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

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, polysaccharides 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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Bibliography

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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. Lentinarin 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. Lentinarin has a molecular weight of about 500 kDa [4], [5].

A multi-center prospective study of lentinarin 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 lentinarin was significantly longer than that of patients without lentinarin treatment (297 days vs. 199 days) [6]. Lentinarin 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 lentinarin treatment was observed [10], [11] as well as an enhanced induction of lymphokine-activated NK cell activity [12]. Addition of lentinarin to maintenance therapy with 5-FU did not exert prognostic benefit on hepatocellular carcinoma patients [13].

Lentinarin 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, lentinarin activated non-specific cytotoxicity of macrophages *in vitro* [14] and *in vivo* [15]. The beneficial effects of lentinarin 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 lentinarin [16]. Combinatory use of 5′-deoxy-5-fluorouridine and lentinarin against AH66 ascites hepatoma cells in rats resulted in a significant inhibition of tumor growth as compared with 5′-deoxy-5-fluorouridine alone. Lentinarin induced pyrimidine nucleoside phosphorylase activity in the tumor and increased the susceptibility of tumor cells to 5′-deoxy-5-fluorouridine [17]. Lentinarin 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)-β-glucans were reported to be more effective on T cells than B cells [19]. It was also suggested that lentinarin 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 lentinarin to human monocytes may initiate the influence of this compound on the immune system and differ between individuals [22].

Intrapertitoneal treatment of mice with lentinarin at a dose of 10 mg/kg affected the number, plastic-adherence, and endogenous peroxidase activity of peritoneal cells. Lentinarin-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 lentinarin (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 lentinarin and IL-2 was found to result in synergistic antitumor and antimetastatic effects in mice against spontaneously metastatic 3-methylcholanthrene-induced fibrosarcoma [26].

Lentinarin is relatively non-toxic. The LD₅₀ values of lentinarin 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 lentinon 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 lentinon [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 poly peptide 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, colon-rectum, 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 post-operative 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 antitumor 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 antitumor 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 HEPC2 with an IC50 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 180 cells with an inhibition rate of more than 93% on day 13. Intraperitoneal pretreatment 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 (Ley, ex Fr.) Karst. (Polyporaceae) [51], Polyporus umbellatus (Pers.) Pries (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 (Zhuiling), 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 astragalan 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 α-glucose, β-galactose, and α-arabinose in addition to trace amounts of pentose with an average molecular weight of 36.3 kDa. The sugar component in both astragalan II and III is D-glucose. The average molecular weights of astragalan II and III are 12.3 kDa and 34.6 kDa, respectively. Astragalan II and astragalan III consist mainly of α(1→4)-linked glucopyranosyl residues and a small amount of α(1→6)-linked glucopyranosyl residues [61]. AG-1 is an α-glucan with α(1→4) and α(1→6) linkages, while AG-2 is an α(1→4)-glucan. The component sugars in AH-1 were identified as galacturonic acid, gluconic acid, glucose, rhamnose, and arabinose, those in AH-2 as glucose and arabinose [62]. Amon-S is composed of α-arabinose, α-galactose, α-galacturonic acid and β-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 astragalan 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]. Astragalan enriched 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 × 10⁶ cells and 100% when the initial tumor load was 1 × 10⁶ 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 immunoregulatory activity of the polysaccharide fraction with an increased local xenogenic graft-versus-host reaction and blastogenic response of lymphocytes in *vitro* were also observed in mononuclear cells from cancer patients [75], [76].

Fructus Lycii (Gouqizhi), the ripe fruit of *Lycium barbarum* L. (Solanaeace), 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 5180 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 as a general tonic. Polysaccharides from the root of C. pilosula are composed of glucose, fructose, galactose, arabino, 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 trichokin 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 trichokin 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, GACA, 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 [3H]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 Ca2+ 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 RPs 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 fibrous vascular 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 RPs from Chinese herbal medicines are e.g. cochinchinin and moromorcin S from the seeds of Momordica cochininchinensis (Lour.) Spreng. (Cucurbitaceae) [124], [125]; cinnamomin and camphor 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. (Phytolaccaeaceae) [130], [131]; ricin from the seeds of Ricinus communis L. (Euphorbiaceae). Senem Momordicae (Mubiezi), the ripe seed of M. cochininchinensis; Senem Ricini (Biniazzi), 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.

References


8. Mio H, Terabe K. Clinical effects of postoperative immunomotherapy with a combination of 5-FU, CDDP and lentian for stage IVb gastric carcinoma and long-term pharmacokinetic studies on CDDP and 5-FU. Gan To Kagaku Ryoho 1997; 24: 337 – 42.


