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Recent Development of Antitumor Agents from Chinese Herbal Medicines. Part II. High Molecular Compounds[3]

Abstract

High molecular compounds from Chinese herbal medicines, including ribosome-inactivating proteins and polysaccharides from both fungi and high plants have been tested for the treatment of malignant diseases. Polysaccharides possessing immunostimulating activities can be used as adjuvants in tumor treatment. The fungi containing such polysaccharides are usually edible mushrooms or tonics in Traditional Chinese Medicine. Parts from high plants such as Radix Astragali and Fructus Lycii containing polysaccharides are mainly used as tonic in Traditional Chinese Medicine. Ribosome-inactivating proteins are a group of proteins exerting cytotoxic activities via inhibition of protein synthesis. Some of the ribosome-inactivating proteins have been used as the cytotoxic part in conjugates with monoclonal antibodies as tumor-targeting drugs. The cytotoxic and antineoplastic mechanisms of the high molecular compounds are rather different from those of the low molecular compounds described in part I.

Dedication

In memory of Prof. Dr. Hans Beyer, Prof. Dr. Roland Pohloudek-Fabini and Prof. Dr. Werner Rothmaler, Ernst-Moritz-Arndt University, Greifswald, Germany

Key words
Antitumor agents · Chinese herbal medicines · high molecular natural compounds

Introduction

Besides the low molecular compounds from Chinese herbal medicines as antineoplastic and cytotoxic agents (see "Recent Development of Antitumor Agents from Chinese Herbal Medicines" Part I, Planta Medica 2003; 69: 97 – 108), some high molecular compounds were isolated from Chinese herbal medicines and were used for the treatment of malignant diseases. Among the high molecular compounds, polysacharides and ribosome-inactivating proteins are of special interest. Polysaccharides from fungi and higher plants exhibiting immunostimulating effects were tested both in experimental and clinical studies. Ribosome-inactivating proteins are a group of proteins which are able to inactivate eukaryotic protein synthesis by attacking the 28S ribosomal RNA. They have been tested experimentally and clinically in the treatment of tumors both as single compounds and as conjugates bound to antibodies. In the present part, a summary of the recent advances in the study and use of polysaccharides and ribosome-inactivating proteins from Chinese herbal medicines for the treatment of tumors is given.

Polysaccharides

Fungal polysaccharides

A number of polysaccharides from fungi were reported to possess immunostimulating and antitumor effects [1], [2]. Lentinan

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1         10         20         30         40         50         60
DVSFRLSGATSSSYGVFISNLRKALPNERKLYDIPLLRSSLPGSQRYALIHLTNYADETI

          70         80         90         100        110        120
SVAIDVTNVYIMGYRAGDTSYFFNEASATEAAKYVFKDAMRKVTLPYSGNYERLQTAAGK

          130        140        150        160        170        180
IRENIPLGLPALDSAITTLFYYNANSAASALMVLIQSTSEAARYKFIEQQIGKRVDKTFL

          190        200        210        220        230        240
PSLAIISLENSWSALSKQIQIASTNNGQFESPVVLINAQNQRVTITNVDAGVVTSNIALL

LNRNNM(A)
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Fig. **1** Primary structure of trichosanthin.

from *Lentinus edodes* (Berk.) Sing. (Tricholomataceae) is one of the most thoroughly studied fungal polysaccharides. *Lentinus edodes* is a common edible mushroom in China and in Japan. Lentinan was first isolated by Chihara [3]. It is a (1→3)-β-glucan highly branched with (1→3)-β- and (1→6)-β-linked glucose residues existing mainly as linear triple-helical structures in aqueous solution. Lentinan has a molecular weight of about 500 kDa [4], [5].

