmaceutical composition may comprise an effective amount of Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of the above in a pharmacologically acceptable medium.

In accordance with the present invention, the active daily dose of Rh2 is 0.1 mg to 5g per day per person, or preferably, 0.5 mg to 2g per day per person; The active daily dose of Rg3 is 0.1 mg to 5g per day per person, or preferably, 0.5 mg to 2g per day per person; The active daily dose of aglycon ginsenoside aPPD is 0.1 mg to 5g per day per person, or preferably, 0.5 mg to 2g per day per person; The active daily dose of aglycon ginsenoside aPPT is 0.1 mg to 5g per day per person, or preferably, 0.5 mg to 2g per day per person; The active daily dose of composition comprising of two or more of Rh2, Rg3, and aglycon ginsenoside aPPD or aPPT is 0.1 mg to 5g per day per person, or preferably, 0.5 mg to 2g per day per person.
In accordance with the present invention, an effective amount of Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or a combination of the above is administered to a subject in order to prevent cancer. The Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or a pharmaceutical composition comprising Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT may be administered in a manner consistent with medical practice.

The dosage of Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of the above to be administered will be dependent upon the size of the subject and can be readily determined by a skilled practitioner. It is to be understood, however, that the dosage and frequency of administration may be adapted to the circumstances in accordance with known practices in the art, for the prevention of cancers.

**Pharmaceutical Kits**

The present invention additionally provides pharmaceutical kits containing Rh2, Rg3, aglycon ginsenosides including aPPD and aPPT, or any combinations of the above in pharmaceutical compositions for use in the prevention of cancers. Individual components of the kit could be packaged in separate containers.

When the components of the kit are provided in one or more liquid solutions, the liquid solution can be an aqueous solution, for example a sterile aqueous solution. In this case the container means may itself be an inhalant, syringe, pipette, eye dropper, or other such like apparatus, from which the composition may be administered to a subject.

The components of the kit may also be provided in dried or lyophilised form and the kit can additionally contain a suitable solvent for reconstitution of the lyophilised components. Irrespective of the number or type of containers, the kits of the invention also may comprise an instrument for assisting with the administration of the composition to a subject. Such an instrument may be an inhalant, syringe, pipette, forceps, measured spoon, eyedropper or any such medically approved delivery vehicle.

To gain a better understanding of the invention described herein, the following examples are set forth. It should be understood that these examples are for illustrative purposes only.

Therefore, they should not limit the scope of this invention in any ways.
**EXAMPLE I  in vitro**

The Inhibitory Effects Of Rh2, Rg3, Aglycon Ginsenosides aPPD And aPPT, And Combinations Of The Above On The Cytochrome P450s CYP3A4, CYP2D6, CYP2C9, CYP2C19 And CYP2B6

**EXPERIMENTAL METHODS**

Test methods are based on the protocols provided by the manufacturer of inhibitor screening kits of the above listed cytochrome p450s. Briefly, recombinant human P450 (SUPERSOMES™) were incubated together with their fluorescent substrates in a buffer containing NADP+, Glucose-6-Phosphate, Magnesium Chloride, Glucose-6-Phosphate Dehydrogenase and the drug substance Rh2, Rg3, aglycon ginsenosides aPPD and aPPT, and a combination of the above (Careseng 2131) at various concentrations for a given time and then the activities of the enzymes CYP3A4, CYP2D6, CYP2C9, CYP2C19 and CYP2B6 were measured at suitable excitation and emission wavelengths. In each experiment, a reaction with a known inhibitor of the tested enzyme was included as a positive control.