115 Zhang JP, Xu CJ, Cao HT, Ji RH, Zhang ZC. In vitro inhibition of tricho-


116 Wang QC, Ying WB, Xie H, Zhang ZC, Yang ZH, Ling LQ. Trichothion-

monoclonal antibody conjugate specifically cytotoxic to human he-


117 Gao HL, Zhou GY, Lu DY, Zhang WY. Trichothian – CEA Mab conjug-

eate cytotoxic to human colon carcinoma. Chin J Immunol 1992; 8: 300 – 3

118 Islam MR, Nishida H, Funatsu G. Complete amino acid sequence of

luffin-α, a ribosome-inactivating protein from the seeds of Luffa cylin-


119 Chen RS, Leung HW, Dong YC, Wong RN. Modeling of the three-di-

mensional structure of luffin-α and its stimulated reaction with the


120 Kataoka J, Hubaka N, Miyano M, Masuta C, Koivai A. Nucleotide se-

quence of cDNA encoding β-luffin, another ribosome-inactivating

protein from Luffa cylindrica. Plant Mol Biol 1993; 19: 887 – 9


novel ribosome-inactivating protein from Luffa cylindrica. Character-


122 Ng TB, Chan WY, Yeung HW. Proteins with abortifacient, ribosome in-

activating, immunomodulatory, antitumor and anti-AIDS activities

from Cucurbitaceae plants. Gen Pharmacol 1992; 23: 579 – 90

123 Poma A, Marzocchi G, Cesare P, Caramignani M, Spano L. Antiprolifera-

tive effect and apoptotic response in vitro of human melanoma cells to

liposomes containing the ribosome-inactivating protein luffin. Bio-


124 Poma A, Miranda M, Spano L. Differential response of human melano-

ma and Ehrlich ascites cells in vitro to the ribosome-inactivating pro-


125 Huang B, Ng TB, Fong WP, Wan CC, Yeung HW. Isolation of a trypsin

inhibitor with deletion of N-terminal pentapeptide from the seeds of

Momordica cochinchinensis, the Chinese drug mubiezhi. Int J Biochem

Cell Biol 1999; 31: 707 – 15


al. Squash trypsin inhibitors from Momordica cochinchinensis exhibit


127 Zhang AH, Tang S, Liu W. Substrate-structure dependence of ribotox-

ins on cleaving RNA in C. camphora ribosome. J Nat Toxins 2001; 10:

119 – 25

128 Xu YZ, Li YJ, Hu HY, Hu R, Wu H, Liu WY. Adenine nucleotide N-glyco-

sidase activity of the A-chain of cinnamomin characterized by H1-nu-


129 Ruan JP, Chen WF, Liu WY. Promotion of ATP and S-140 to ribosome

inactivation with camphorin, cinnamomin, and other RNA N-glycosi-


130 Li XD, Chen WF, Liu WY, Wang GH. Large-scale preparation of two

new ribosome-inactivating proteins-cinnamomin and camphorin

from the seeds of Cinnamomum camphora. Protein Expr Purif 1997;

10: 27 – 31

131 Kurinov IV, Mao C, Irvin JD, Uckun FM. X-ray crystallographic analysis

of pokeweed antiviral protein-II after reductive methylation of lysine


132 Ferens WA, Hovde CJ. Antiviral activity of shiga toxin 1: suppression of

bovine leukemia virus-related spontaneous lymphocyte proliferata-


133 Schlick J, Dulieu P, Desvoyes B, Adam P, Radom J, Jouvenot M. Cytoto-

xic activity of a recombinant GnR-H-PAP fusion toxin on human tum-

or cell lines. FEBS Lett 2000; 472: 241 – 6

134 Waurzyńska B, Schneider EA, Tumer N, Yanishevsky Y, Gunther R,

Chełstrom LM, et al. In vivo toxicity, pharmacokinetics, and anti-lym-

phic activity of TXU (anti-CD7)-pokeweed antiviral protein immu-


135 Zhong RK, van De Winkel JG, Thepen T, Schultz LD, Ball ED. Cytotoxic-

ity of anti-cd64-ricin a chain immunotoxin against human acute

myeloid leukemia cells in vitro and in scid mice. J Hematother Stem

Cell Res 2001; 10: 95 – 105

136 van Oosterhout YV, van Ernst JL, Bakker HH, Preijers FW, Schatten-

berg AV, Ruiters DJ, et al. Production of anti-CD3 and anti-CD7 ricin

A-immunotoxins for a clinical pilot study. Int J Pharm 2001; 221:

175 – 86

137 Schindler J, Sausville E, Messmann R, Uhr JW, Vitetta ES. The toxicity of
deglycosylated ricin A chain–containing immunotoxins in patients

with non-Hodgkin’s lymphoma is exacerbated by prior radiotherapy:

a retrospective analysis of patients in five clinical trials. Clin Cancer

Res 2001; 7: 255 – 8


Combination chemotherapy followed by an immunotoxin (anti-B4-

blocked ricin) in patients with indolent lymphoma: results of a phase


139 Messmann RA, Vitetta ES, Headlee D, Senderowicz AM, Figg WD,

Schindler J, et al. A phase I study of combination therapy with immu-

notoxins IgG-HD37-deglycosylated ricin A chain (dGA) and IgG-RBF4-

dGA (Combotox) in patients with refractory CD19 (+), CD22 (+) B cell


Treatment to refractory Hodgkin’s lymphoma patients with an anti-


141 Grossbard ML, Multani PS, Freedman AS, O’Day S, Gribben JC, Rhuda

C, et al. A Phase II study of adjuvant therapy with anti-B4-blocked ric-

in after autologous bone marrow transplantation for patients with


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