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Application of monoclonal antibody against ginsenoside in ginseng research: a review

Yui Sasaki[1], Koichi Shimizu[1], Hitoshi Watanabe[2], Yukihiro Shoyama[3*]

[1]Association for Health Economics Research and Social Insurance and Welfare, Tokyo 105-0003, Japan; [2]Center for Environment, Health and Field Sciences, Chiba University, Kashiwa 277-0882, Japan; [3]Faculty of Pharmacy, Nagasaki International University, Nagasaki 859-3298, Japan.

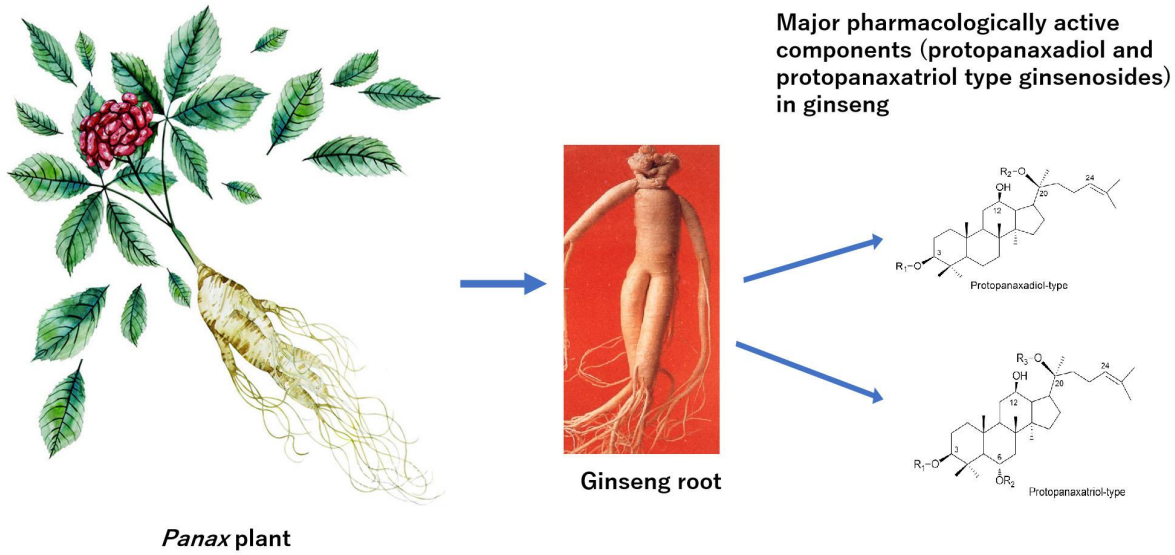
*Corresponding to: Yukihiro Shoyama. Faculty of Pharmacy, Nagasaki International University, 2825-7 Huis-Ten Bosch, Sasebo, Nagasaki 859-3298, Japan. E-mail: shoyama@niu.ac.jp.

Highlights

Newly developed Eastern blotting can stain only ginsenosides in the crude extract, and double Eastern blotting suggests type of ginsenosides and sugar number for raff structure of ginsenosides. Immunoaffinity expands one-step ginsenoside isolation to include knockout extract which make evident the really pharmacological activity of hapten molecule in the crude extract.

Tradition

Ginseng was listed in the Chinese book *Shennong's Classic of Materia Medica* (Shennong, 200 C.E. and 250 C.E.) and introduced historically as a most important medicine in almost all medicinal books until today and had been cultivated from 5th century in China. More than 200 ginsenosides which are most typical constituents in ginseng, have been isolated to demonstrate wide and complicated pharmacological activities. Although chromatographic analysis solved the quality control of ginseng, some new innovation is desired for scientific evaluation of ginseng.



Abstract

Panax genus belonging to a family of Araliaceae grow in Asia (9 species) and in North America (2 species). Especially *Panax ginseng* was listed in Chinese medical book, *Shennong's Classic of Materia Medica* approximately 2 thousand years ago and *Panax* species are now one of the most important natural medicine. Since Panax species contain approximately 260 ginsenosides, its quality control and pharmacological movement of ginsenoside in body are not enough understanding. Monoclonal antibodies against ginsenosides were prepared and set up the enzyme linked immunosorbent assay system for the quality control of natural product. Furthermore, we developed Eastern blotting system using monoclonal antibodies resulted that protopanaxadiol and protopanaxatriol group ginsenosides can be separately stained by two corresponding monoclonal antibodies, respectively. It became evident that the ginseng slice was stained by Eastern blotting system. Histochemical staining of ginseng can make clear the ginsenoside-Rb1 distribution in cells and tissues. Double Eastern blotting system facilitated by two monoclonal antibodies like anti-ginsenoside-Rb1 and anti-ginsenoside-Rg1 monoclonal antibodies gives several information such as sugar number, structure, and qualitative/quantitative evaluation. Immunoaffinity column combined with monoclonal antibodies succeeded one-step isolation of ginsenoside and make it possible to prepare knockout extract of which only antigen molecule was removed suggesting the pharmacological and biological value of antigen molecule in the crude extract.