A multi-center prospective study of lentinan used in combination with cytostatic agents in patients with advanced unresectable and recurrent gastric cancer revealed survival prolongation and improvement in life quality. Median survival time of patients treated with lentinan was significantly longer than that of patients without lentinan treatment (297 days vs. 199 days) [6]. Lentinan at a daily *i.v.* dosage of 2 mg per patient combined with 5′-deoxy-5-fluorouridine [7], cisplatin, 5-FU [8] and tegafur [9] was effectively used for postoperative therapy of gastric cancer. An increase of more than 50% IL-1β production in the peripheral blood of gastric cancer patients associated with lentinan treatment was observed [10], [11] as well as an enhanced induction of lymphokine-activated NK cell activity [12]. Addition of lentinan to maintenance therapy with 5-FU did not exert prognostic benefit on hepatocellular carcinoma patients [13].

Lentinan exhibited significant immunostimulating effects and showed antitumor activities also in experimental animals, augmenting helper T cell-mediated cytotoxic T cell activity, NK cell activity and humoral immune responses. Moreover, lentinan activated non-specific cytotoxicity of macrophages *in vitro* [14] and *in vivo* [15]. The beneficial effects of lentinan on cellular immune function were also observed in cyclophosphamide-treated mice [5]. Among the cytokine genes, the mRNA levels of IL-1α, IL-1β, TNF-α, IFN-γ and macrophage colony-stimulating in mouse peritoneal cells and splenocytes were markedly induced by lentinan [16]. Combinatory use of 5′-deoxy-5-fluorouridine and lentinan against AH66 ascites hepatoma cells in rats resulted in a significant inhibition of tumor growth as compared with 5′-deoxy-5-fluorouridine alone. Lentinan induced pyrimidine nucleoside phosphorylase activity in the tumor and increased the susceptibility of tumor cells to 5′-deoxy-5-fluorouridine [17]. Lentinan decreased glutathione-S-transferase GST-II and GST-III contents in colon 26 adenocarcinoma tissues transplanted into mice and enhanced the susceptibility of tumor to cisplatin [18]. β-(1→3)-Glucans were reported to be more effective on T cells than B cells [19]. It was also suggested that lentinan affects the tumorous vascular system resulting in the induction of hemorrhagic necrosis which is dependent on T cells in the tumor [20], [21]. In addition, the binding of lentinan to human monocytes may initiate the influence of this compound on the immune system and differ between individuals [22].

Intraperitoneal treatment of mice with lentinan at a dose of 10 mg/kg affected the number, plastic-adherence, and endogen peroxidase activity of peritoneal cells. Lentinan-stimulated peritoneal macrophages exerted cytotoxicity against several murine and human metastatic tumors, including Lewis lung carcinoma and two human melanomas [23]. Peritoneal carcinomatoses induced in BDIX rats by *i.p.* injections of syngeneic cells from a colon carcinoma cell line, treated with lentinan (five *i.p.* injections, 2 days apart at a daily dose of 2 mg/kg, starting on day 14 after tumor cell injection) significantly inhibited the growth of carcinomatoses and increased the life span of the animals [24]. Oral administration of *L. edodes* mycelia to mice suppressed postoperative liver metastasis of primary colorectal cancer and increased the survival period, elevating the activities of NK cells and macrophages in the liver and increasing IL-1β levels in liver and spleen [25]. Combined use of lentinan and IL-2 was found to result in synergistic antitumor and antimetastatic effects in mice against spontaneously metastatic 3-methylcholanthrene-induced fibrosarcoma [26].

Lentinan is relatively non-toxic. The LD_{50} values of lentinan in mice (ICR) and rats (CD) are essentially the same regardless of

species and sex and are estimated to be 250 – 500 mg/kg by *i.v.* administration and greater than 2500 mg/kg by *i.p.*, *s.c.*, and oral administration. No remarkable toxic signs specific to lentinan were observed after i.p., s.c., and oral treatment whereas cyanosis, convulsion and death were observed in animals administered i.v. with higher dosages of lentinan [27].

