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Effects of Korean Ginseng Saponin Fraction on the Biosynthesis of Spermidine and Spermine from Rat Prostate and Testis

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Abstract : To study effects of Korean ginseng (*Panax ginseng* C. A. Meyer) total saponin fraction on spermidine and spermine metabolism in rat reproductive systems, we administrated the saponin fraction to rats for 2 years. Then, we determined the activities of S-adenosylmethionine decarboxylase (SAMDC), the quantitation of the enzyme protein and the amounts of spermidine and spermine contents in prostate and testis. In young sexually immature stage, administration of Korean ginseng saponin fraction showed no effect on SAMDC activities. The stimulatory effect on the activities of SAMDC gradually increased and reached maximal activities in test groups of prostate and testis at sexually mature stage. The amounts of SAMDC protein in test groups were paralleled by the changes of SAMDC activities in test groups, indicating that all of the increased activity occurring in administration of ginseng saponin fraction was not due to the activation of SAMDC activity but to the increase in enzyme protein. However, the spermidine and spermine contents of test groups showed small increase in compared to that of control groups. From these results, we suggest that administration of ginseng saponin fraction alter the spermidine and spermine metabolism in sexually mature and aged reproductive systems in rats.

Key words: S-adenosyl methionine decarboxylase, spermidine, spermine, ginseng.

Introduction

Mammalian cells are known to contain significant amounts of polyamines, putrescine, spermidine, and spermine, which play different roles in various tissues.[1] Although the physiological function of these amines is still not well understood at the molecular level, recent studies have shown that their concentrations are tightly regulated and that normal cellular growth, multiplication, and differentiation require polyamines.[1,2]

Since the discovery of spermine in human seminal plasma by van Leeuwenhoek, the research on physiological roles of spermidine and spermine in male reproductive tract system has been discussed in numerous papers.[3,4,5] Most of the information obtained comes from laboratory experiments with animals which have been studied particularly the polyamine metabolism in prostate, semen and testis.

Human seminal plasma is very rich in spermine, which is derived almost exclusively from the secretion of the prostate gland, which is added to semen at the time of ejaculation. Under normal circumstance, many other mammalian body fluids (including blood plasma, saliva, pancreatic juice, and sweat) contain only traces of polyamines in comparison with the prostatic secretion in man and rat.[6-8] The functions of polyamines in seminal plasma remain enigmatic, despite a plethora of hypothesis.[9,10] Nevertheless, investigations on the prostate gland, whose development and secretory functions are utterly dependent on androgenic hormones, have uncovered the de novo biosynthesis of polyamines and the nature and endocrine regulation of the enzyme reactions that produce spermidine and spermine.[11-13]

In the testis of sexually mature mammals, the sperm production takes place and is regulated by androgens, pituitary follicle stimulating hormone (FSH) and pituitary luteinizing hormone (LH). In addition, it has been found that the spermatozoa contained much more spermine than putrescine or spermidine and their concentrations also increased during spermatid differentiation and the production of mature spermatozoa.[14-16] However, it is quite clear from all of a few meager and in some cases contradictory data[17-19] that the relationships of spermine to testicular functions and their hormonal regulation needs to be examined much more thoroughly and incisively.

Korean ginseng, *Panax ginseng* C. A. Meyer, has a wide range of pharmacological properties including antifatigue, antiinflammatory, antistress and antitumor actions, mild normalizing effects of blood pressure and carbohydrate metabolism. In addition, it has been supposed to have enhancing effects on the function of the reproductive system.[20-23] Although many evidences have been accumulated on the enhancing effects of *Panax ginseng* on these tissues during the past 50 years, their reports resulted from the observation of animal behavior[20] and the clinical aspect of patients with dysfunction of these organs.[21-23] Thus the physiological and biochemical approach to elucidate the mechanism of ginseng effect on these systems is required intensively.