Keywords: *Panax* species, Ginsenoside, Monoclonal antibody, Pharmacological activity, Eastern blotting, Knockout extract

***Author contributions*:**

Yui Sasaki and Koichi Shimizu collected and discussed the comprehensive data on monoclonal antibodies against natural products having small molecule. Hitoshi Watanabe collected data and discussed about the biological activity and metabolism of ginsenosides. Yukihiro Shoyama prepared the manuscript from draft to final version. All authors read several times, corrected and improved the manuscript.

***Competing interests*:**

The authors declare no conflicts of interest.

***Acknowledgments*:**

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***Abbreviations*:**

G, ginsenoside; PPD, protopanaxadiol; PPT, protopanaxatriol; TLC, thin-layer chromatography; GC-MS, gas chromatography coupled with mass spectrometry; HPLC, high-performance liquid chromatography; THCA, anti-tetrahydrocannabinolic acid; LC-MS, liquid chromatography coupled with mass spectrometry; PAb, polyclonal antibody; MAb, monoclonal antibody; ELISA, enzyme linked immunosorbent assay; BSA, bobbin serum albumin; MALDI-tof-MS, matrix assisted laser desorption/ionization time of flight mass spectrometry.

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Background

Panax genus belonging to a family of Araliaceae consists 11 species, 9 growing in Asia and 2 in North America. Among them *Panax ginseng*, *Panax quinquefolius* and *Panax notoginseng* are cultivated and three major species in the world medical market. Ginseng, *P. ginseng* was listed in 120 safety medicines of the Chinese book *Shennong's Classic of Materia Medica* (Shennong, 200 C.E. and 250 C.E.). Ginseng has been introduced historically as a most important medicine in almost all medicinal books in China, Korea and Japan until today. Latin name of ginseng was named by Russian botanist, Carl Anton Meyer in 1843. The genus name comes from "cure all" in Greek, and species name, ginseng means Chinese pronunciation of Renshen. This species is characterized from the other *Panax* species like root form resembling to human body and origin limiting in northern China, Korea and a part of Siberia. The shape of *P. ginseng* is changed depending on the cultivation period like the number of palmate leaves which is 1, 2, 3, 4, 5 and 6, respectively cultivated for 1 to 6 years although the number of leaflet in palmate leaf is 5 except 1 year old plant (Figure 1). It is suggested that *P. ginseng* had been started to cultivate from 5th century in China. In *Panax* species several components like ginsenoside(G)s, triterpene saponins, polyacetylenes, polysaccharides, phenolics, alkaloids, lignans and peptides are contained [1]. Yang et al. [2] reviewed that 257 dammarane-type triterpene, 14 octillol-type triterpenes and 18 oleanana-type triterpenes were isolated from *Panax* species. Among them G is the typical component in *Panax* species and has a wide pharmacological spectrum like analgesic, anti-febric, central nervous system suppression, sleep induction, promotion of cholesterol biosynthesis, promotion of neutral lipid synthesis, promotion of adrenal cortex hormone secretion, anti-fatigue, improvement of memory and learning, promotion of synthesis of DNA and RNA and central nervous system excitation [3]. G is biosynthesized by the pathway of terpenoid, via farnesyl group from squalene. Han et al confirmed that cytochrome P450 catalyzes the pathways of protopanaxadiol (PPD) from dammarenediol-II [4] and protopanaxatriol (PPT) from PPD [5]. Regarding G biosynthetic enzyme gene, several investigations have been performed on the correlation studies of biosynthetic enzyme activity, gene expression and G concentration [6], G profile and related gene expression [7] and expression of G biosynthesis related gene [8]. Dammarane-type and octillol-type triterpenes are closely related their biosynthetic pathways. Furthermore, dammarane-type triterpenes are divided into two Gs; PPD and PPT having sugars attached to hydroxyl group at C-3 and C-20 or at C-6 and C-20, respectively.

