行政院國家科學委員會專題研究計畫成果報告

人參皂苷 Rb1 及多醣體對牛黃體細胞類固醇生成作用之探討

計畫編號：NSC 89-2313-B-002-061
執行期限：88 年 8 月 1 日至 89 年 7 月 31 日
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一、中文摘要

促黃體活性係指某物質具有提升黃體細胞功能的作用，因此該物質兼具促性腺素的作用。根據文獻記載，藥用植物的抽出物中，尚未發現有直接促黃體作用。很幸運地，本研究室經多年之研究，證實萃取自人參花與葉多糖體，確實具有此活性，為所有文獻中第一次的發現；而人參根之多醣體及人參皂苷 Rb1，則無此作用。

關鍵詞：人參、根、花、葉、促黃體、人參皂苷、多醣體

Abstract

Luteotropic activity means that some specific function of materials can increase the progesterone production from luteal cells. It also implies those materials containing gonadotropins which stimulate the gonadoal functions of animals. Nearly no materials from plants have been proved directly to have such activity except those hormones found in animal tissues themselves. The present essay describes briefly the processes of investigations and provides the first evidence for the luteotropic activity of polysaccharides isolated from ginseng flowers and leaves, and a possible application in reproductive clinical uses. However, ginsenoside Rb1 and polysaccharides of ginseng roots do not alter the activities.

Keywords: ginseng, root, flower, leaf, luteotropic, ginsenoside, polysaccharide.

二、Purpose：

In recent years some scientists have investigated the functions of ginseng leaves and found that the leaves posses the same properties as the roots such as anti-ulcerative, immune enhancing activities etc. (Gao *et al.*, 1989; Sun *et al.*, 1992, 1994; Yamada, 1994; Hu *et al.*, 1995; Huh *et al.*, 1998). That suggests ginseng leaves to replace the roots in some aspects of clinical use. Even more recently the ginseng flower was also focused on to develop its use especially on the nutraceutical sector. Some commercial products already appeared on the market in Korea, Japan, China and Taiwan. However, very few papers concerning the research works and clinical use of ginseng flower were published in the scientific media meanwhile.

Several pharmacological activities have been reported in polysaccharides isolated from Chinese medicinal herbs (Yamada, 1994). Most of those activities have been observed in pectic polysaccharides. Recently we found that ginseng polysaccharides isolated from flowers and leaves have luteotropic activity (Lin *et al.*, 1998a; Wu *et al.*, 2000). In this paper, these new findings are presented.

Roots and flowers of *Panax ginseng* C. A. Meyer were cultivated and harvested in Korea and their dried products were bought in Taipei, Taiwan. Some other crude polysaccharides of ginseng leaves

and roots were obtained from Prof. H. Yamada, Kitasoto Institute, Tokyo, Japan. M199, the culture medium were purchased from Pharmacia Co. Ltd. Ovine luteinizing hormone (NIDDK-oLH-26) was kindly provided by the National Hormone and Pituitary Program, USA. The bovine luteal tissues were obtained from Heng-Chun Station, Taiwan Livestock Research Institute. Most of the chemical compounds such as ginsenoside Rb1, bovine serum albumin, collagenase, desoxyribonuclease I etc. were obtained from Sigma Chemical Co. The frozen-thawed bovine luteal cell culture system and the enzyme immunoassay of progesterone were adopted from Yuan *et al.* (1994). Data were expressed as mean±S.D.. The differences between the control and the treatment in these studies were tested for statistical significance by using Student's t-test and Duncan's multiple range test. A value of $P<0.05$ was considered a statistically significant difference.

三、Results and Discussion

Experiment 1: A preliminary study of crude extracts isolated from ginseng roots and flowers, ginsenoside Rb1 on luteotropic activities:

Flowers are the reproductive organs of plants. For humans, flowers present themselves in many ways: we can enjoy their beauty and elegance, add them to our food for nutritious reasons and even use them as medical herbs to treat our diseases.