As shown in Table 3, in the presence of the known inhibitors the activities of the P450 enzymes were decreased, which demonstrated the testing system worked properly.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Inhibitor</th>
<th>Inhibitor Concentration (uM)</th>
<th>Percentage of Enzyme Activity Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4</td>
<td>KTZ</td>
<td>5</td>
<td>3.0</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Quinidine</td>
<td>0.25</td>
<td>1.8</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>SFZ</td>
<td>5</td>
<td>5.74</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>TCP</td>
<td>250</td>
<td>2.83</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>TCP</td>
<td>250</td>
<td>4.76</td>
</tr>
</tbody>
</table>

*Table 3: Demonstration of inhibition of CYP3A4, CYP2D6, CYP2C9, CYP2C19, and CYP2B6 by their known inhibitors.*

**EXPERIMENTAL RESULTS**

Fig.1 is a graph of the activity of cytochrome P450 enzyme CYP3A4 in the presence of 0 to 60ug/ml of Rh2, Rg3, aPPD, aPPT, and Careseng 2131 (a combination of Rh2, aPPD and aPPT). The activity of CYP3A4 is inhibited in a dose dependent manner in the presence of these compounds.
Fig. 2 is a graph of the activity of cytochrome P450 enzyme CYP2D6 in the presence of 0 to 60ug/ml of Rh2, Rg3, aPPD, aPPT and Careseng 2131 (a combination of Rh2, aPPD and aPPT). The activity of CYP2D6 is inhibited in a dose dependent manner in the presence of these compounds.

Fig. 3 is a graph of the activity of cytochrome P450 enzyme CYP2C9 in the presence of 0 to 60ug/ml of Rh2, Rg3, aPPD, aPPT, and Careseng 2131 (a combination of Rh2, aPPD and aPPT). The activity of CYP2D6 is inhibited in a dose dependent manner in the presence of these compounds.

Fig. 4 is a graph of the activity of cytochrome P450 enzyme CYP2C19 in the presence of 0 to 60ug/ml of Rh2, Rg3, aPPD, aPPT, and Careseng 2131 (a combination of Rh2, aPPD and aPPT). The activity of CYP2C19 is inhibited in a dose dependent manner in the presence of these compounds.

Fig. 5 graph of the activity of cytochrome P450 enzyme CYP2B6 in the presence of 0 to 60ug/ml of Rh2, Rg3, aPPD, aPPT, and Careseng 2131 (a combination of Rh2, aPPD and aPPT). The activity of CYP2B6 is inhibited in a dose dependent manner in the presence of these compounds.

**EXAMPLE II  in vivo**

The Inhibitory effects of Rh2, Rg3, aglycone ginsenosides aPPD and aPPT, and combinations of the above on the cytochrome P450s-dependent carcinogens

AFB1, a AFB1, a mycotoxin produced by Aspergillus Flavus, is considered to be an important factor for liver cancers in Africa and Southeast Asia. It is activated into AFB1 exo-8,9-epoxide by cytochrome P450s (P450 3A4, etc). AFB1 can also be activated into tumour on rat by its P450s. In 1972, Vesselinovitch et al. discovered that AFB1 causes liver tumors in mice when administered to newborn animals, and this model is now well established.
EXPERIMENTAL METHODS

Male Fischer F344 rats obtained from Charles River Co. (Montreal, Quebec, Canada), were housed under negative pressure with a 12 h light–dark cycle, with a temperature range of 19–23°C and humidity of 40–60%.

AFB1 (Sigma, USA), Ginsenoside Rh2, Rg3, aPPD, and Composition (Careseng 2131) were obtained from Pegasus Pharmaceuticals Group Inc. (Richmond, B.C. Canada). Diet was freshly prepared every week. Food and water were provided ad libitum.

Experimental Design for Treatment Schedules:

AFB1+Treatments

10 Group 1: Control group:

Group 2: Rh2 group:

Group 3: Rg3 group:

Group 4: aPPD group:

Group 5: Composition group:

15 20 wks 20wks

Method:

For the carcinogenesis studies, 1-wk-old male F344 rats were randomly divided into five groups. Control group received the diet only for 20 weeks. During the first 20 weeks, all other groups (except control group) received diet with a daily dose of 20mg/kg of ginsenoside Rh2, Rg3, aPPD, and Careseng 2131 separately. Starting from the 21st week to 40th week, an additional i.p. injection dosage of 1.5 mg/kg b.w. Aflatoxin B1 dissolved in DMSO (dimethyl
sulfoxide) was given to all groups. At the 40th week, all animals were terminated and subjected to a complete autopsy.