The quality of natural products variation depends on species differences, growing places, cultivation condition and harvest season resulted that almost all countries developed own pharmacopoeia for the confirmation of quality control against natural products which induce chromatographic analyses like thin-layer chromatography (TLC), capillary electrochromatography [9], gas chromatography coupled with mass spectrometry (GC-MS) [10], high-performance liquid chromatography (HPLC) and

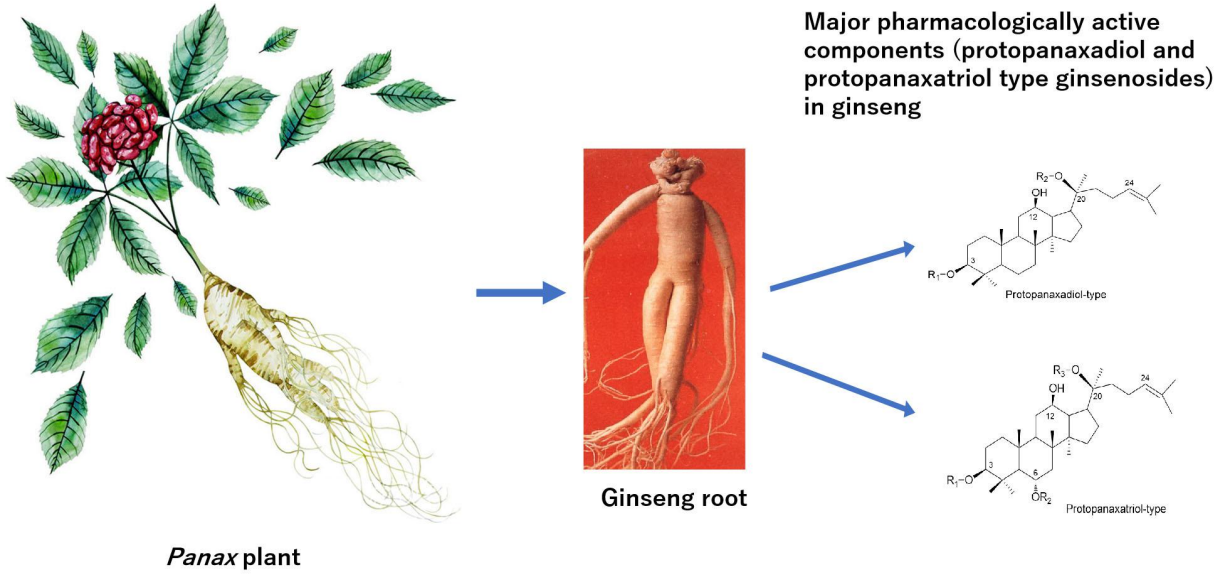

Figure 1 Whole plant, medicinal part and active compounds of *Panax ginseng*

coupled with MS (LC-MS) [11], and LC-MS/MS [12] coupled with an evaporative light scattering detector [13]. Wide knowledges regarding on chemistry, physics, biology and biochemistry were incorporated resulted that the rapid progress of biotechnology and molecular biosciences bring polyclonal antibody (PAb) against natural products until 1970s. Although during 1980s many monoclonal antibodies (MAbs) [14] have been accumulated on medicinal field, few limited except targeted drugs such as morphine [15] in natural products. The preparation of MAbs for natural product like terpenoids [16–19], alkaloids [20, 21], phenolic compounds [22–25], plant saponins [26–31], increased in 1990s and recently reached to variety of natural product as an important assay technique as indicated in Table 1.

Since Gs have wide majority [2], their quality control in *Panax* species have been expanded like TLC, gas liquid chromatography, HPLC, LC-MS and the enzyme linked immunosorbent assay (ELISA) system using MAb. In our ongoing study for the quality control of bioactive medical products, we continued to prepare MAbs as listed in Table 1 for natural products including ginsenosids [28–31]. Recently Yan et al. [32] and Wang et al. [33] reviewed detail about MAbs against natural products. From the survey of MAbs against Gs, G-Rb1 [28], Rg1 [29], Re [30] and notoginsenoside R1 [31] and G-Rg3 [34] have been established during 2000 to 2020. Furthermore, we established a new staining system on membrane using individual MAb following Northern, Southern and Western blotting and named as Eastern blotting. This newly developed methodology well functioned for quantitative and qualitative control of *Panax* species. Moreover, immunoaffinity concentration of antigen by affinity column combined with MAb can be used for quality control of small amount of G. In this case the eluate fraction by washing solution showed the antigen removing profile from the crude extract resulted to be called as knockout extract which can be used for confirmation of really active component. These methodologies also will be discussed in this review.

