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OVERVIEW ON SIGNIFICANCE OF PECTIN IN HEALTH CARE

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ABSTRACT

Pectin, a naturally occurring polysaccharide, has in recent years gained increasingly in importance. The benefits of natural pectin are also more and more appreciated by scientists and consumer due to its biodegradability. Pectin is the methylated ester of polygalacturonic acid. It is commercially extracted from citrus peels and apple pomace under slightly acidic conditions. Pectins are divided into two major groups on the basis of their degree of esterification. The association of pectin chains leads to the formation of the three dimensional networks that is to gel formation. The pectin, by itself or by its gelling properties, was employed in immunostimulating activity, anti-metastasis activity, anti-ulcer activity and anti-nephrosis activity.This review will discuss the significance of pectins on health care.

KEYWORDS: Pectin, Biodegradability, Immunostimulating activity, Anti-metastasis activity, Anti-ulcer activity.

INTRODUCTION

Many plants have long been known to have medicinal effect therefore have been used as medicines especially in traditional medicines. Even today, about 75% of the world population still relies on plant, plant extracts and other tools of traditional medicines in basic health

need. While incidences of numerous globally rampant infections had been reduced by the development of the modern medicines such as antibiotics.[1] Furthermore, pressing medical problems such as non-specific, constitutional or psychosomatic diseases have also increased. Disillusion with modern medicine has on occasion brought about by severe adverse effects of synthetic compounds. Because of these circumstances traditional medicinal herbs have begun to attract worldwide attention. Herbal extracts contain substances with both low and high molecular weight. Although biologically active substances of the former compounds in medicinal herbs including Kampo (Japanese herbal) medicines have been studied, they cannot account for all of the clinical effects achieved. Pharmacological activities have been observed in fractions with high molecular weights from boiled water extracts of the medicinal herbs.Of the high molecular weight substances, various pharmacological activities such as immunostimulating activity; complement activating activity, mitogenic activity, Fc receptor up-regulation on macrophages, anti-ulcer activity, anti-metastasis activity and anti-nephritis activity have been observed in pectin polysaccharides isolated from medicinal herbs.[2-6]

Pectin is a structural polysaccharide which forms an important component of middle lamella and primary cell wall of higher plants. Pectin have been used as important food fiber, ubiquitous nutritional factor and gelling agent in food. Pectic polysaccharides comprise a family of rhamno-galacturonans and several neutral oligosaccharides (arabino and galacto-oligosaccharides) and polysaccharides (arabinans, galactans, arabino-galactans) which are believed to be covalently attached to the rhamno-galacturonan backbone primarily through the rhamno-pyranosyl residues.[7-9] They are usually extracted from walls by digestion with endo-polygalacturonase (E.C. 3.2.1.15), exo-polygalacturonase (E.C. 3.2.1.67), pectin lyase (E.C. 4.2.2.10) and pectin esterase (E.C. 3.1.1.11).[10-12] The present paper deals with significance of pectins and pecticpolysaccharides in health care.

Anti-metastasis activity

Prostate cancer is the most commonly diagnosed cancer in U.S. men and is the second leading cause of male cancer deaths.[13] Approximately 50% of patients diagnosed with prostate cancer have disease that has or will metastasize to the skeletal system. At present, there is no effective curative therapy and very little palliative therapy for patients with metastatic disease.[14] The process of tumor cell metastasis requires that cells depart from primary tumors, invade the basement membrane, travels through the blood stream, interact with the vascular endothelium of the target organ and proliferate to form secondary tumor

colonies.[15] It is generally that many stages of this metastatic cascade involve cellular interactions mediated by cell surface components, such as carbohydrate binding proteins, which include galectins.[16]

Tumor galactose binding proteins mediate cellular recognition by linking oligosaccharides with terminal D-galactoside residues on adjacent cells. Platt and Raz tested the effect of citrus pectin (CP) on modulation of the lung colonization of B16-F1 melanoma cells, and found that conjunction of modified citrus pectin (MCP), which was prepared by alkaline treatment and mild acid hydrolysis of the pectin, with B16 melanoma cells resulted in a marked inhibition (>90%) of their ability to colonize the lungs of the mice receiving the injection, however natural pectin resulted in a significant increase in the appearance of tumor colonies in the lung.[17] Oral administration of MCP also inhibited spontaneous metastatic colony formation of rat prostate adenocarcinoma MAT-LyLu cells.[14]MCP inhibited MAT-LyLu cell adhesion to rat endothelial cells in time and dose-dependent manners. From these observations, they concluded that oral intake of MCP acts as a potent inhibitor of spontaneous prostate carcinoma metastasis in the rats.[14] Because galectin-3 is present in human prostate adenocarcinoma cell line and MCP interferes with cell-cell interactions mediated by cell surface galectin-3 molecules,[14] it was suggested that the inhibition of cancer metastasis might be caused by the action of galactoserich ramified region in MCP against cell-cell interactions.