Krestin (PSK) [28], a protein-bound β-glucan containing approximately 25% protein; and PSP [29], a polysaccharide peptide, were isolated from CM-101 strain and COV-1 strain, respectively, of *Coriolus versicolor* (Fr.) Quel. (Polyporaceae). PSP possesses a molecular weight of approximately 100 kDa. Glutamic and aspartic acids are abundant in its polypeptide component, its polysaccharide component is composed of monosaccharides with α-(1→4)- and β-(1→3) glucosidic linkages. The presence of fucose in PSK and rhamnose and arabinose in PSP distinguishes the two protein-bound polysaccharides, which are otherwise chemically similar [1].

In Japanese trials carried out since 1970, krestin significantly extended the five-year survival in cancers of the stomach, colonrectum, esophagus, nasopharynx, lung (non-small cell), and in a HLA B40-positive breast cancer subset [1]. However, a retrospective study on 963 Japanese patients with gastric cancer of postoperative survival treated with or without PSK showed no significant differences as compared to untreated patients at any stage [30]. PSP, subjected to Phase II and Phase III double-blind trials in China, significantly extended five-year survival time in esophageal cancer. Moreover, it significantly improved life quality, provided substantial pain relief, and enhanced immune status in 70% – 97% of patients with cancers of the stomach, esophagus, lung, ovary, and cervix [1].

In experimental studies, krestin was reported to exert a preventive effect on spontaneously developed carcinogenesis as well as on carcinogen- or radiation-induced tumors [31]. Krestin prolonged the survival time of SD rats bearing mammary tumors induced by *N*-methyl-*N*-nitrosourea, when given at a dose of 250 mg/kg twice a week for 3 weeks [32]. Moreover, it inhibited the growth of LLC-WRC-256 (Walker 256 fibrosarcoma) cells, H4-II-E (rat hepatoma) and of H4-II-E-C3 (rat hepatoma) cells *in vitro* at a concentration of 500 μg/ml [33]. Krestin enhanced the cytotoxicity of cisplatin toward H4-II-E and human ovarian cancer cells [34] and stimulated the production of IL-1 by human peripheral blood mononuclear cells [35]. The combined use of krestin with granulocyte-colony-stimulating factor, granulocyte/macrophage-colony-stimulating factor and IL-3 led to an improved recovery of myelosuppression in BDF1 mice induced by an *i.v.* administration of 5-FU (150 mg/kg) [36].

Obviously, krestin has also antimetastatic effects since it was reported to suppress pulmonary metastasis of methylcholanthrene-induced sarcomas, human prostate cancer DU145M and mouse melanoma cells and lymphatic metastasis of mouse leukemia P388. The survival time of tumor-bearing animals in spontaneous metastasis models was prolonged. Krestin also inhibited metastasis of rat hepatoma AH60C, mouse colon cancer colon 26, and mouse leukemia RL male 1 in artificial metastasis models. The antimetastatic effect of krestin is suggested to depend on different mechanisms: suppressed tumor invasion, adhesion and production of cell matrix-degrading enzymes, suppression of tumor cell attachment to endothelial cells by the inhibition of tumor cell-induced platelet aggregation, suppression of tumor cell migration after extravasation through the inhibition of tumor cell motility, and, finally, suppression of tumor growth after extravasation through the inhibition of angiogenesis, modulation of cytokine production, and augmentation of effector cell functions [37], [38], [39].

In addition, krestin was reported to enhance selenium-dependent and selenium-independent glutathione peroxidase (GST) activity, and to increase selenium-dependent glutathione peroxidase and GST-P mRNA expression in mouse peritoneal macrophages [40]. Krestin also enhanced manganese superoxide dismutase activity and its mRNA expression in mouse peritoneal macrophages [41]. Krestin was further reported to up-regulate inducible nitric oxide synthase (iNOS) gene expression and nitric oxide production in mouse peritoneal polymorphonuclear leukocytes. The stimulated production of nitric oxide in combination with IFN-γ may regulate the immune system *in vivo* [42].