Table 1 Monoclonal antibodies against natural product

Component plant	Plant resource	Reference
Forskolin	*Coleus forskolii*	[16]
Crocin	*Crocus sativus*	[17]
Paeoniflorin, albiflorin	*Paeonia lactiflora*	[18]
Artemisinin, artesunate	*Artemisia annua*	[19]
Morphine, thebaine, codeine	*Papaver somuniferum*	[15, 20]
Berbarine	*Coptis japonica*	[21]
Sennoside A, B	*Rhem spp.*, *Senna spp.*	[22, 23]
Tetrahydrocannabinolic acid	*Cannavis sativa*	[24]
Gindgolic acid	*Ginkgo biloba*	[25]
Saikosaponin a	*Bupleurum falcatum*	[26]
Glycyrrhizin	*Glycyrrhiza spp.*	[27]
Ginsenoside Rb1, Rg1, Re	*Panax spp.*	[28–30]
Notoginsenoside R1	*Panax notoginseng*	[31]
Ginsenoside Rg3	*processed ginseng*	[34]
Ginsenoside Rh1	*Panax spp.*	[32]
Puerarin	*Pueraria lobata*	[32]
Paclitaxel	*Taxus brevifolia*	[32]
Naringin	*Citrus spp.*	[32]
Solamargine	*Solanum spp.*	[32]
Daidzin	*Glycine max*	[32]
Baicalin	*Scutellaria baicalensis*	[32]
Mitragynine	*Mitragyna speciosa*	[32]
Plumbagin	*Plumbago indica*	[32]
Aconitine	*Aconitum spp.*	[32]

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Methodology

Synthesis of hapten-carrier protein conjugate

Although bigger molecular compounds can be immunized directly, small molecular component like G is impossible immunizing without carrier protein conjugate. We cleavage sugar moiety by sodium periodate to create aldehyde function in a molecule which is combined to carrier protein like bobbin serum albumin (BSA) resulting in hapten-BSA conjugate as previously reported [35] for Gs [28–31], saikosaponin [26], glycyrrhizin [27] and crocin [17].

Determination of hapten number in hapten-carrier protein conjugate by matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-tof-MS)

The first important step is confirmation of hapten number in hapten-carrier protein conjugate because previously we had no suitable methodology for its confirmation. In order to confirm the hapten number we induced MALDI-tof-MS in 1993 [37–39] resulting in the MS spectrum of G-Rb1-BSA conjugate [28]. The molecular peak appeared at centering around 80,000 as a radar peak which means that hapten number were 5 to 20 [28]. On the other hand, the conjugate of marihuana compound, THC-BSA showed a sharp molecular peak at 72,000 M^+ indicating that the hapten number is approximately 13 [36]. The same evidence was found for those of forskolin [37] and opium alkaloids [38] too because the preparation method of hapten-carrier protein conjugate was same as previously published carbodiimide method [39]. From above evidence MALDI-tof-MS can easily confirm the possibility of immunization. Recently Yan et al. reviewed that MALDI-tof-MS determined the hapten number in naringin-BSA conjugate and supported our finding [32].

Immunization, hybridization and purification of MAb

Mice were injected intraperitoneally with hapten-carrier protein conjugate and immunized. Splenocytes were isolated and fused with mouse myeloma cells using polyethylene glycol method [40]. Hybridomas producing MAb reactive to the hapten were cloned by the limited dilution method [41]. Cultured medium of cloned hybridoma was subjected to the affinity column and absorbed IgG was eluted with diluted acetic acid containing salt [20].

Cross reactivity of MAb against Gs

Cross-reactivity is the most important factor for the value of antibody. The cross-reactivity of anti-G-Rb1MAb against G-Rc and -Rd having a protopanaxadiol moiety including a di-glucose group attached at C-3 hydroxy group and different sugar moiety attached to C-20 hydroxy group are weak as 0.024% and 0.020% respectively comparing with G-Rb1 (100%). G-Re and -Rg1 which possess PPT moiety have no cross-reactivity similar to those of triterpene saponin like glycyrrhizin and saikosaponin. From these results it becomes evident that a di-glucose group attached at C-3 hydroxy group is necessary for the reactivity of anti-G-Rb1 MAb [28]. In other word the glucose moiety attached at C-3 position may open by oxidation of sodium periodate to give aldehyde resulting conjugation with carrier protein.

The cross-reactivity of anti-G-Rg1 MAb against G-Rg1, -Re are 100 and 3.3%, respectively [29], and nothing for G-Rb1 and -Rc. Structurally differences between above two groups are PPT and PPD type, respectively. From these results the sugar moiety at C-6 position was necessary, but not related to the sugar moiety at C-20 position.

Anti-G-Re MAb has cross-reactivity against G-Rg1 (70.94%) and -Rd (76.23%) respectively and nothing for G-Rb1 and -Rc [30]. It becomes evident that C-20 glucose may be immunized and functioned as an epitope in the structure of Gs.