Anti-tumor activity

The polysaccharide fraction from the roots of *Angelica acutiloba* was found to have a potent antitumor activity against ascetic form of Sarcoma-180, IMC carcinoma and MethA fibro sarcoma as well as the solid tumor MM-46 tumor.[18] Purified active polysaccharide, AR-4E-2 was characterized to contain rhamno-galacturonan moiety in which 2,4-disubstituted rhamnoseresidues were attached to 4-substituted galacturonic acid through position 2 of rhamnose. AR-4E-2 also contained highly branched 3,5-arabinan and (l-4)-galactan.[18]

Anti-nephrosis activity

Salviaemiltiorrhizae radix (SMR) has been used in China for the treatment of various renal diseases or lesions in blood circulation. Guoji et al. reportedthat SMR suppressed urinary protein excretion and improved levels of serum albumin, cholesterol and lipid peroxide in rats which had amino nucleoside (puromycin) induced nephrosis.[19] They purified the active component in a hot-water extracts of SMR, and certain acidic polysaccharide containing 80%

galacturonic acid, probably pectin, was found to reduce urinary protein excretion in rats with the experimental nephrosis by oral or intramuscular administration.[19] Electron microscopical analysis also revealed that the extent and the severity of lesions of the epithelial cells in glomerulus were significantly less in the rats treated with this acidic polysaccharide. Because the effect on experimental nephrosis was decreased by a reduction of carboxyl groups, it was suggested that pectin may stabilize and restore the glomerular basement membrane structure by its polyanionic nature due to galacturonan region, and this may contribute to the improvement of puromycin-induced nephrosis.

Application for hepatic drug delivery

Delivery of diagnostic or therapeutic agents to hepatocytes has often been achieved by attachment of the agent to carrier molecules that bind the asialoglycoprotein receptor.[20] Pectins consist of ramified region which substituted arabinansandarabinogalactans. Groman et al. demonstrated that intravenous injection of radiolabelled arabinogalactan (4mg/kg) from the tree Larix*Occidentalis* in rats resulted in 52.5% of the dose being present in the liver, while prior injection of asialofetuin (100mg/kg) reduced hepatic radioactivity to 3.54%.[21] When the titratedarabinogalactan was injected, radioactivity cleared from the liver with a half-life of 3.42 days.[21] The arabinogalactan produced no adverse reactions in single intravenous dose (mouse, 5000mg/kg) and repeat dose toxcity studies (rats, 500mg/kg/day, 90days). This arabinogalactan had highly branched structure and consisted mainly of terminal 6-1inked and 3, 6-disubstituted-galactopyranosyl and terminal a-arabinofuranosyl and arabinopyranosyl residues. Therefore it indicates that arabinogalactan is suitable as carrier for delivering diagonostic or therapeutic agents to hepatocytes via the asialoglycoprotein receptor.

Vaccine for typhoid fever

Capsular polysaccharide (Vi) is an essential virulence factor and protective antigen of *Salmonella typhi*.[22] Vi is a linearhomopolymer of (1-4)D-GalpANAc.[23] Field trials in Nepal and in Republic of South Africa showed that a single injection of Vi conferred about 70% protection against typhoid fever in older children and in adults.[24] Its protective action is to elicit a critical level of serum antibodies and the Vi conjugates elicited significantly higher levels of serumantibodies than did Vi alone. Because high molecular weight of Vi causes the conjugates to be poorly soluble and standardization is very difficult, Szu et al. attempted to apply citrus pectin as animmunogen instead of Vi.[24] Pectins were mainly composed of (1-4)-

linked a-D-galacturonic acid with small of neutral sugars. Therefore, they prepared O-acetylated pectin (OAcPec) which has different structure from Vi. Pectin did not react with Vi antiserum in double immune diffusion, but OAcPec formed a line of identity with Vi. OAcPec is not immunogenic as like Vi, but OAcPec conjugated to tetanus toxoid elicited Vi antibodies in mice, and reinjection elicited a booster response. These observations indicates that the use of pectin as an immunogen for prevention of a systemic infection caused by capsulated pathogen (*S. typhi*) provides a novel approach to improve the preparation and immunogenicity of polysaccharide-based vaccines.[24]

Immunostimulating activity

Fc-receptor up-regulation on macrophages

Combination of circulating antigens and antibodies to form immune complexes is a normal phenomenon, and normally this immune complex eliminates by reticuloendothelial system. But, if the excessive quantities are formed, immune complex deposits to several tissues, in which case the complement system is activated, resulting in anaphylatoxin formation and then several inflammations occur. Therefore it has been considered that this deposition of immune complex may be one of the causes of auto immune disease development. A primary function of mononuclear phagocytic cells is to bind immune complex through Fc and complement receptors, followed by subsequent endocytosis and degradation.