Table 1. Daily feeding

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mice</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>AFB1 dose per mouse per day (mg/kg)</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Dose taken per mouse per day (mg/kg)</td>
<td>0</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

At autopsy, livers were grossly examined and nodules larger than 1 mm in diameter were counted as tumors. After fixation in 10% formaldehyde solution, each liver lobe will be completely cut into 2.0 mm thick slices and processed for light microscopy. Diagnosis of hepatocellular adenomas and carcinomas was made microscopically based on established diagnostic criteria (2). Hepatocellular adenomas were at most 5–10 mm in diameter and clearly demarcated. Relatively small and monotonous tumor cells proliferate, forming a thin trabecular pattern. Hepatocellular carcinomas showed a thick trabecular growth pattern with dilated sinusoids.

Group differences were assessed for statistical significance using the χ² test for incidences of benign and malignant lesions and the t-test for numbers of tumors per mouse and diameters. Results are presented in form of table 2.

**EXPERIMENTAL RESULTS**

Table 2 shows the prevention effects of different ginsenosides on Aflatoxin B1-induced tumorigenesis.

<table>
<thead>
<tr>
<th>Group Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Control</td>
<td>Rh2</td>
<td>Rg3</td>
<td>αPPD</td>
<td>Careseng 2131</td>
</tr>
<tr>
<td>No. of mice in experiment</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>No. of tumor-bearing mice (%)</td>
<td>7(70%)</td>
<td>2(20%)</td>
<td>4(40%)</td>
<td>1(10%)</td>
<td>1(10%)</td>
</tr>
<tr>
<td>No. of carcinoma-bearing mice (%)</td>
<td>1(10%)</td>
<td>1(10%)</td>
<td>2(20%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
This example shows the preventative effects of different ginsenosides on P450s-dependent carcinogen AFB1 on F344 rats.

These above P450s are known to produce carcinogens. Therefore, inhibition of these enzymes reduces the production of carcinogens in the human body. Therefore, when P450s are inhibited, they prevent or reduce the chances of developing cancer. The production of carcinogens in the body is widespread and occurs in almost all of the organs, such as in skin, lung, breast, prostate, colon, rectal, pancreatic, bladder, kidney, uterine, bile duct, mouth, throat, stomach, ovarian, brain, thyroid, cervical, esophageal, laryngeal, and testicular. Therefore, many types of cancer are therefore prevented.

As will be apparent to those skilled in the art in the light of the foregoing disclosure, many alterations and modifications are possible in the practice of this invention without departing from the spirit or scope thereof. Accordingly, the scope of the invention is to be construed in accordance with the substance defined by the following claims.
WHAT IS CLAIMED IS:

1. A method of preventing cancer in humans or other animals comprising administering an effective amount of Rh2, Rg3, aglycon ginsenosides, or any combination thereof, to the humans or other animals to inhibit activity of cytochrome P450 enzymes in the humans or other animals.

2. The method according to claim 1 wherein the aglycon ginsenosides comprise aPPD, aPPT, PAM-120, PBM-100, PBM-110, or any combination thereof.

3. The method according to claim 1, wherein the aglycon ginsenosides comprise aPPD, aPPT, or any combination thereof.

4. The method according to claim 1, wherein the cytochrome P450 enzymes that are inhibited by the method comprise CYP3A4, CYP2D6, CYP2C9, CYP2C19, CYP2B6, or any combination thereof.

5. The method according to claim 1, wherein the method is administered to humans at high risk of developing cancer.

6. The method as claimed in claim 1, wherein the method is administered to humans who have previously had cancer.

7. The method according to claim 1, wherein the method is used to prevent cancers selected from the group consisting of non-Hodgkin’s lymphoma, melanoma, leukemia, multiple myeloma, Hodgkin’s Disease, skin, lung, breast, prostate, colon, rectal, pancreatic, bladder, kidney, uterine, bile duct, mouth, throat, stomach, ovarian, brain, thyroid, cervical, esophageal, laryngeal, and testicular cancer.