In the case of G-Rb1 the full measuring range of the assay extends from 20 to 400 ng/mL [28]. G-Rb1 can be analyzed specifically and highly sensitive in the crude ginseng extract as nano order. The concentration of G-Rb1 in traditional Chinese medicine and in the extract of *Panax* species can be analyzed without pretreatment by anti-G-Rb1 MAb and confirmed the good correlation with that of HPLC.

Among our MAb studies a few unique examples will be discussed. As described above we prepared anti-tetrahydrocannabinolic acid (THCA)-MAb and reported that the MAb had wide cross-reactivities against almost all marihuana compounds [24]. Therefore, the anti-THCA-MAb was used for the analysis of related marihuana compounds as a forensic purpose [42]. Furthermore, we confirmed that all metabolites of THC in rats can be detected using the assay system of anti-THCA-MAb [43]. From these data it is easily suggested that C-2' hydroxyl group, C-4' alkyl group and C-6' hydroxyl group or C-6' alkyl ether are immunized resulting that almost all cannabinoids can cross-react with anti-THCA MAb [24]. Previously we succeeded to prepare a typical anti-solamargine MAb which has wide-reactivity against all solasodine glycosides [44] resulting in suitable for analysis of total amount of solasodine glycosides [45]. In this case since the sugar moiety in all solasodine glycosides is attached at only C-3 position, such wide-reactivity may be appeared. On the other hand, the structural differences on E and F rings sensitively reflect the cross-reactivity resulted that tomatine and tomatigine have nothing of reactivity [45].

Gs in body-absorption, bioavailability, pharmacokinetics and metabolism

Gs are structurally complex compounds having a typical triterpene, dammarane skeleton and sugar moiety which mainly combine 2 position, at C-3 or C-6 and C-20 position in a molecule. From this complication approximately 260 ginsenosides have been reported [2] resulting in the difficulty of metabolism for ginsenosides in body. However, since many investigations regarding pharmacokinetic studies have been accumulated recently, the turnover of G including analytical system will be discussed in this section.

The absorption mechanism of G-Rg1 and notoginsenoside R1 in *P. notoginseng* were investigated using the in situ intestinal perfusion rat [46]. Phospholipid complex of notoginsenoside R1 and G-Rg1 which are major component in *P. notoginseng* were orally investigated in rats to confirmed that bioavailability of them was increased remarkedly [47]. Lai et al. found that G-Rh1 was metabolized to PPT in vivo and in vitro [48]. The metabolic pathway of G-Rg2 which has potent biological activity was investigated in rat liver microsomal incubate proposing 4 metabolites which were suggested by ultra-performance liquid chromatography coupled with quadrupole fragmentation analysis [49]. Wei et al. investigated the metabolites of notoginsenoside R1, G-Rg1 and G-Rb1 by Zebrafish using HPLC-electrospray-MS resulted that hydroxylated Gs and de-sugar Gs were major metabolites [50]. More recently G-Rc having potential pharmacological activity was administrated in rat and its pharmacokinetics was determined by LC-MS system [51]. Pharmacokinetics of G-Rb1 and compound K were performed by oral administration of red ginseng extract using LC-MS/MS system [52]. Jin et al. optimized LC-MS conditions for clear separation of 13 Gs to investigate pharmacokinetic properties of their Gs including metabolism in humans [53]. Won et al. reviewed that Gs are metabolized into two groups, PPD-type and PPT-type Gs which are metabolized by mainly de-sugar pathways individually and finally reaches to PPD or PPT, respectively although there are minor metabolic pathways [54]. All above biological investigations on Gs have been performed by LC-MS owing to good separation and high sensitivity. However, since these assay systems should have troublesome pretreatment of fluid samples, we selected MAbs for pharmacokinetics of G-Rb1 and -Rg1 as simple, quick and without complicated pretreatment. The full measuring range of the assay for anti-G-Rb1 MAb extends from 20 to 400 ng/mL [55]. On the other hand, the concentration of G-Rg1 can be analyzed between 0.3 to 10 μg/mL [55]. The correlation coefficient was calculated from fitting a straight line analyzed by ELISA and HPLC method. There were good correlations for G-Rb1 and -Rg1 between assay values from both methods [56]. Therefore, it is possible to study a large number of animal samples and a small sample size in vivo with these systems. Within 1 h after G-Rb1 administration in rats, the serum G-Rb1 concentration decreased quickly from about 100 μg/mL to a level of 55 μg/mL, followed by further declination at lower rate even 72 h after the injection, G-Rb1 at a concentration at 6.88 μg/mL was detected. On the other hand, G-Rg1 exhibited quite a different pharmacokinetic pattern. The serum concentration of G-Rg1 rapidly decreased to an undetectable level after injection during several minutes resembling previous report [56]. The discrepancy in the metabolic rate between G-Rb1 and -Rg1 in rat blood may be responsible to the different molecule structure of the two Gs as substrates of β-glucosidase in the blood.