Therefore the binding of immune complex to these cells is an important functional parameter for immune complex clearance, and the enhancing substance for this binding has a possibility that is able to treat auto-immune diseases. Matsumoto etal. developed a new photometric micro assay for immune complex binding to macrophages in a homologous system.[25] When the ability of immune complex clearance through Fc receptor of macrophages was tested by this assay, acidic pectic polysaccharide, bupleuran2IIb, from *Bupleurumfalcatum* was found to have a potent activity.[26]

Bupleuran 2IIb remarkably enhanced GAG binding through Fc receptor of macrophages. When bupleuran 2IIb was administrated to the mice, immune complex was clearanced from thecirculating blood by dose dependent manner.[27]However, bupleuran 2IIb did not affect to the carbon clearance. These results suggest that bupleuran 2IIb specifically potentiate function of macrophage in clearance of immune complex through Fc receptor (FcR). Bupleuran 2IIb, which is a homogenous polysaccharide, and has a molecular weight of

23,000, consists of highly methyl esterified and less esterified galacturonan regions, ramified region and KDO containing region.[28-30]

The ramified region consists of rhamno-galacturonan core which is substituted with several neutral carbohydrate side chains such as arabino and galacto-oligosaccharides, arabinogalactan or arabinopyranan. KDO-containing region resembles to rhamnogalacturonan II (RG-II) which was first reported in plant cell wall by Darvill et al.[30] This region consisted of galacturonan substituted with rare sugars in plant cells such as KDO, DHA, apiose, aceric acid, 2-methylfucose and 2-metylxylose. When bupleuran 2IIb was treated with endo-polygalacturonase, the enzyme resistant ramified region, PG-1, showed a potent activity, but the activities of KDO-containing region and oligogalacturonides were weak or negligible. This result suggest that the ramified region consisting of rhamnogalacturonan core with neutral carbohydrate chains is important for the binding activity. Scatchard analysis indicated that bupleuran 2IIb enhanced FcR expression on macrophage cell surface but did not increase affinity of the receptors. Bupleuran 2IIb-stimulated cells showed enhanced expression of both FcRI and FcRII m-RNA, which were measured as PCR products.[31] When macrophages were incubated with bupleuran 2IIb, a rapid increase of intracellular Ca^{2+} level was observed by a fluorescence image analysis using the calcium-sensitive dye, Fura-2.[32] However, signal transduction through protein kinases C and A did not involve to the expression of activity. These observations suggest that bupleuran 2IIb may be bind to the macrophage through the specific polysaccharide receptor for this ramified region, then this stimulation enhances FcRgene-expression by through signal transduction due to the increase of intracellular Ca^{2+}, and then FcR protein may be up-regulated on the macrophage surface.

Complement activating (anti-complementary) activity

The complement system consists of over 20 serum proteins including 9 complement components (C1-C9) and their regulators. The complement proteins are activated by a cascade mechanism of classical or alternative pathways. The classical pathway is activated by the binding of C1 to the Fc region of immune complexes containing IgM and IgG antibodies and is followed by further activation. On the other hand alternative pathway is directly activated from C3 by some activators such as lipopolysaccharide, and is followed by further activation. Complement activation appears to be intrinsically associated with several immune reactions such as the activation of macrophages and lymphocytes.[33] Several complement

activating polysaccharides have been discovered in the medicinal herbs such as the roots of *Angelica acutiloba Kitagawa*, the leaves of *Artemisia princeps* PAMP, the roots of *Bupleurumfalcatum L*, the roots and leaves of *Panax ginseng*, the roots of *Glycyrrhizauralensis Fisch* and berries of *Viscum album*var. album.[34-35]