The ginseng flower blossoms once a year. Recently it has been used commercially in the nutraceutical sector for tonifying the body functions. Still very few scientific papers concerning the clinical use of ginseng flower have yet been published. Therefore we evaluated its possible abilities by testing its luteotropic activity, which is an indicator for reproductive functions.

Results showed that hot-water extracts of ginseng flower (GF-1) could significantly increase the progesterone secretion of bovine luteal cells *in vitro* in a dose response manner (1–100 μg/ml) during 4 h incubation whereas the ginseng root (GR-1) extract could not. After 24 h incubation the stimulation effect of GF-1 was reduced in comparison with oLH treatment. This results implied that the mechanism of ginseng flower may involve the acute steroidogenic synthesis only (Lin *et al.*,1998a).

Surprisingly, the roots of ginseng did not express any enhancing effect on the steroidogenesis in luteal cells in the present study. The question now is: how does it work in the sexual or adrenal system (Rim 1979; Lin *et. al.*, 1995). One of the possible ways of ginseng root actions may involve the pituitary system. Odani *et al.* (1986) demonstrated that the ginsenosides enhanced the ACTH secretion of rat pituitary cells *in vitro*.

Next step in our study was to have the GF-1 fraction undergo molecular cutting between 10,000 daltons using molecular sieving technology and then perform further extraction for the lipid soluble substances. Those extracts from GF-1 were also examined for their luteotropic activities. Only the fractions of a molecular weight larger than 10,000 daltons possessed luteotropic activities indicating that the polysaccharides of ginseng flower may involve this action like those from ginseng leaves (Wu *et al.*, 1998). Therefore further purification of crude polysaccharides from ginseng flower (GF-2) was done and additionally also the residue of methanol soluble fraction (GF-MeOH) was collected. Data also demonstrated that only GF-2 could stimulate the progesterone production on bovine luteal cells whereas the GF-MeOH did not. In addition, ginsenoside Rbb1 with 1, 10, 100 ug/ml did not alter the progesterone production from the cells. In conclusion, crude polysaccharides of ginseng flowers possess significant luteotropic activities whereas the hot water extracts of ginseng roots and ginsenoside Rb1 do not have that activity

(Wu *et al.*, 2000)

Experiment 2: Isolation of luteotropic action of polysaccharide from leaves and roots ginseng.

As mentioned previously, some polysaccharides obtained from roots and leaves share the same pharmacological actions such as GL-2 (from leave) and GR-3 (from root)for enhancing activity of immune complexes binding to macrophages (Sun *et al.*, 1994). However, there is a considerable discrepancy between the two compounds isolated from flowers and root for luteotropic activities in the above experiment. For this further investigation, we used partial-identified polysaccharides obtained from the Kitasoto Institute to re-evaluated the biological activities of ginseng polysaccharides. Data showed that the luteotropic activity was only found in the polysaccharides isolated from the water- and alkaline-fractions of ginseng leaves. In other words, the water-fraction from roots and the alkaline-fractions from roots did not show significant activities. Again, this study was not to prove luteotropic activity presented in ginseng roots and their polysaccharides . How does ginseng roots work on animal reproductive system (Lin *et al.*, 1998b)? Further works involving each aspect of reproductive system shall be totally examined for the evidences that ginseng roots can tonic the sex function. The order of the activities of these fractions was weakly acidic fraction (GL-4) > strongly acidic polysaccharide (GL-3) > neutral fraction (GL-5) > crude fraction (GL-2) > alkaline fraction (GLA-2).

Experiment 3: Chemical properties of GL-4 affecting on luteotropic activity.

As shown above, GL-4 is one of the most potent polysaccharides which can increase the progesterone production from bovine luteal cell *in vitro*. The chemical properties of GL-4 have been illustrated by Gao *et al.* (1989) and contain 66.4% carbohydrate, 13.8% uronic acid and 31.3% protein. In this experiment, we determined the active composition of GL-4 for luteotropic action. GL-4 was digested with pronase (Kaken Kagaku Co. Ltd, Japan) or subjected to periodate oxidation (NaIO4) as previously described (Gao *et al.* 1989). The non-dialyzable portions were lyphilized and tested on the luteotropic activity by using the bovine luteal cell system. The luteotropic action of GL-4 did not change significantly after deproteinization, but the activity was lost after periodate oxidation which destroys the carbohydrate structure. It suggested that the carbohydrate portion of GL-4 contributes to the expression of the luteotropic activity.