8. The method according to claim 6, wherein the humans have previously developed non-Hodgkin’s lymphoma, melanoma, leukemia, multiple myeloma, Hodgkin’s Disease, skin, lung, breast, prostate, colon, rectal, pancreatic, bladder, kidney, uterine, bile duct, mouth, throat, stomach, ovarian, brain, thyroid, cervical, esophageal, laryngeal, and testicular cancer.
9. The method as claimed in claim 1, wherein the Rh2, Rg3, aglycon ginsenosides, or any combination thereof, are administered in a non-purified form having less than 50% purity.

10. The method as claimed in claim 1, wherein components of the combination of any of Rh2, Rg3, or aglycon ginsenosides comprise a concentration between 0.2% to 99% of each component in the combination formula.

11. The method as claimed in claim 1, wherein Rh2, Rh3, the aglycon ginsenosides, or any combination thereof are administered in a dosage between 0.1mg to 5g per person per day.

12. The method as claimed in claim 11, wherein Rh2, Rh3, the aglycon ginsenosides, or any combination thereof are preferably administered in a dosage between 0.5mg to 2g per person per day.

13. The method as claimed in claim 1, wherein Rh2, Rh3, the aglycon ginsenosides, or any combination thereof are administered as a pharmaceutical composition.

14. The method as claimed in claim 1, wherein Rh2, Rh3, the aglycon ginsenosides, or any combination thereof are administered as a non-pharmaceutical composition.

15. The method as claimed in claim 14, wherein the non-pharmaceutical composition comprises a nutraceutical, a food, a health food, a natural health product, a functional food, a nutritional supplement, a dietary supplement, an herbal supplement, an herb, an alternative medicine product, or a naturopathic product.

16. The method according to claim 13, wherein the pharmaceutical composition is administered in a form selected from the group consisting of an orally administrable form, an injectable form, and a topically applicable form.

17. The method according to claim 14, wherein the non-pharmaceutical composition is administered in a form selected from the group consisting of an orally administrable form, a parenterally administrable form, an inhalable form, and a topically applicable form.
18. The method according to claim 16, wherein the orally administrable form is selected from the group consisting of a tablet, a powder, a suspension, an emulsion, a capsule, a granule, a troche, a lozenge, a pill, a liquid, a spirit, a syrup, an elixir and a drink.

19. The method according to claim 17, wherein the orally administrable form is selected from the group consisting of a tablet, a powder, a suspension, an emulsion, a capsule, a granule, a troche, a lozenge, a pill, a liquid, a spirit, a syrup, an elixir and a drink.

20. The method according to claim 16, wherein the topically applicable form is selected from the group consisting of a drop, a paste, an ointment, a liquid, a powder, a plaster, a suppository, an aerosol, a liniment, a lotion, an enema and an emulsion.

21. The method according to claim 17, wherein the topically applicable form is selected from the group consisting of a drop, a paste, an ointment, a liquid, a powder, a plaster, a suppository, an aerosol, a liniment, a lotion, an enema and an emulsion.

22. A pharmaceutical composition for use in preventing cancers in humans or other animals according to the method as claimed in claim 13 comprising an effective amount of Rh2, Rg3, aglycon ginsenosides, or any combination thereof to inhibit the activity of cytochrome P450 enzymes in the humans or other animals, and a pharmaceutically acceptable carrier, diluent, recipient or vehicle.

23. The pharmaceutical composition as claimed in claim 22, wherein components of the combination of any of Rh2, Rg3, or aglycon ginsenosides comprise a concentration between 0.2% to 99% of each component in the combination formula.

24. The pharmaceutical composition as claimed in claim 22, wherein the cytochrome P450 enzymes inhibited by the pharmaceutical composition comprise CYP3A4, CYP2D6, CYP2C9, CYP2C19, CYP2B6, or any combination thereof.

25. The pharmaceutical composition as claimed in claim 22, wherein the pharmaceutical composition is administered in a form selected from the group consisting of an orally administrable form, a parenterally administrable form, an inhalable form, and a topically applicable form.
26. The pharmaceutical composition as claimed in claim 25, wherein the orally administrable form is selected from the group consisting of a tablet, a powder, a suspension, an emulsion, a capsule, a granule, a troche, a lozenge, a pill, a liquid, a spirit, a syrup, an elixir and a drink.