Six kinds of complement activating pectins (AR-2IIa, 2IIb, 2IIc and 2IId) and pecticarabinogalactans (AGIIa, AGIIb-1) have been purified from the hot water extract of the roots of*Angelilcaacutiloba*.[36-38]AGIIa and AR-2IId showed the most potent complement activating activity. AGIIb-1 showed moderate activity and others were weak. These four pectins commonly consisit of over 90% a-(1-4) linked galacturonan and a small amount of ramified region which contains rhamnogalacturonan core with neutral carbohydrate side chains such as arabinan, galactan and arabinogalactan which are assumed to be linked to position 4 of rhamnose.[38] Digestion withendo-a-(1-4)-polygalacturonase after deesterification gave ramified region as an enzyme resistant fraction and several oligogalacturonides.[38] The ramified region from each pectin had a more potent complement activating activity than the corresponding original pectin, but the oligogalacturonides had weak or negligible activities.[39] These results suggest that for AR-2IIa, 2IIb, 2IIc and 2IId, ramified region might be essential for expression of the activity since each ramified region from four pectins showed similar potent activity. Because (l-3,6)-linked long galactosyl chains and oligosaccharides containing (1-6)-galactose, which were obtained from ramified regions, showed potent or significant activity, for AR-2IIa, 2IIb, 2IIc and 2IId, the neutral carbohydrate side chains attached to the rhamnogalacturonan core might be essential for expression of the activity.[39]

Although AR-2IIa-IIc activated complement through the classical pathway but not the alternative pathway, all ramified regions (PG-1) activated complement through both pathways. AR-2IId had a different methyl-ester distribution in the galacturonan region compared to the other three pectins.[40]These results suggest that this galacturonan moiety may modulate the activation of an alternative pathwayby the ramified region, and this modulation may be controlled by the distribution of the methyl-ester on the polygalacturonan moiety. Both arabinogalactans, AGIIa and AGIIb-1, were characterized to contain arabino-3, 6-galactans by structural analysis.[36-38]Thesecarbohydrate structures seem to be common for complement activation by pectins and pectic polysaccharides. Acidic arabinogalactan from berries of *Viscumalbum* also strongly activated.

Other immunostimulating activity

Wagner et al. obtained immunostimulating pectic polysaccharides from plant cell culture of *Echinacea purpurea.*[5] From the extracellular polysaccharide mixture, acidic arabinogalactan (*Echinacea*polysaccharide II) was purified.[41]*Echinacea* polysaccharide II, which has a molecular weight at 75,000 was effective in activating macrophages to cytotoxicity against tumor cells [42] and in vitro as well as in vivo against microorganisms such as *Leishmaniaenriettii* and *Candida albicans*. This polysaccharide induced macrophages to produce tumor necrosis factor (TNF-a), interleukin-1 (IL-1), interferon-2 and oxygen radicals. *Echinacea* polysaccharide II consists of arabino-3, 6-galactan part, rhamnogalacturonan part and arabinan part.[5]therefore it is suggested that the polysaccharide may be certain ramified region of pectic polysaccharide. Compounds of plant origin which modify immunological responses have also been shown to influence natural cytotoxicity against tumor cells. *Viscum album* extracts (Iscador-M) contain a component which strongly increases the cytolytic activity of peripheral blood mononuclear cells (PBMCs) from human.[43]The rapid formation of conjugates between effector cells and tumor cells in the presence of *V.album*extracts appears to involve bridging by*V.album*rhanmogalacturonan which enhances the cytotoxicity of human NK cells.[44] Pre-incubation of NK cells with the rhamnogalacturonan did not activate the potential. Therefore, a synergistic effect of NK cell-tumor cell bridging by the rhanmogalacturonan, together with the activation of NK cytotoxicity by physiological response modifiers such as interleukin-2 might offer a new basis for the effective treatment of cancer.[44]

Mitogenic polysaccharide was obtained from the roots of *Glycyrrhizauralensis.*[34]Endo-a-(1-4)-polygalacturonase digestion indicates, this mitogenic polysaccharide has a pectic nature and the enzyme resisitant ramified region showed more potent mitogenic activity than the original polysaccharide. This mitogenic polysaccharide also contained RG-II like region. Several arabino-3,6-galactans, which were obtained from *Panaxnotoginseng* and *Saposhinikoviadivaricata*, were observed to activate the reticuloendothelial system (RES) in vivo by carbon clearance test.[45-46]Pectins from *Glycyrrhizauralensis*, *Euchommiaulmoides* and *Angelica acutiloba*also had RES activating activity, and arabino-3,6-galactan rich pectin showed more potent activity.[47-49]