Experiment 4: A mechanism of luteotropic activity of GL-4.

From dose response and time course studies, we found that the GL-4, around 1-100 μg/ml, stimulated progesterone production from the luteal cell in a dose response manner both at 4 and 24 hr incubation periods, and the stimulation index between GL-4 and oLH was around 1/1000 folds i.e. 10 μg of GL-4 was approximately equivalent to 10 ng of oLH. The other difference noted was the response curve of GL-4 was not parallel to that of oLH and the less stimulation of GL-4 at 24hr incubation compared with the control data. These findings indicated that the mechanism of GL-4 on luteal cells is different from that of oLH which is mainly mediated by the c-AMP pathway (Miller, 1988).

In order to understand the luteotropic action of GL-4, actinomycin D, a RNA synthesis inhibitor, and cycloheximide, a protein synthesis inhibitor on the GL-4 action were examined. The luteal cells were cultured with 10 μg/ml GL-4 in the presence of various concentrations of actinomycin D or cycloheximide for evaluating the mechanism GI-4 of on steroidogenesis. These compounds were purchased from

Sigma Co. and tested with 0.1-10 μg/ml. The data showed that only the cycloheximide with 1-10 μg/ml blocked the progesterone production from the luteal cells induced by GL-4 whereas the actinomycin D with 0.1-10 μg/ml was not have such inhibitory action. It clearly indicated that GL-4 the luteotropic activity of GL-4 is merely through the enhancing present protein synthesis for steroidogenesis and is not through the *de novo* protein synthesis system. Conversely, the enhancing immune complex activity induced by GL-4IIb2, a further purified polysaccharides from GL-4, was blocked by actinomycin D and cycloheximide (Sun *et al.*, 1994). This discrepancy must be clarified in near future.

Experiment 5: Effects of GL-4 on serum testosterone concentration in male mice.

The luteotropic activity of GL-4 proved by a serial *in vitro* study was further examined by using male mice for an *in vivo* study. Male ICR mice, weighing 30-40 gm were purchased from the animal house of National Taiwan University and kept 5 heads in each cage in a controlled animal house with temperature between 23-26 ℃ and lighting between 6:00 am to 8:00 pm. Purina chow and water were provided *ad libitum*. The mice were divided into groups of ten animals each. Each group was treated with intraperitoneal injection of GL-4 at 4 or 24 hr before sacrifice. Human chorionic gonadotropin (HCG) with 10 IU/kg, b.w. was used as the positive control. Their sera were collected and stored at -20 ℃ until assayed for the testosterone by an enzyme immunoassay (Cayman Co., U.S.A.).

Results showed that the serum testosterone concentrations in male mice were significantly increased in a dose dependent fashion by being injected with GL-4 at a dose between 25 to 50 mg/kg, Interestingly, similar results were obtained in the enhancing immune complexes when mice were injected with GL-4IIb (Sun *et al.*, 1994).

Conclusion

1. The present studies provide the first evidence for the luteotropic activity of polysaccharides isolated from flowers and leaves and a possible application for reproductive clinical uses in future.
2. The hot-water extract and crude polysaccharides isolated from ginseng roots and ginsenoside Rb1 do not have the luteotropic activity.
3. The GL-4, pectic polysaccharides isolated from leaves of ginseng, contains two different biological activities i.e. luteotropic activity and enhancing immune complex activity.

四、Self-evaluation

We shave spent about 3 years for the projects which finally shown in the text. A future study to identify the structure of ginseng polysaccharides must be carried out soon by some experts. We do need the help for Taiwan experts and financial support.

五、References

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