Eastern blotting system for quantitative analysis of Gs

Zhao et al. recently found that only G-Rg1 was detected in brain tissue through blood brain barrier after oral administration of ginseng by immunohistochemistry using anti-G-Rg1 PAb [57]. We also determined the movements of G-Rb1 and -Rg1 in blood by newly developed histochemical staining system, Eastern blotting assay system as discussed previously [55] using MAbs against G-Rb1 and -Rg1. Furthermore, as indicated above we formed anti-crocin MAb [17] and confirmed the incorporation of crocin into PC-12 cells by histochemical staining [58] in the first stage of saffron investigations related to protection of neuronal cell death [59]. From these results MAbs against Gs makes it possible to clear further histochemical analysis though lower concentration. This is one more characteristic comparing with that of LC-MS system.

Naming of Eastern blotting was started from an immunostaining of glycyrrhizin in licorice extract using anti-glycyrrhizin MAb [27] via called as Western blotting of G in ginseng [60]. The pathway of Eastern blotting is following. Gs were applied to TLC plates and developed with suitable solvent system. One developed TLC plate was sprayed with H_2SO_4 and detected. The other TLC plate was covered with a polyvinylidene difluoride membrane sheet. After heating the blotted polyvinylidene difluoride membrane was treated with $NaIO_4$ solution which cleavage Gs followed by BSA, resulting in a G-BSA conjugate on a polyvinylidene difluoride membrane. The conjugate was treated with anti-G-Rb1 MAb and peroxidase-labeled goat anti-mouse IgG, and finally exposed to substrate, 4-chloro-1-naphthol-H_2O_2 solution [60] as shown in Figure 2. In the case of anti-G-Rg1 MAb the procedure was the same with that of anti-G-Rb1 except substrate, 3-amino-9-ethylcarbazole.

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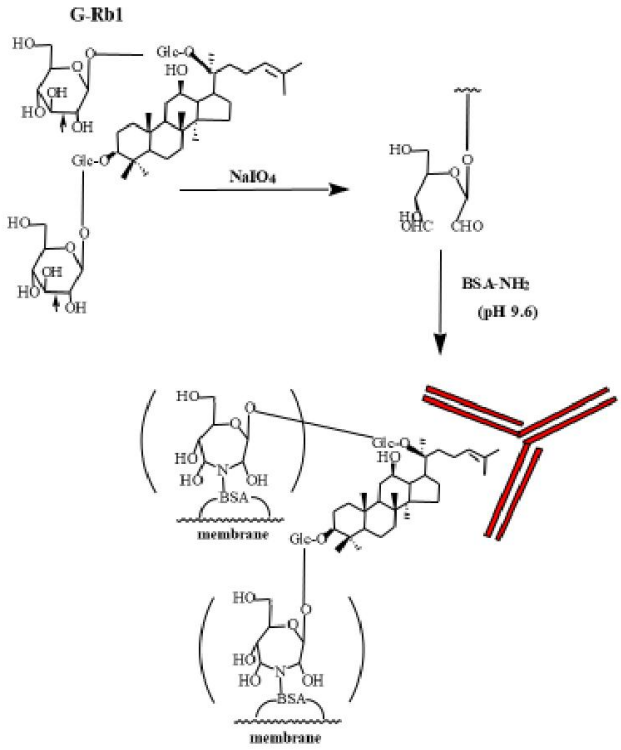

Figure 2 Pathway of Eastern blotting using anti-ginsenoside Rb1 monoclonal antibody.

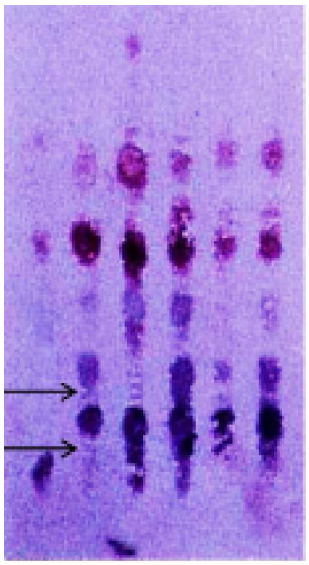

Figure 3 Double Eastern blotting of several ginseng extract by anti-ginsenosides Rb1 and Rg1 monoclonal antibodies (author's unpublished data). Lines are *Panax japonicus*, *P. quinquefolium*, *P. notoginseng*, fibrous ginseng, red ginseng and white ginseng from left. Two arrows show minor ginsenosides in *P. quinquefolium*.