PSP did not show cytotoxicity against tumor cell lines and mouse peritoneal macrophages *in vitro* at concentrations of 2.5 – 10 μg/ml. It activated the transcription of TNF gene in these cells, indicating that PSP exerted an immunomodulatory effect on the defensive cells [43]. Both mouse lymphocytes and macrophages were activated by preparations of polysaccharide from cultured mycelia and culture medium of *C. versicolor* [29]. PSP as a biological response modifier induced the production of IFN-α, IFN-γ, IL-2, and induced T cell proliferation. It counteracted the depressive effect of cyclophosphamide on leukocyte count [44] and showed a restorative effect against spleen injury in mice induced by γ-irradiation [45]. At a daily *i.p.* dose of 25 mg/kg to mice for 5 days, PSP antagonized the inhibition of IL-2 production by cyclophosphamide from activated T lymphocytes and restored the suppressed T cell-mediated delayed type hypersensitivity response to normal values. The lymphocyte proliferation *in vitro* was also stimulated by incubation with PSP at concentrations of 100 – 800 μg/ml. Moreover, PSP increased the phagocytic functions of the host reticulo-endothelial system [46]. PSP given s.c. to BALB/c mice inoculated *s.c.* with H238 tumor, a murine tumor transformed by herpes virus type 2, at a dose of 2 mg per mouse for 10 days significantly inhibited tumor progression when given immediately after tumor cell injection [47].

A refined polysaccharide peptide fraction from PS was reported to concentration-dependently inhibit the proliferation of the human hepatoma cell line $HEPG_2$ with an IC_{50} value of 243 μg/ml after an exposure of three days. However, little or no inhibitory effects of the refined polysaccharide peptide were observed on the proliferation of normal human fetal hepatocytes. In tumor-bearing nude mice, 5 days after inoculation with S180 cells, *i.v.* administration of refined polysaccharide peptide significantly suppressed the growth of S180 cells with an inhibition rate of more than 93% on day 13. Intraperitoneal preptreatment of nude mice with the refined polysaccharide peptide for two weeks before S180 cell inoculation led to a lower incidence of tumor growth than in control mice without polysaccharide pretreatment. The tumor size of the control group was about 3 – 5 times bigger than that of animals

with polysaccharide pretreatment. The refined polysaccharide peptide did not cause any pathological lesions in vital organs of rabbits such as heart, liver, spleen, lung and kidney [48].

Further fungal polysaccharides with immunostimulating and antitumor activities have been isolated from *Auricularia auricula* (L. ex Hook) Underw. (Auriculariaceae) [49], *Armillariella tabescens* (Scop. ex Fr.) Sing (Tricholomataceae) [50], *Ganoderma lucidum* Leys. ex Fr.) Karst. (Polyporaceae) [51], *Polyporus umbellatus* (Pers.) Fries (Polyporaceae) [52], [53], [54], *Poria cocos* (Schw.) Wolf (Polyporaceae) [55], [56] and *Tremella fuciformis* Berkely (Tremellaceae) [57], [58], [59]. Ganoderma (Lingzhi); the fruiting body of *G. lucidum*; Poria (Fuling), the sclerotium of *P. cocos*; and Polyporus (Zhuling), the fungal body of *P. umbellatus*, are all listed in the Chinese Pharmacopoeia.