In the present report, we examined the effect of ginseng total saponin fraction on the spermine and spermidine *de novo* biosynthesis of prostate and testis during the administration period in rats and reported these effects for the first time. First, we observed the change of S-adenosylmethionine decarboxylase (SAMDC) activity which is responsible for spermidine and spermine biosynthesis. Second, we determined the quantitation of SAMDC protein using radioimmunoassay method. Finally, the spermidine and spermine contents of these systems were measured during the administration period. From all these data, we could infer the effect of ginseng saponin fraction on the regulation of spermidine and spermine biosynthesis in prostate and testis. Besides, we hope that our results would be helpful to understand both the roles of these amines and the phamacological effect of ginseng saponin fraction on the development and maturation in these systems.

Materials and Method

1. Chemicals

The Korean ginseng total saponin fraction[21] was kindly supported by Korea Tobacco and Ginseng Cooperation. L-[1-^{14}C] Ornithine (54 mCi/mmol), S-[Carboxy-^{14}C]-adenosyl-L-methionine (58.9 mCi/mmol) were purchased from Amersham. Ornithine, pyridoxal-5-phosphate, dithiothreitol (DTT), dansylchloride were purchased from Sigma Chemical Co. (St. Louis, MD). All other chemicals were of reagent grade and were obtained from Sigma unless otherwise stated.

2. Animal

Four-week-old male rats, weighing 40~50 gs, were used. The test groups of rats (SD strain) was administrated the solution of Korean ginseng saponin fraction (40 mg/l) for 2 years by replacing the drinking water in the control groups. The number of a group in this experiment was 10.

3. The preparation of enzyme solution

The prostate and testis were removed from the test and the control groups of rats. These tissues were sliced fine and then homogenized with buffers containing 25 mM Tris (pH 7.0), 1 mM dithiothreitol and 0.1 mM EDTA at 4(C, respectively. The homogenized solution was centrifuged at 13,000×g for 30 min and the supernatant was used for enzyme solution.

4. Assay of ornithine decarboxylase and S-adenosylmethionine decarboxylase

Ornithine decarboxylase activity was measured by measuring the release of $^{14}CO_2$ from [1-^{14}C]ornithine. The assay medium contained 25 mM Tris buffer (pH 7.0), 1mM dithiothreitol, 0.1 mM EDTA, 50 μM [1-^{14}C]ornithine (40 mCi/mmol) and 0.1 mM pyridoxal-5-phosphate. One unit of enzyme activity was defined as 1 nmole CO_2 released in a 60 min incubation at 37°C. S-adenosyl-

methionine decarboxylase was measured with S-adenosyl[Carboxy-^{14}C]methionine. The assay medium contained 25 mM Tris buffer (pH 7.0), 1 mM dithiothreitol, 0.1 mM EDTA, 50 μM S-adenosyl[Carboxy-^{14}C]methionine (55 mCi/mmol) and 2.5 mM putrescine. One unit of enzyme activity was defined as 1 nmole co_2 released in a 60 min incubation at 37℃. Both decarboxylase activities were expressed per mg soluble cytosol protein solution. The protein concentration was measured according to the method of Lowry.[24]

5. Radioimmunoassay of SAMDC protein

The labeled antigen was prepared by incubating a total volume of 1ml containing 34 units of the purified rat SAMDC with 0.125 (M methyl-3H-S-adenosylmethionine (80 Ci/mmol), 150 mM sodium cyanoborohydride, 25 mM Tris buffer (pH 7.0), 150 mM NaCl, 2.5 mM putrescine, 2.5 mM dithiothreitol, and 0.1 mM EDTA at 37℃ for 60 min. The unbound labeled S-adenosylmethionine was removed by dialysis twice against 2 liters of 25 mM Tris (pH 7.0), 150 mM NaCl, 2.5 mM putrescine, 2.5 mM dithiothreitol, and 0.1 mM EDTA for 12hr at 4℃. Approximately 2.9 (Ci of labeled SAMDC was obtained. The RIA procedure were carried out in buffer A consists of 25 mM Tris (pH 7.0), 150 mM NaCl, 2.5 mM putrescine, 2.5 mM dithiothreitol, 0.1 mM EDTA and 0.1% bovine serum albumin. The sample (50 μl) was mixed with 100 μl of antiserum (diluted 100-fold) and incubated for 1 hr at room temperature. Labeled SAMDC (50 μl; 8000 dpm) was then added, and after being thoroughly mixed, the sample was incubated for 12 hr at 4℃. After the incubation, 20 (l of 10% bacterial protein A adsorbent was added and mixed, and the mixture was incubated for 1 hr at 4℃. The pellet was collected by centrifugation at 13,000×g for 1 min wash once by resuspension in 0.2 ml of buffer A, dissolved in 0.3 ml of NCS tissue solubilizer, mixed with 10 ml of formula 949 liquid scintillation fluid, and counted for radioactivity. Calibration curves were constructed by using preparations of purified SAMDC in which the amount of enzyme present was calculated by protein determination method.