27. The pharmaceutical composition as claimed in claim 25, wherein the topically applicable form is selected from the group consisting of a drop, a paste, an ointment, a liquid, a powder, a plaster, a suppository, an aerosol, a liniment, a lotion, an enema and an emulsion.

28. The pharmaceutical composition as claimed in claim 22, wherein the composition is administered in a dosage between 0.1mg to 5g per person per day.

29. The pharmaceutical composition as claimed in claim 28, wherein the composition is preferably administered in a dosage between 0.5mg to 2g per day.

30. A pharmaceutical kit comprising one or more pharmaceutical compositions as claimed in claim 22 for use in preventing cancer in humans or other animals by inhibiting cytochrome P450 enzymes in the humans or other animals.

31. The pharmaceutical kit as claimed in claim 30, wherein the one or more pharmaceutical compositions are individually packaged.

32. The pharmaceutical kit as claimed in claim 30, wherein the kit comprises an instrument for administering the one or more pharmaceutical compositions.

33. A non-pharmaceutical composition useful for preventing cancers in humans or other animals according to the method as claimed in claim 13 comprising an effective amount of Rh2, Rg3, aglycon ginsenosides, or any combination thereof to inhibit the activity of cytochrome P450 enzymes in the humans or other animals, and an acceptable carrier, diluent, buffer, solvent, oil, cream, or edible material.

34. The non-pharmaceutical composition as claimed in claim 33, wherein the form of the non-pharmaceutical composition is selected from the group consisting of a nutraceutical, a food, a health food, a natural health product, a functional food, a
nutritional supplement, a dietary supplement, an herbal supplement, an herb, an alternative medicine product, or a naturopathic product.

35. The non-pharmaceutical composition as claimed in claim 33, wherein components of the combination of any of Rh2, Rg3, or aglycon ginsenosides comprise between 0.2% to 99% of each component in the formula.

36. The non-pharmaceutical composition as claimed in claim 33, wherein the pharmaceutical composition is administered in a form selected from the group consisting of an orally administrable form, a parenterally administrable form, an inhalable form, and a topically applicable form.

37. The non-pharmaceutical composition as claimed in claim 36, wherein the orally administrable form is selected from the group consisting of a tablet, a powder, a suspension, an emulsion, a capsule, a granule, a troche, a lozenge, a pill, a liquid, a spirit, a syrup, an elixir and a drink.

38. The non-pharmaceutical composition as claimed in claim 36, wherein the topically applicable form is selected from the group consisting of a drop, a paste, an ointment, a liquid, a powder, a plaster, a suppository, an aerosol, a liniment, a lotion, an enema and an emulsion.

39. The non-pharmaceutical composition as claimed in claim 33, wherein the composition is administered in a dosage between 0.1mg to 5g per person per day.

40. The non-pharmaceutical composition as claimed in claim 39, wherein the composition is preferably administered in a dosage between 0.5mg to 2g per day.
Effects of careseng and its major components on CYP3A4

% CYP3A4

Concentration (µg/ml)

Fig. 1
Effects of careseng and its major components on CYP2D6

Fig. 2
Effects of careseng and its major components on CYP2C9

Fig. 3
Effects of careseng and its major components on CYP2C19

Fig. 4
Effects of careseng and its major components on CYP2B6

Fig. 5
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC*: A61K-31/704; A61P-35/00; A61K-35/78; A61K-31/575

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K; A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of database, and, where practicable, search terms used)
STN, Delphion, Questel-Orbit, Canadian Patent Database (keywords: "ginsenoside" and "cancer"; narrowed by & "prevent")

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>US 2003/0185910 A1 Yun et al. 02-10-2003</td>
<td>1-40</td>
</tr>
<tr>
<td>X</td>
<td>CA 2,470,402 Park 17-07-2003</td>
<td>1-40</td>
</tr>
<tr>
<td>X</td>
<td>WO 03/010182 Huang et al. 06-02-2003</td>
<td>22-40 1-21</td>
</tr>
<tr>
<td>X, Y, P</td>
<td>WO 2004/056371 Huang 08-07-2004</td>
<td>22-40 1-21</td>
</tr>
<tr>
<td>X, P, Y</td>
<td>WO 2004/056372 Huang 08-07-2004</td>
<td>22-40 1-21</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. [X] Patent family members are listed in annex. [X]