Polysaccharides from higher plants

Radix Astragali (Huangqi), the root of *Astragalus membranaceus* Bge. var. *mongholicus* (Bge.) Hsiao or *Astragalus membranaceus* (Fisch.) Bge. (Fabaceae), listed in the Chinese Pharmacopoeia, is used as a general tonic in Traditional Chinese Medicine since ancient time. A series of polysaccharides such as astragalans I – III, AG-I, AG-II, AH-I, AH-II, Amem-P and Amon-S were isolated from the roots of *A. membranaceus* or of *A. membranaceus* var. *mongholicus* [60], [61], [62], [63]. Astragalan I is composed of D-glucose, D-galactose, and L-arabinose in addition to trace amounts of pentose with an average molecular weight of 36.3 kDa. The sugar component in both astragalans II and III is D-glucose. The average molecular weights of astragalans II and III are 12.3 kDa and 34.6 kDa, respectively. Astragalan II and astragalan III consist mainly of $\alpha(1\rightarrow4)$-linked glucopyranosyl residues and a small amount of $\alpha(1\rightarrow6)$-linked glucopyranosyl residues [61]. AG-1 is an α-glucan with $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ linkages, while AG-2 is an $\alpha(1\rightarrow4)$-glucan. The component sugars in AH-1 were identified as galacturonic acid, glucuronic acid, glucose, rhamnose, and arabinose, those in AH-2 as glucose and arabinose [62]. Amon-S is composed of L-arabinose, D-galactose, D-galacturonic acid and D-glucuronic acid, in addition to small amounts of *O*-acetyl groups and a peptide moiety [63].

An extract of the root of *A. membranaceus* was reported to exert immunostimulating activities in various test systems enhancing the NK activity of lymphocyte effector cells and acting synergistically with partially purified human IFN-α [65]. Another extract of the root was reported to significantly enhance the activity of IL-2, of B cell growth factor and IL-6 *in vitro* and of phytohemagglutinin-induced proliferation of T lymphocytes from patients with IgG subclass deficiency [66]. The administration of a polysaccharide fraction of *A. membranaceus*, which consists mainly of astragalans I and II, to mice at a daily dose of 1 g/kg *i.p.* for 6 days stimulated humoral immune functions and restored immunosuppression of mice caused by prednisolone and cyclophosphamide [67], [68]. After *i.p.* administration, the extract increased weight and cell number of mouse spleen, elevated the response of mouse spleen against sheep red blood cells, and stimulated the phagocytic activity of peritoneal macrophages [69]. Astragalans enhanced the secretion of TNF from human peripheral blood mononuclear cells *in vitro* [70].

The aqueous extract of the root of *A. membranaceus* was reported to inhibit the DNA synthesis in ovarian mucinous cystadenoma and ovarian papillary cystadenoma cells in a dose- and time-dependent manner [71]. Ten days following tumor cell transplantation an i.p. treatment with the extract inhibited the growth of murine renal cell carcinoma in BALB/c mice. The cure rate was 57% when the initial tumor load was 2×10^5 cells and 100% when the initial tumor load was 1×10^5 cells. Splenocytes from mice transplanted with renal carcinoma cells responded less favorably to IL-2 in generating lymphokine-activated killer cells; this depression was restored after treatment with *Astragalus* extract. It was suggested that *A. membranaceus* may exert its antitumor effect *via* augmentation of phagocyte and lymphokine-activated NK cell activities [72], [73]. The root of *A. membranaceus* was reported to be used as clinical adjuvant in radiotherapy or chemotherapy of cancer, such as small cell lung cancer [74]. A significant immunorestorative activity of the polysaccharide fraction with an increased local xenogeneic graft-versus-host reaction and blastogenic response of lymphocytes *in vitro* were also observed in mononuclear cells from cancer patients [75], [76].

Fructus Lycii (Gouqizi), the ripe fruit of *Lycium barbarum* L. (Solanaceae), is a further Chinese herbal medicine containing polysaccharides with immunostimulating and antitumor activities. It is listed in the Chinese Pharmacopoeia and is used as a general tonic. A number of peptidoglycans has been isolated from the fruit [77], [78], [79]. The polysaccharide content in different samples of *L. barbarum* ranges from 5% to 8% [80]. The fruit extract extensively increased the expression of the IL-2 receptor (α- and β-chains) on the membrane of tonsillar mononuclear cells [81]. *Lycium barbarum* polysaccharides increased the weight of spleen and thymus, and reticuloendothelial phagocytosis in normal mice [82]. They enhanced the IL-2 activity from aged mice (16 months) to the same extent as that of adult mice (2 months) [83].

