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Plant Glycosides are Natural Prodrugs - Role of Human Intestinal Flora -

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Characteristics of traditional medicines are the prescription composed of several natural sources, depending on the physical constitution and symptoms of patients, and the oral route of administration to human, not to be injected and not to be medicines to rats and mice. Hundreds of pharmacological and biochemical researches in vitro have been widly preformed and reported, using extracts or isolated components of natural medicines and experimental animals, neglecting the three criteria above described of traditional medicine.

All the components from traditional medicines inevitably contact with microflora in the gastrointestinal tract after oral administration. Some of them are transformed by bacterial enzymes and then absorbed through intestinal membrane to portal vein and modified or conjugated in the liver, excreted through the bile into digestive tract and metabolized again by intestinal bacterial enzymes and reabsorbed, so called, enterohepatic circulation of the compounds. Especially glycosides of natural origins are water-soluble, being difficult to be absorbed from intestine and staying long periods of time, suggesting that glycosides itself are low in bio availability and pharmacologically inactive. Based on this hypothesis, we have been working for these twenty years on the metabolism of glycosides of various plant origins by human intestinal microflora, the isolated bacteria or a mixture of identified bacterium from all human origin. In summary, plant components, glycosides, are in general hydrolyzed by human intestinal flora to their corresponding aglycones.[27,28] We have identified the responsible bacterium capable of hydrolyzing such glycosides (Table I) : sennoside (senna and rhubarb), glycyrrhizin (GL) (licorice), barbaloin (aloe), baicalin (scutellariae radix), ginsenoside (panax ginseng). We have also isolated the respective β-glycosidase from each bacterium and elucidated the metabolic pathways of these glycosides.

Glycosides are of no effect to germ-free animal

When glycyrrhizin (GL) was orally administered to germ-free rats, the aglycone, glycyrrhetic acid (GA), was not detected in sera and intestinal contents. However, when GL was orally administered to gnotobiotic rats associated with Eubacterium sp. strain GLH, which produces GL-hydrolase, GA was detected in sera and intestinal contents at concentrations similar to that in conventional rats. On the other hand, GL was not detected in sera of any of the rat groups. Using these groups of rats with liver injury caused experimentally by carbon tetrachloride, GL was effective in conventional and gnotobiotic rats but not effective in germfree rats. These results showed that E.sp.strain GLH was necessary for the therapeutic action of GL.

Barbaloin, a popular laxative component from aloe, is transformed to an anthrone derivative, aloe-emodin anthrone, by Eubacterium sp. strain BAR obtained from human feces. Barbaloin was ineffective in the rat where fecal barbaloin-metabolizing ability is very weak. However, barbarloin showed strong diarrheal action in gnotobiotic rats associated with human E.sp. strain BAR. Animal-specific differences in laxative effect of barbaloin are due to species differences in intestinal bacteria.

Sennoside A, a famous laxative, was ineffectual by its injection, but effectual by it oral administration to conventional rats. However it was completely invalid to germ free rats, but became effective to gnotebiotic rats associated with two strains, Bidobacterium sp. SEM and Peptostreptococcus intermedius, the former produces β-glycosidase specific for sennoside and the latter produces C-C reductase (NADH-flavin reduction) for the aglycone, Sennidin. The final diarrheal compound is rheinanthrone, which is oxidized rapidly by oxygen. Therefore, sennoside is an effectively designed natural prodrug, which is activated by anaerobic metabolism in the lower part of intestine. The individual differences of laxative action of sennoside may be depend upon the specific β-glycosidase activity and therefore, previous oral administration of probiotics which produce sennoside-β-glycosidase may be

Table 1. Glycosidase isolated from human intestional bacteria

Glycoside	Pharmacological Action	Metabolite	The enzyme isolated from the bacterium
Sennoside A[1,4,9,20,26]	Laxative	Rheinanthrone	β-glucosidase Bifidobacterium sp. SEN C-C reductase Pepotostreptcoccus intermedius
Glycyrhizina[2,3,7,8,12,14]	Anti£linflammation	Glycyrhetic acid	β-glucuronidase Eubacterium sp. GLH
Barbaloin[15,16,17,19,25]	Laxative	Aloe-emodin-anthrone	β-glucosidase Eubacterium sp. BAL
Baicalin[27]	Anti-allergy	Baicalein	β-glucuronidase Widely distributed
Geniposide[18,21,24]	Cholagogue	Genipin	β-glycosidase Widely distributed
Paeoniflorin[5,6,10,11,13]	Sedative	Paeonimetabolin	β-glucosidase esterase Widely distributed
Saikosaponin	Anti-inflammation	Saikosapogenin	fucosidase Eubacterium sp.A-44
GinsenosideRb$_1$[22,23]	Psychiotropic	Compound K	β-glycosidase Eubacterium sp. A-44

effective to the constipation.