6. Determination of polyamine content

The polyamines were isolated and their contents were determined by a slight modification of the method Goren.[25] The tissue materials (1 g) were homogenized with 1 vol of 5% (w/v) perchloric acid and centrifuged at 13,000×g for 20 min. Acid-soluble polyamines contained in the supernatant fraction were dansylated with 2 vols of dansylchloride (5 mg/ml) and 1 vol of Na_2CO_3 for 16 hr at room temperature. Then, 1 vol of proline (100 mg/ml) was added to the sample and incubated for 30 min to remove the free dansylchloride. The dansylpolyamines were extracted with 3 vols of benzene and 2 vols of extracts were spotted on the thin layer chromatography plate(TLC, silicagel 60 plate). The plates were developed with chloroform : triethylamine (5 : 1 v/v). TLC plates were illuminated with UV lamp to identify the dansylpolyamine band in comparison to standard polyamine. Their bands were scrapped with razor and extracted with 4 ml ethylacetate. The extracts were measured by spectrofluorometer at 350 nm (excitation) and 500 nm (emission).

Result and Discussion

S-adenosylmethionine decarboxylase (SAMDC) which is responsible for the spermidine and spermine biosynthesis was ubiquitous and important enzyme for the cell proliferation and differentiation in most tissues. However, the specific activities of this enzyme in various tissues were very different.[26,27] In our experiments, the specific activities of this enzyme in cruding prostate extracts were 50~150 pmole $^{14}CO_2$/hr/mg protein and these values were much higher than that of any other organs[26,27] such as brain (20~40 pmole $^{14}CO_2$/hr/mg protein), liver (4~30 pmole $^{14}CO_2$/hr/mg protein), spleen (5~10 pmloe $^{14}CO_2$/hr/mg protein) and colon (15~30 pmole $^{14}CO_2$/hr/mg protein). Meanwhile, the activities in testis were 5~20 pmole $^{14}CO_2$/hr/mg protein which is similar to that of other organs.[26,27]

Experiments were performed with cytoplasmic

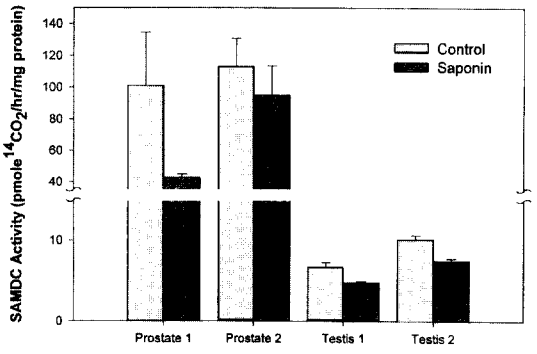

Fig. 1. Effects of Korean ginseng saponin fraction on S-adenosylmethionine decarboxylase activity in prostate and testis at young sexually immature rats administrated water (white bars) and ginseng saponin fraction (filled bars). Prostate 1, prostate at 1 month; Prostate 2, prostate at 2 month; Testis 1, testis at 1 month; Testis 2, testis at 2 month.