The polysaccharides from *L. barbarum* fruits significantly enhanced the membrane PKC activity of rabbit red blood cells at a concentration of 100 μg/ml [84]. At concentrations of 50 – 400 μg/ml, the polysaccharide increased both the cellular cAMP and cGMP levels in mouse lymphocytes in a concentration-dependent manner [85]. At daily *i.p.* doses of 5 – 10 mg/kg to mice for 7 days, the polysaccharide increased spleen lymphocyte proliferation induced by Con A. The number of plaque-forming cells in the spleen in sheep red cell-immunized mice was increased by the polysaccharide [86]. A single *i.p.* injection of the fruit polysaccharides at a dose of 10 mg/kg induced proliferation of splenocytes in 12 months old mice, the lymphokine-activated killer cell activities being significantly higher than that of control animals. Combined use of polysaccharides with IL-2 *i.p.* resulted in a synergistic induction of lymphocyte-activated killer cell activities (LAK) from aged mice *in vitro* [87]. Furthermore, the polysaccharide was reported to partially or completely reverse the immunosuppressive effect of cyclophosphamide in mice [88].

In a clinical trial, 79 patients with advanced cancer were treated with LAK/IL-2 combining with *L. barbarum* polysaccharides: initial results from 75 evaluable patients indicated that objective regression of cancer was achieved in patients with malignant melanoma, renal cell carcinoma, colorectal carcinoma, lung cancer, and nasopharyngeal carcinoma. The response rate was 41%,

while that of patients treated with LAK/IL-2 alone was 16%. Patients treated with LAK/IL-2 plus polysaccharides showed a significantly longer mean remission period and a more pronounced increase in NK cell and LAK cell activity [89]. The polysaccharide also significantly increased the T lymphocyte blastogenic and phagocytic rate of macrophages in 171 cancer patients after radiotherapy [90].

Experimental studies showed that the polysaccharides enhanced the antineoplastic activity of irradiation and carmustine treatment in G422 tumor-bearing mice [91]. Furthermore, they exhibited radiosensitizing effects in mice transplanted with Lewis lung cancer [92]. The polysaccharide given to mice bearing S180 tumors significantly decreased tumor weight in a dose-dependent manner and increased the number of splenocytes, and it promoted the activated T cells, NK cell activity and TNFβ levels in tumor bearing mice [93].

Radix Codonopsis Pilosulae (Dangshen), the root of *Codonopsis pilosula* (Franch.) Nannf., *Codonopsis pilosula* Nannf. var. *modesta* (Nannf.) L. T. Shen or *Codonopsis tangshen* Oliv. (Campanulaceae), listed in the Chinese Pharmacopoeia, is known to contain polysaccharides and to exhibit immunostimulating and antitumor activities. It is used in Traditional Chinese Medicine mainly as a general tonic. Polysaccharides from the root of *C. pilosula* are composed of glucose, fructose, galactose, arabinose, mannose, rhamnose, xylose, and ribose in different ratios [94].

The extract of the root of *C. pilosula* was reported to enhance T-lymphocyte activity, but not NK cell activity of human lymphocytes *in vitro* and to stimulate the production of immunoglobulin by B-cells and of IL-1 by monocytes [95]. The extract of the root of *C. pilosula* was also reported to stimulate the immunological functions in normal and in cyclophosphamide-suppressed mice [96] as well as in tumor-bearing mice treated with cyclophosphamide [97]. The extract of the root of *C. pilosula* used as an adjuvant in 76 cancer patients during radiotherapy reduced the immunosuppressive effect of radiation [98].