The finger printing analysis of several ginsengs by Eeastern blotting can visualize plant metabolites which are distinguished separately into PPD and PPT types by staining color. An Eastern blotting profile of several Gs using anti-G-Rb1 MAb indicated the staining of PPD type Gs like G-Rb1, -Rc and -Rd. On the other hand, the staining pattern by anti-G-Rg1 MAb showed the detection of only G-Rg1 and -Re. Further we succeeded to detect the double Eastern blotting which can stain two types of G separately. In this case it is possible to confirm that reddish color indicated PPT type and blush color showed PPD type Gs [61] as shown in Figure 3 (author's unpublished data). This system might be applied for the metabolic research of Gs as already discussed. Moreover, National Institutes of Health analyzer can be used for staining band resulted that the qualitative analysis of metabolites is available.

Since Rf value reflects the sugar number in a molecule, the combined information can suggest the whole structure of G. For example, when *P. japonicus* extract was performed by an immunoaffinity column conjugated with anti-G-Rb1 MAb to separate two PPD type Gs (compound 1 and compound 2) [62]. Compound 1 and compound 2 were suggested to have 3 and 5 sugar moieties in a molecule, respectively comparing with the authentic samples resulting that compound 1 and compound 2 were determined as chikusetsusaponins III and VI, respectively. Furthermore, Eastern blotting analysis promotes to find unknow G. Small amount of two unknow Gs were detected by fingerprinting of Eastern blotting in American ginseng resulting in isolation of two new Gs named as quinquenoside Ja and quinquenoside Jb from American ginseng root by using double Eastern blotting system [63]. Moreover, G-Rb1 was isolated from *Kalopanax pictus* [64] using Eastern blotting assay system although nothing of G-Rb1 was believed. From above evidence MAbs can be used properly depending on research purposes.

Previously we prepared anti-aristolochic acid MAb and developed its Eastern blotting [65]. On the bases of antigen antibody technology surrounding aristolochic acid which is known as a strong toxin for kidney, the human kidney cells were cultured in aristolochic acid adding medium and then separated cells combined with aristolochic acid by coprecipitation method with anti-aristolochic acid MAb, separated and removed MAb and finally analyzed the combined peptide by LC-MS-MS after digestion. The target peptide was determined as a kind of structural protein, alpha-actinin-4 [66]. This methodology easily suggests that anti-G MAbs can be applied for the determination of target protein against Gs and for the evidence of real pharmacologically active Gs in a body.

The other application of Eastern blotting can open the staining of ginseng root slice by anti-G-Rb1 MAb

[67]. In this case the distribution of PPD type Gs can be detected by Eastern blotting resulted that phloem contained higher concentration of PPD type Gs, xylem much lower and epidermis is almost nothing as indicated in Figure 4.

More detail information of Gs in *P. ginseng* cells and tissues was investigated by microscopic analysis as shown in Figure 5 (author's unpublished data). The water-soluble substance such as G-Rb1 can be detected by an immunofluorescent technique applied to sections of materials fixed with a high concentration of glutaraldehyde and embedded in acryl resin, LR white. This immunostaining technic can be used for the localization of soluble substance such as Gs. In this method green fluorescein dye-conjugated secondary antibodies are not needed because acryl resin-embedded materials are powerfully fluorescent. This autofluorescence was reduced by red fluorescein. Furthermore, autofluorescence of glutaraldehyde fixation could be eliminated by sodium borohydride treatment. Therefore, very weak autofluorescence was detected in immunostaining control sections, especially in chloroplasts and vacuolar protein granules. Strong immunostaining for G-Rb1 was observed in the leaves, rhizomes and roots in *P. ginseng* plant [68]. This finding indicated the same phenomenon with distribution of G in tissues. From this result the leaves are also a good resource for production of Gs as reported [69]. Moreover, it is suggested that Gs in leaves might be moved into rhizomes and accumulated in winter season.

Immunoaffinity isolation of G-Rb1 from ginseng extract and preparation of knockout extract

Anti-G-Rb1 MAb was treated by sodium periodide resulting in sugar opened MAb which was coupled with affi-gel Hz hydrazide gel to give immunoaffinity gel [70]. When the crude extract of *P. ginseng* was applied to the immunoaffinity column and completely washed by the washing solution which contained no Gs although the other constituents were eluted. G-Rb1 appeared as a single spot in the eluate by the eluting solution containing MeOH. The washing fraction contained all components of *P. ginseng* extract except G-Rb1 as indicated in Figure 6. This is the first success of knockout extract preparation and following to glycyrrhizin in licorice extract [71] although the isolation of antigen compound by immunoaffinity column was investigated previously for forskolin [72] and solasodine glycosides [73]. We succeeded to confirm the value and pharmacological activity of knockout extract regarding glycyrrhizin in licorice extract [71, 74] as further new approaches though the knockout extract of Gs in *Panax* species which is equivalent to the washing fraction has not been yet investigated.