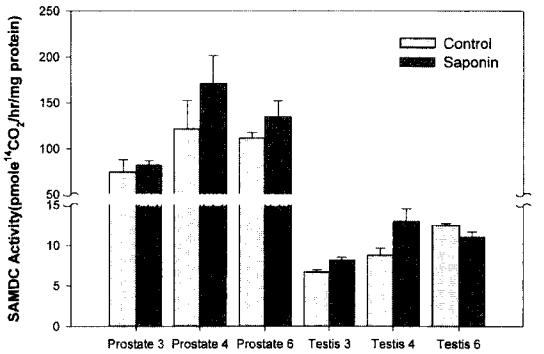

Fig. 2. Effects of Korean ginseng saponin fraction on S-adenosylmethionine decarboxylase activity in prostate and testis of sexually mature rats administrated water (white bars) and ginseng saponin fraction (filled bars). Prostate 3, prostate at 3 month; Prostate 4, prostate at 4 month; Prostate 6, prostate at 6 month; Testis 3, testis at 3 month; Testis 4, testis at 4 month; Testis 6, testis at 6 month.

extracts prepared from either prostate or testis of young sexually immature (1-to 2-month-old) rats (Sprague-Dawley strain). During this age, SAMDC activities of control groups were high and increased gradually for 6 month (Fig. 1, 2). However, administration of ginseng total saponin fraction[21] resulted in the reduction of their activities in prostate and testis. For 1 month administration, SAMDC activities of test groups in prostate and testis reduced 60% and 30% in comparison to control groups, respectively (Fig. 1). At 2 month, these reduced activities were somewhat restored and 20% and 15% in that of control groups, respectively(Fig. 1). However, these results indicated that ginseng saponin fraction had an inhibitory effects on SAMDC activities in prostate and testis at young sexually immature age.

The highest SAMDC activities of control groups were achieved at sexually mature age (3- to 4-month old) and maintained till 6-month-old age in prostate (Fig. 2). In testis, SAMDC activities also increased and reached their maximum at 6-month-old age (Fig. 2). During this administration period, their activities of test groups were higher than that of control groups (Fig. 2). In prostate, SAMDC activities of test groups at 3, 4 and 6 month

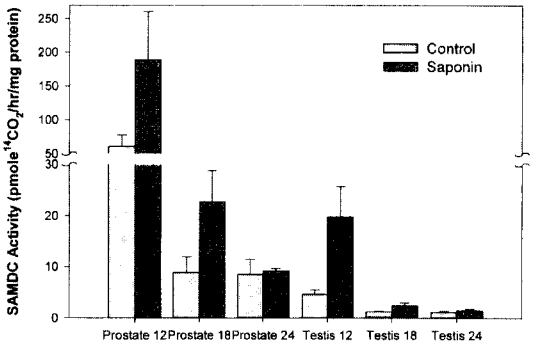

Fig. 3. Effects of Korean ginseng saponin fraction on S-adenosylmethionine decarboxylase activity in prostate and testis of aged rats administrated water(white bars) and ginseng saponin fraction (filled bars). Prostate 12, prostate at 12 month; Prostate 18, prostate at 18 month; Prostate 24, prostate at 24 month; Testis 12, testis at 12 month; Testis 18, testis at 18 month; Testis 24, testis at 24 month.

were increased 110%, 140% and 120% as compared to the enzyme activities of control groups, respectively. In testis, the activities of test groups were 120%, 150% and 95%. Therefore, at sexually mature age, ginseng saponin fraction showed the increasing effect on the SAMDC activities.

In aged rats, SAMDC activities of control groups in both of prostate and testis showed age-related

declines (Fig. 3). At 18 month of age, prostate and testis SAMDC activities of control groups were diminished 90% and 85%, respectively, relative to the enzyme activities of 4- and 6-month-old rats (Fig. 2, 3). However, maximum enhancement of SAMDC activities in prostate and testis by administration of ginseng saponin fraction was achieved during this period (Fig. 3). In prostate, SAMDC activities of test groups at 12, 18 and 24 month were increased 300%, 250% and 110% as compared to the enzyme activities of control groups, respectively. In testis, the activities of test groups were 400%, 200% and 130%.