Ribosome-Inactivating Proteins (RIP)

Ribosome-inactivating proteins (RIP), widespread throughout the plant kingdom, especially from Cucurbitaceae, are a group of proteins able to inactivate eukaryotic protein synthesis by attacking the 28S ribosomal RNA [99]. One of the first isolated, purified and sequenced RIP was trichosanthin from the root of *Trichosanthes kirilowii* Maxim. (Cucurbitaceae). Radix Trichosanthis (Tianhuafen), the dried root of *Trichosanthes kirilowii* or *Trichosanthes rosthomii* Harms is listed in the Chinese Pharmacopoeia and is used clinically to terminate pregnancy. Besides Radix Trichosanthis, also Fructus Trichosanthis (Gualou), the ripe fruit, Pericarpium Trichosanthis (Gualoupi), the pericarp of the ripe fruits; and Semen Trichosanthis (Gualouzi), the ripe seed of *T. kirilowii* or *T. rosthomii*, are listed in the Chinese Pharmacopoeia.

Trichosanthin is a relatively simple linear polypeptide composed of 246(7) amino acid residues with a C-terminus of Asn-Asn-Met or Asn-Asn-Met-Ala with a molecular weight of about 27 kDa [100]. X-ray diffraction and Raman spectroscopy of trichosanthin revealed 8 segments of an α-helix with about 85 amino acids, and 13 strands of β-sheet structure with about 70 amino acids, as well as some extended chains. The α-helices are in the center of the molecule and are surrounded by β-sheets [101].

α-Kirilowin, β-kirilowin and trichokirin are further RIP from the seed of *T. kirilowii*. β-Kirilowin possessing a molecular weight of 27.5 kDa did not show cross-reactivity with trichosanthin. Sequence comparison of the first 10 residues of β-kirilowin with trichosanthin and trichokirin indicated 60 – 70% identity [102]. α-Kirilowin was reported to have a molecular weight of 28.8 kDa. The amino acid composition of α-kirilowin grossly resembled β-kirilowin and other RIP. The *N*-terminal sequence of α-kirilowin was identical to that of β-kirilowin, at least in the first ten residues [103].

Trichosanthin inactivated ribosomes and arrested protein synthesis by removing a specific adenine from 28S rRNA. Experiments on the binding modes of trichosanthin with oligonucleotides GAG, GAGA, and CGAGAG as substrates showed that all the oligoribonucleotides can dock into the active cleft of trichosanthin without unfavorable contacts [104], [105]. Positions 120 – 123 of the native trichosanthin molecule may play a critical role in maintaining the inhibition of protein biosynthesis [106], [107]. Fragments corresponding to amino acids 1 – 72 and 153 – 246 are supposed to be the antigenic sites [108].

Trichosanthin was found to inhibit the growth of trichoblastic cells. It is therefore used for the treatment of abnormal growth of trichoblastic cells such as hydatiform and malignant moles and choriocarcinomas [109]. Studies on the *in vitro* cytotoxicity of trichosanthin showed that it selectively damaged choriocarcinoma and melanoma cells whereas hepatoma cells were resistant to the treatment. Cytotoxicity profiles of trichosanthin differed from those of anti-cancer drugs which interfere with DNA metabolism. The *N*-terminal peptides composed of amino acid residues 1 – 15 and 16 – 30 caused increases in concanavalin A (ConA) stimulated incorporation of ^{3}[H]thymidine into normal spleen cells at a concentration of 5 μg/ml. These peptides also showed growth inhibitory effects against L1210 leukemic cells *in vivo* [110].

Clinical trials using trichosanthin for the treatment of hydatidiform mole were successful in 44 of 52 patients (85%) including 38 complete remissions (73%) and 6 incomplete remissions (12%) [111]. *In vitro* studies revealed that trichosanthin induced the generation of reactive oxygen species in human choriocarcinoma cell line (JAR cells). The formation of reactive oxygen species induced by trichosanthin was dependent on the presence of extracellular Ca^{2+} and involved in the apoptosis of JAR cells [112]. Trichosanthin also induced apoptosis of HeLa cells [113].