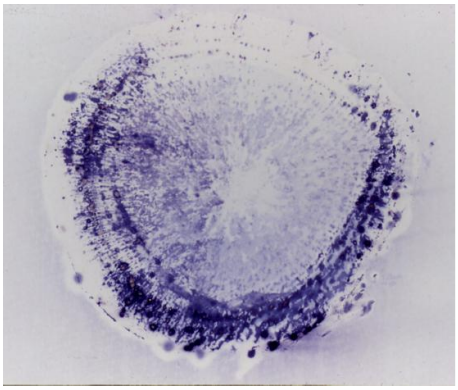

Figure 4 Eastern blotting of ginseng slice by anti-ginsenoside Rb1 monoclonal antibody.

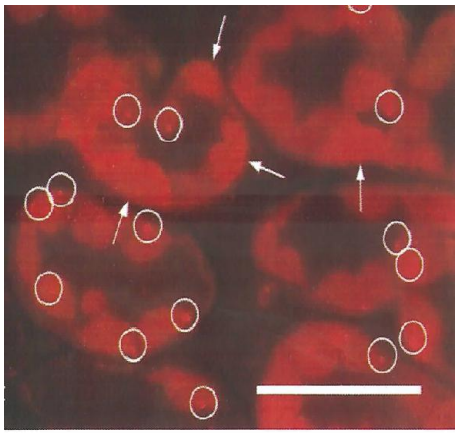

Figure 5 Histochemical staining of ginsenoside Rb1 in ginseng leaf tissues using anti-ginsenoside Rb1 monoclonal antibody (author's unpublished data). Chloroplasts (arrows) and peroxisomes (circles) of parenchymal cells are strong stained but vacuoles are not.

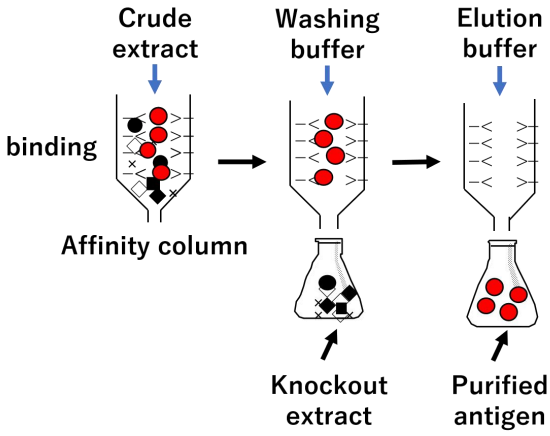

Figure 6 Scheme of affinity isolation and preparation of knockout extract.

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Conclusion

This review discussed the application of MAbs having specific affinities for Gs. Reproducible, highly specific and quantitative analytical assay system like ELISA can contribute the quick and accurate quality control of *Panax* species, but also for many crude drugs prescribed in traditional Chinese medicine. Eastern blotting system newly named as fourth methodology following Northern, Western and Southern blotting, can be apply for Gs used as an on-membrane immunostaining and more specific and sensitive assay system compared with the other methodology not limited for Gs but also for pharmacologically active natural products such as solasodine glycosides, sennosides, aristrochic acid, saikosaponin, glycyrrhizin and liquiritin. Furthermore, the histochemical study of ginseng using Eastern blotting is possible to search the distribution of Gs in the sliced plant section. Double Eastern blotting system facilitated by two MAbs like anti-G-Rb1 and anti-G-Rg1 MAbs yielded different types of information such as sugar numbers in a molecule and structural differences such as type of PPD and/or PPT which can promote the structure elucidation, actually this assay system succeeded to isolate new Gs and elucidate their structures, and further qualitative, quantitative and pharmacologically active judgements of *Panax* species. Immunoaffinity separation of Gs is the first time for small molecule natural product although it is common for bigger molecule compounds such as peptides and proteins. In addition, we succeeded to prepare the knockout extract which removes only antigen molecule from the crude extract using immunoaffinity column. The knockout extract can confirm the pharmacological and biological value of antigen molecule in the crude extract. Moreover, the combination of several knockout extract in traditional Chinese medicine prescription might be able to solve the pharmacological activity of traditional Chinese medicine.

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