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international-type search 24 January 2005 (24-01-2005)
Date of mailing of the international-type search report 03 March 2005 (03-03-2005)

Name and mailing address of the ISA/CA
Commissioner of Patents
Canadian Patent Office-PCT
Ottawa/Gatineau K1A 0C9
Facsimile No. 1-819-953-9358

Authorized officer Chris Evans (819) 934-2323

Form PCT/ISA/210 (second sheet) (January 2004)
<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X, P</td>
<td>CA 2,431,806 Huang</td>
<td>11-12-2003 22-40</td>
</tr>
<tr>
<td>Y, P</td>
<td></td>
<td>1-21</td>
</tr>
<tr>
<td>X</td>
<td>US 5,776,460 Kim et al.</td>
<td>07-07-1998 22-40</td>
</tr>
<tr>
<td>X</td>
<td>Chang et al., &quot;In Vitro Effect of Standardized Ginseng Extracts and Individual Ginsenosides on the Catalytic Activity of Human CYP1A1, CYP1A2, and CYP1B1&quot;, Drug Metabolism and Disposition, 2002, 30, 378-384</td>
<td>1-40</td>
</tr>
</tbody>
</table>
# INTERNATIONAL SEARCH REPORT

## Box No. II  
Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

<table>
<thead>
<tr>
<th>No.</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Claims Nos.: 1-21 because they relate to subject matter not required to be searched by this Authority; namely: Claims 1-21 define methods of treatment of the human or animal body by therapy, which are excluded subject-matter under Rule 39.1(iv), PCT. Despite this, a search has been performed based on the alleged effects of the compositions used in the defined methods.</td>
</tr>
<tr>
<td>2.</td>
<td>Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:</td>
</tr>
<tr>
<td>3.</td>
<td>Claims Nos.: because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).</td>
</tr>
</tbody>
</table>

## Box III  
Observation where unity of invention is lacking (Continuation of item 3 of first sheet)

<table>
<thead>
<tr>
<th>No.</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.</td>
</tr>
<tr>
<td>2.</td>
<td>As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.</td>
</tr>
<tr>
<td>3.</td>
<td>As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:</td>
</tr>
<tr>
<td>4.</td>
<td>No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:</td>
</tr>
</tbody>
</table>

**Remark on Protest**

<table>
<thead>
<tr>
<th>No.</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>The additional search fees were accompanied by the applicant’s protest.</td>
</tr>
<tr>
<td>2.</td>
<td>No protest accompanied the payment of additional search fees.</td>
</tr>
</tbody>
</table>

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2004)
<table>
<thead>
<tr>
<th>Patent Document Cited in Search Report</th>
<th>Publication Date</th>
<th>Patent Family Member(s)</th>
<th>Publication Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WO003086438 A1</td>
<td>23-10-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR2003059984 A</td>
<td>12-07-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO003056929 A1</td>
<td>17-07-2003</td>
</tr>
<tr>
<td>WO03010182</td>
<td>06-02-2003</td>
<td>BR20205792 A</td>
<td>22-07-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA2454799 A1</td>
<td>06-02-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP1414843 A1</td>
<td>06-05-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US2003087835 A1</td>
<td>08-05-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US2003087836 A1</td>
<td>08-05-2003</td>
</tr>
<tr>
<td>WO2004056371</td>
<td>08-07-2004</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>WO2004056372</td>
<td>08-07-2004</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>CA2431806</td>
<td>11-12-2003</td>
<td>CA2390290 A1</td>
<td>11-12-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO003103682 A1</td>
<td>18-12-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO2004056379 A1</td>
<td>08-07-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AT6017696 A</td>
<td>30-12-1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN1091604 B</td>
<td>02-10-2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE69615181 D1</td>
<td>18-10-2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP8331864 A1</td>
<td>01-04-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP11501322 T</td>
<td>02-02-1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO9640181 A1</td>
<td>19-12-1996</td>
</tr>
</tbody>
</table>