Baicalin, monoglucuronide of baicalein, was reported to be absorbed easily and intact from intestinal tract, because baicalin was detected in blood after the oral administration. However, baicalin was not detected in sera, when the compound was orally administered to germ free rats. Therefore, baicalin is hydrolyzed by glucuronidase of intestinal bacteria to the aglycon, which is absorbd into the intestinal mucosal cells and there glucuronized again by the glucuronyl transferase to transport to portal vein.

Intestinal metabolism of ginsenoside

Ginsenosides have been widely regarded as the principal components responsible for several pharmacological activities such as neurologic, metabolic, blood circulating agents and so on. However, most of them have been performed by in vitro assay systems or injection in vivo studies, neglecting the intestional metabolism of the compound by bacterial flora of humans.

Ginsenoside Rb$_1$, was incubated under anaerobic condition at 37°C with human fresh fecal suspension. Sugar moieties successively were hydrolyzed to form ginsenoside Rd at 8 hr. incubation, -F and compound K (CK)24 hr, which was further hydrolyzed to the aglycone, 20(S)-protopanaxadiol(20(S)Ppd). (Fig. 1 and Chart 1), Ginsenoside Rg$_1$ was also hydrolyzed more slowly under the same conditions to Rh$_1$ and then the aglycone, 20(S)-protopanaxatriol. All the final products was 20(S)-type, which shows the hydrolysis proceeded without any isomerization. We screened 31 species obtained from human intestinal flora, all of which were β-glycosidase positive for p-nitorophenyl β-glycoside as substrate, but only Eubacterium A-44 had the ability to metabolyze Rb$_1$. The strain is

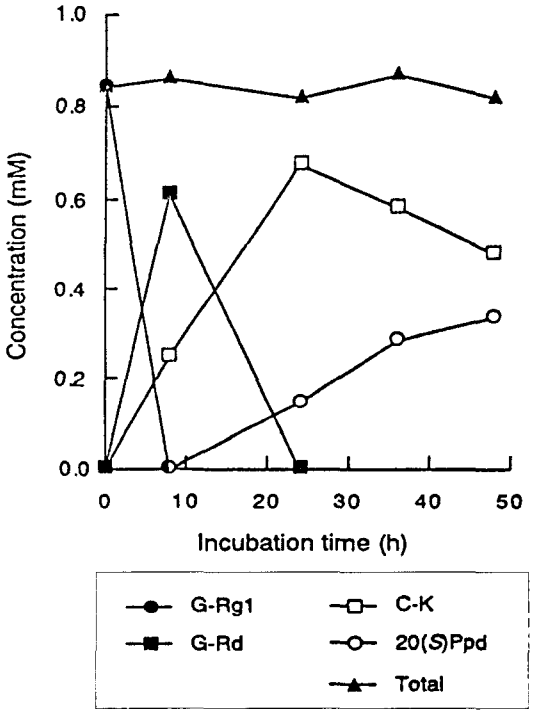

Fig. 1. Metabolism of G-Rb$_1$ by human intenstinal flora.

strictly anaerobic rod-form bacteria belonging to major genus of human intestinal flora. This strain hydrolyzed successively Rb$_1$-Rd-F3-CK-20(S)Ppd and the hydrolase was purified from this strain, by extraction, $(NH_4)_2$ SO_4 fractionation, Butyl-Toyopearl column chromatography, Sephacryl S-300-and hydroxyapatite column chromatography procedures, to 105times purity from the cell extract. The purified enzyme catalyzed all the hydrolysis of Rb$_1$ to CK. Hasegawa *et al*[33] recently reported that the intestinal bacterium, Prevolella oris, also was able to hydrolyze Rb$_1$ to CK (They named M1).