Anti-ulcer activity

During a study of the polysaccharides from Chinese herbs, potent anti-ulcer activity was observed in the acidic polysaccharide fraction (BR-2) from *Bupleurumfalcatum*, and the active polysaccharides, bupleuran 2IIb and 2IIc were purified.[50]Hundred mg/kg ofbupleuran 2IIb and 2IIc showed the significant anti-ulcer activity against HCl-ethanol induced ulcero genesis in mice. This activity was almost same with positive control, sucralfate. The activity of bupleuran 2IIc was higher than that of sucralfate. Bupleuran 2IIc, which has a molecular weight of 63,000 consists of 85.8% of galacturonan region comprising of 70% of a-(l-4)-linked galacturonic acid, 30% of carboxymethylatedgalacturonic acid and branched galacturonic acid.[51]Bupleuran 2IIc also contained ramified region which consisted of rhamnogalacturonan core and several arabino and galactooligosaccharide side chains attached to either 2-1inked rhamnosyl residue through 4-linked galacturonic acid or 2-1inked rhamnose directly in the rhamnogalacturonan core. RG-II like region was also contained in bupleuran 2IIc as a minor region.[51-52]

The oral administration of BR-2 at doses 50 to 200mg/kg prevented the formation of gastric lesions induced by HCl-ethanol by dose dependent manner.[53]Theintraperitoneal and the subcutaneous administrations of BR-2 also dose dependently reduced this gastric lesion. These results suggested that BR-2 exerts through not only a local action but also a systemic action in the stomach. BR-2 also inhibited a variety of acute and chronic experimental ulcer models such as ethanol induced ulcer, indomethacin-HC1 induced ulcer, pyrolus ligated ulcer, water-immersion stress ulcer and acetic acid induced ulcer by oral administration.[54] The collective results suggested that the major mechanism of mucosal protection by BR-2 may be due to its anti-secretory activity on acid and pepsin, its increased protective coating and its radical scavenging effects but not involved in the action of endogenous prostaglandins and mucus synthesis.[53]

The ramified region seemed to be one of active site in bupleuran 2IIc. Therefore anti polysaccharide antibody was made by immunization of its ramified region (PG-1) to the rabbits. Then highly sensitive ELISA method using the purified antibody was developed in order to detect the active polysaccharide. This method is very useful for quality control of the active polysaccharide and for study of absorption to the body and pharmacodynamics of the polysaccharide.[54] In this ELISA system, anti-bupleuran2IIc-PG-1 antibody, which was purified by Protein G-Sepharose, was coated as first antibody on the micro titer plate, and the

ramified region was detected by the biotynylatedanti-bupleuran 2IIc-PG-1, which was purified by both Protein-G Sepharose and bupleuran 2IIc- PG-1 immobilized Sepharose, and the enzyme labelled streptavidin. Bupleuran 2IIc-PG-1 at concentrations greater than lng/well could be measured by this two site sandwich ELISA method. Immunohistograph showed that lymph-follicle in payer's patch and liver both were stained with anti-bupleuran 2IIc-PG-1 antibody specific IgG after oral administration of bupleuran 2IIc. Bupleuran 2IIc-PG-1 was also detected in the liver homogenate one week after oral administration by sandwich ELISA method. These results indicate that at least a part of bupleuran 2IIc was absorbed to the body after the oral administration.

Ginseng leaves also contained a unique anti-ulcer polysaccharide.[55] The most active anti-ulcer polysaccharide, GL-BIII, had a molecular weight at 16,000 and 56.6% of neutral sugar and 33.1% of uronic acid were contained. As major component sugars, rhamnose, arabinose, galactose, galacturonicacid and glucuronic acid were detected. Although most of phamacologically active pectins consist of ramified region, galacturonan region and RG-II like region, GL-BIII was endo -polygalacturonase resistant and contained only ramified region which consists of rhamnogalacturonan core attached long neutral carbohydrate side chains composed of 6-1inked glucosyl, 2-1inked mannosyl and other sugar residues and short side chains composed of arabinosyl and galactosylresidues.[55] Oral administration of GL-BIII showed a potent anti-ulcer activity against HC1/ethanol induced gastric ulcer of mice by dose dependent manner.

CONCLUSION

Pectins and pectic polysaccharides involve in several pharmacological activities. Each pharmacological activity of the pectins may depend on their fine chemical structure of characteristic structural units such as galacturonan, ramified region (rhamnogalacturonan core substituted arabinan and/or arabinogalactan) and rhamnogalacturonan II (RG-II) like region in pectin molecules. Many pharmacological activities have been appeared in the ramified region therefore detailed structural analysis of neutral carbohydrate side chains will be required in order to elucidate exact essential carbohydrate sequence for the expression of activity. Most of pharmacologically active pectins contained RG-II like region therefore it also has a possibility to involve in some phamacological activity. Even if natural pectin had no activity, chemical andenzymic modification of pectin may provide useful product for

health care. Present observations suggest that application of pectins on health care brings many possibilities of benefits for human being.

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