SAMDC activities are very highly regulated as they respond to a wide variety of stimuli affecting cell growth and differentiation and their regulation was occurred at a multitude of levels, including translational and post-translational, and have a very rapid turnover rate with a half-life of often less than 1h.[28-30] In order to provide more information on the increase of SAMDC activities, we determined the amount of SAMDC protein during the administration period using competitive radioimmunoassay method. As shown in Fig. 4 and 5, the amounts of SAMDC protein in prostate and testis control groups showed age related-decrease and the administration of ginseng saponin fraction increased their amounts in these systems (Fig. 4, 5). The increases in amounts of protein were paralleled by the increases of SAMDC activities in test

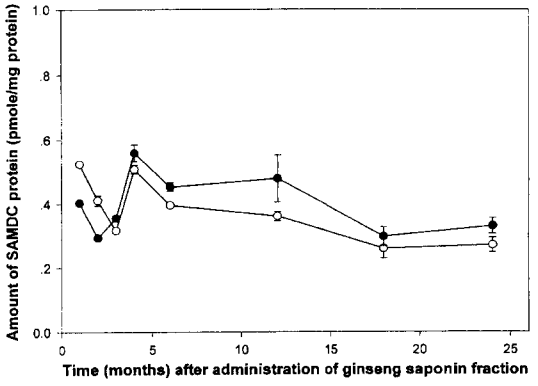

Fig. 4. Effects of Korean ginseng saponin fraction on the amounts of S-adenosylmethionine decarboxylase protein in prostate of rats administrated water (○) and ginseng saponin fraction (●).

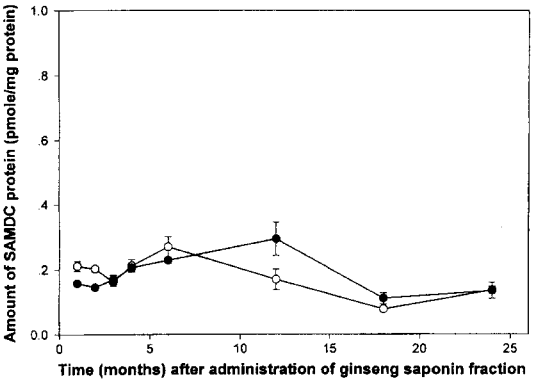

Fig. 5. Effects of Korean ginseng saponin fraction on the amounts of S-adenosylmethionine decarboxylase protein in testis of rats administrated water (○) and ginseng saponin fraction (●).

groups. From all these results, we suggest that all of the increased activity occurring in test groups was not due to the activation of SAMDC activity by ginseng saponin fraction but to the increase in amount of enzyme protein (Fig. 4, 5).

Ornithine decarboxylase (ODC) is a key enzyme of putrescine biosynthesis, which appears to be to serve as a precursor of spermidine and spermine in mammalian. Parenthetically, we also examined the changes of ODC activity when administrated ginseng saponin fraction. In prostate,

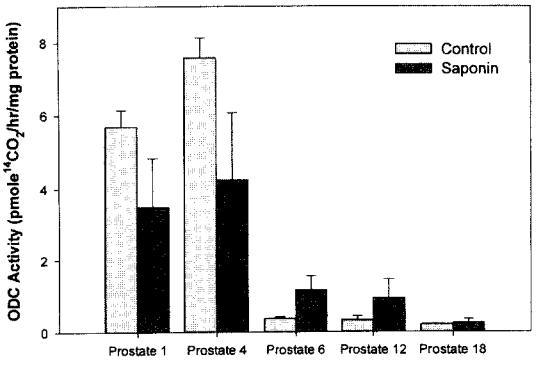

Fig. 6. Effects of Korean ginseng saponin fraction on ornithine decarboxylase activity in prostate of aged rats administrated water(white bars) and ginseng saponin fraction (filled bars). Prostate 1, prostate at 1 month; Prostate 4, prostate at 4 month; Prostate 6, prostate at 6 month; Prostate 12, prostate 12 month; Prostate 18, prostate at 18 month;