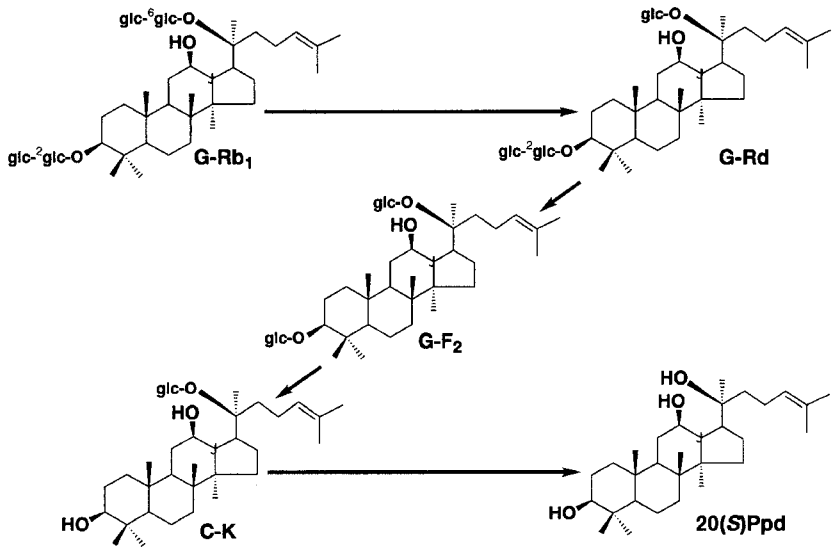

Chart 1. Metabolism of G-Rb_1 by intestinal bacterial flora from rat and man.

Plasma concentration of metabolites

Kanaoka *et al*[23] reported the enzyme linked immunoassay method (EIA) of Rb_1, ten years ago, but Rb_1 was not detected in sera any time during one day after the oral administration of red ginseng powder to human. And Rb_1 was not detected in sera and any organ of rats after the oral administration of considerably large amounts of Rb_1. Therefore Rb_1 was not absorbed at all intact after its oral intake and we developed new EIA method for CK, based on the metabolic pathway of Rb_1 by the intestinal bacterial enzyme described above, Fig. 2 shows plasma CK concentration for 24 hr after the oral administration of Rb_1 to rats. At 7 hr, the concentration was the highest and decreased gradually during one day. When administered to germ-free rats, plasma CK was not detected, but when administered to gnotebiote rats associated with Eubacterium sp. A-44 originally obtained from human feces, plasma CK was detected in the same pattern as conventional rats. When CK was orally administration to rats, CK was rapidly absorbed to the highest level after 30 min, decreased immediately and not detected after ten hr. When red-ginseng powder was orally intaked to three human, CK was detected at 8 and 12 hr after , but the concentrations were individually different, probably the deviation of intestinal β-glycosidase activity.

Ginsenoside Rb_2, Rg_3 and other saponins were reported to be effective to invasion and metastasis of cancer, by in vitro system or injection to animal studies, which neglect metabolism by intestional bacterial flora, absorption and serum concentration of the metabolites of ginseng saponins. Recently Sahekiís group reported anti-metastatic

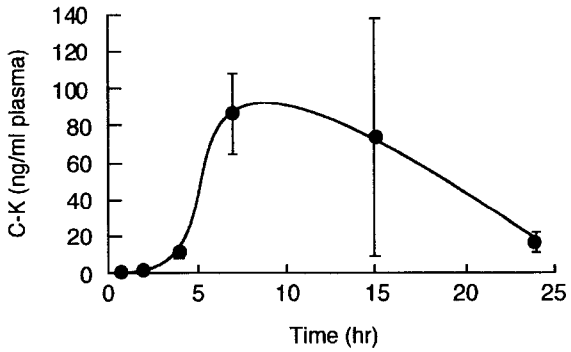

Fig. 2. Plasma C-K concentration after oral administraction of G-Rb_1 to rats.

effect of M1(=CK)[34] and fatty acid ester of M1.[35]

Ginsenosides must be reevaluated as prodrugs in view points of ADMET in the beginning of the 21st century.

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