The conjugation of trichosanthin to monoclonal antibodies formed an immunotoxin able to inhibit the growth of human melanoma and hepatoma cells *in vitro*. The cytotoxicity of the conjugate to M21 human melanoma cells was 2000-fold higher than that of a mixture composed of antibody and trichosanthin [114]. The cytotoxicity of the immunotoxin composed of trichosanthin and monoclonal antibody was 500-fold higher in inhibiting the growth

of human hepatoma cells as compared to free trichosanthin [115]. The *in vitro* cytotoxicity of the conjugated immunotoxin versus human colon carcinoma (LoVo) cells was approximately 150-fold higher than that of free trichosanthin or 2000-fold higher than that of the conjugate of trichosanthin with normal mouse immunoglobulin IgG. *In vivo* experiments showed that the immunotoxin also effectively inhibited human colon carcinoma xenografts in nude mice and prolonged the life span of tumor-bearing animals without obvious toxic effects to host mice [116].

Luffins, such as luffin-α [117], [118], luffin-β [119] and luffin-S [120] are RIPs from the fruit and seed of *Luffa cylindrica* (L.) Roem. (Cucurbitaceae). Luffins are basic glycoproteins in nature and possess a molecular weight of approximately 30,000. They are reported to exhibit abortifacient, antitumor, ribosome inactivating and immunomodulatory activities [121]. Luffin-α consists of 248 amino acid residues and possesses a relative molecular weight of 27,021 Da, excluding the attached sugar chains present at each Asn residue of positions 28, 33, 77, 84, 206, and 227. Retinervus Luffae Fructus (Sigualuo), the fibrovascular bundle of the ripe fruits of *L. cylindrica*, is listed in the Chinese Pharmacopoeia. Luffin-α has been observed to inhibit the proliferation and to induce apoptosis of human melanoma cells *in vitro* [122], [123].

Further known RIPs from Chinese herbal medicines are e.g. cochinchinin and momorcochin S from the seeds of *Momordica cochinchinensis* (Lour.) Spreng. (Cucurbitaceae) [124], [125]; cinnamomin and camphorin from the seeds of *Cinnamomum camphora* (L.) Sieb. (Lauraceae) [126], [127], [128], [129]; pokeweed antiviral proteins from the leaves and seeds of *Phytolacca americana* L. (Phytolaccaceae) [130], [131]; ricin from the seeds of *Ricinus communis* L. (Euphorbiaceae). Semen Momordicae (Mubiezi), the ripe seed of *M. cochinchinensis*; Semen Ricini (Bimazi), the ripe seed of *R. communis*; Radix Phytolaccae (Shanglu), the root of *P. americana* and *P. acinosa* are listed in the Chinese Pharmacopoeia. Pokeweed antiviral proteins [132], [133] and ricin [134] are used for the preparation of immunotoxin with monoclonal antibody. Ricin immunotoxins have been entered in clinical trials mainly in the treatment of lymphomas [134], [135], [136], [137], [138], [139], [140].

Conclusion

High molecular compounds from Chinese herbal medicines, including ribosome-inactivating proteins and polysaccharides from both fungi and high plants have been tested for the treatment of malignant diseases. Polysaccharides possessing immunostimulating activities can be used as adjuvants in tumor treatment. The fungi containing such polysaccharides are usually edible mushrooms or tonics in Traditional Chinese Medicine. Parts from high plants such as Radix Astragali and Fructus Lycii containing polysaccharides are mainly used as tonic in Traditional Chinese Medicine. Ribosome-inactivating proteins are a group of proteins exerting cytotoxic activities *via* inhibition of protein synthesis. Some of the ribosome-inactivating proteins have been used as the cytotoxic part in conjugates with monoclonal antibodies as tumor-targeting drugs. The cytotoxic and antineoplastic mechanisms of the high molecular compounds are rather different from those of the low molecular compounds described in part I (see footnote 3, p. 193). Obviously, because of genetic factors that influence enzyme levels accounting for sometimes striking differences in metabolism and pharmacokinetics of drugs, results obtained in clinical studies carried out in China or Japan are not transferable to 100% to the European population. Since many of the clinical studies referred to in this paper have been carried out on Asians, the outcome of such studies in Caucasians or Africans might not be the same.

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