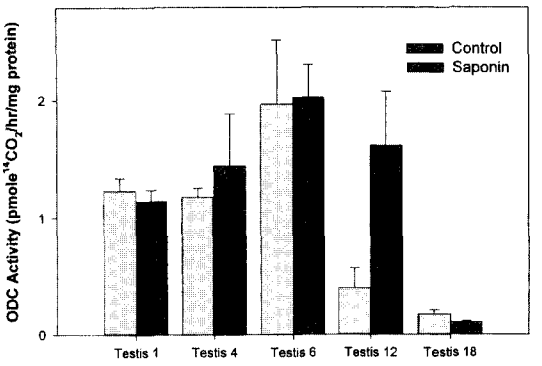

Fig. 7. Effects of Korean ginseng saponin fraction on ornithine decarboxylase activity in testis of aged rats administrated water(white bars) and ginseng saponin fraction (filled bars). Testis 1, testis at 1 month; Testis 4, testis at 4 month; Testis 6, testis at 6 month; Testis 12, testis at 12 month; Testis 18, testis at 18 month.

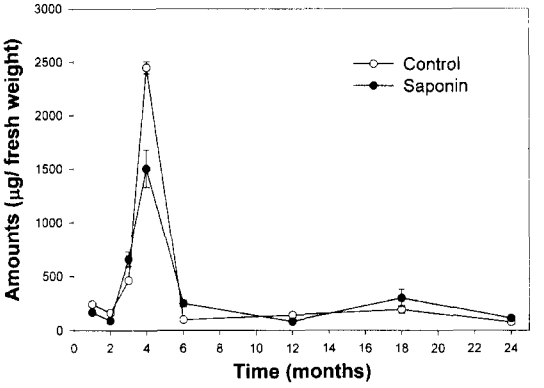

Fig. 8. Effects of Korean ginseng saponin fraction on amounts of spermidine in prostate of rats administrated water (○) and ginseng saponin fraction (●).

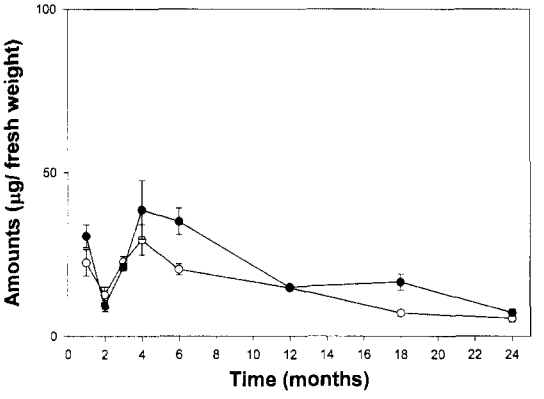

Fig. 9. Effects of Korean ginseng saponin fraction on amounts of spermidine in testis of rats administrated water (○) and ginseng saponin fraction (●).

ODC activities of test groups were reduced about 50% at 1- and 4-month period as compared to the enzyme activities of control groups (Fig. 6). However, these activities were increased 250% and 200% at 6 and 12 months, respectively (Fig. 6). In testis, those activities of test groups were similar to that of control groups till 6 months and then increased maximally at 12 months (Fig. 7).

The spermidine and spermine contents in both of control and test groups were showed in Fig. 8~11. At sexually mature age, rat prostate contained more spermidine contents (150~2400 μg/fresh weight) than spermine contents (100~600 μg/fresh weight) and both of these values were much higher than that of any other tissues.[28~30] In contrast, testis contained more spermine contents (40~120 μg/fresh weight) than spermidine contents (20~50 μg/fresh weight), which are well within the range of spermidine and spermine contents in other tissues.[28~30] The changes in their contents of control groups reflected the age related changes of SAMDC activities in prostate and testis (Fig. 8~11). However, the spermidine and spermine contents of test groups showed small increase in compared to that of control groups (Fig. 8~11) with the exception that the spermidine contents of test groups in testis were increased evidently (Fig. 9). Since we could determine the only free polyamine contents in those organs, we could not exclude the possibility that administration of ginseng saponin fraction might change the bound polyamine contents in these system. Besides, the following speculation are left : Increase in amounts of spermidine and spermine due to increase in SAMDC activity might be excreted into semen which had higher concentration of polyamine than that of the control. Thus, these results could indicate that administration of ginseng saponin fraction resulted in small increase of free spermidine and spermine contents in prostate and testis.

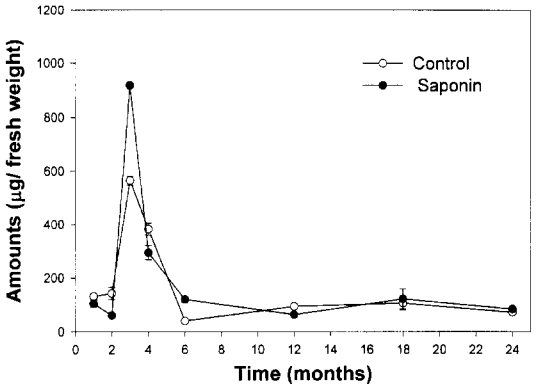

Fig. 10. Effects of Korean ginseng saponin fraction on amounts of spermine in prostate of rats administrated water (○) and ginseng saponin fraction (●).

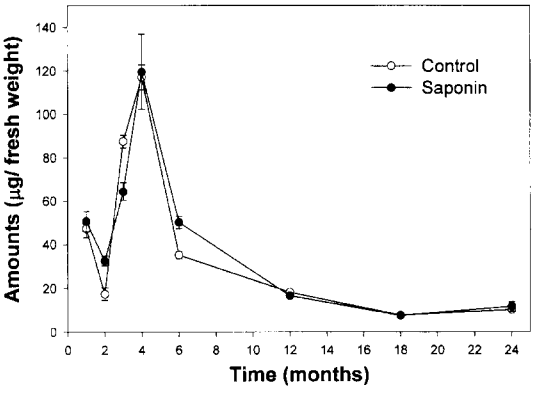

Fig. 11. Effects of Korean ginseng saponin fraction on amounts of spermine in testis of rats administrated water (○) and ginseng saponin faction (●).

A variety of hypothesis attributing functional significance to the polyamines in male reproductive system have been promulgated, but many of them are not based on compelling evidence : 1) Polyamines stimulating the sperm motility and the acrosome reaction.[31,32] 2) Spermine has been proposed to be the active antibacterial reagent in prostate fluid or semen.[33] 3) Polyamines have been postulated related to the regulation of the postejacultory coagulation of semen.[34] In this report, although we could not make clear that ginseng saponin fraction related to these significant role in male reproductive system, we suggested that administration of ginseng saponin fraction alter the spermidine and spermine metabolism in sexually mature and aged reproductive systems in rats.

요 약

고려 인삼 총 saponin 분획이 흰쥐의 생식기관인 전립선과 고환에서 spermidine 과 spermine 대사에 미치는 영향을 관찰하고자 흰 쥐에 인삼 총 saponin 분획을 2년 동안 계속 복용하여 이들 장기에서 spermidine과 spermine의 합성에 중요한 효소인 S-adenosylmethionine decarboxylase(SAMDC)의 활성변화, 세포 내 이 효소 양의 변화, 그리고 spermidine과 spermine의 함량을 측정하였다. 인삼 saponin 분획에 의해 SAMDC 활성변화는 생식능력이 발달하지 않은 어린 흰쥐에서는 별 효과를 보이지 않다가 3개월 이후부터 SAMDC의 활성을 점차적으로 증가시켜 점차 나이가 들어감에 따라 뚜렷한 활성 변화를 보였다. 세포 내의 효소의 함량변화 역시 활성변화와 비슷한 양상을 보여 인삼 saponin 분 획에 의하여 단순히 효소활성을 촉진시키는 것이 아니라 효소의 함량이 증대됨을 보여주었다. 그러나 spermidine과 spermine 함량은 약간 증가함을 보여주었다. 이와 같은 결과를 종합해보면 인삼 saponin 분획이 생식능력이 있는 쥐와 복용기간이 길어질수록 spermidine과 spermine의 생합성 능력이 촉진되고 있음을 알 수 있었다.

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