in vivo *20*: 341-346 (2006)

Effects of VPS Extract of *Coriolus versicolor* on Cancer of the Large Intestine Using a Serial Sacrifice Technique*

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Abstract. *VPS, a hot water extract of the Coriolus versicolor mushroom, was given at a 2% dose level in the diet of female Swiss Webster CFW outbred mice in a serial sacrifice experiment. The mice were also administered either 1,2-dimethylhydrazine dihydrochloride (1,2-DMH) as ten weekly subcutaneous (s.c) injections of 20 μg/g body weight or physiological saline (PS) as ten weekly (s.c) injections of 0.01 ml/g body weight. The animals were sacrificed at 26 weeks or 35 weeks after the first injection of 1,2-DMH or PS. The number of mice with large intestinal tumors and the total number of these tumors were: Group 1 (1,2-DMH), 29 and 438; Group 2 (VPS + 1,2-DMH), 29 and 344; Group 3 (VPS + PS), 0 and 0; and Group 4 (PS), 1 and 1, in the mice sacrificed at 26 weeks. The corresponding tumor incidences in mice sacrificed at 35 weeks were: Group 1 (1,2-DMH), 30 and 323; Group 2 (VPS + 1,2-DMH), 29 and 521; Group 3 (VPS + PS), 1 and 2; and Group 4 (PS), 0 and 0. Histopathologically, the tumors were diagnosed as polypoid adenomas and adenocarcinomas of the cecum, colon and rectum. Contrary to expectations, the VPS treatment enhanced the development of large intestinal tumors induced by 1,2-DMH in animals sacrificed at 35 weeks after the first injection of the carcinogen.*

The mushroom *Coriolus versicolor* (CV) was recorded, in the Compendium of Materia Medica by Li Shi Zhen during the Ming Dynasty in China, as being beneficial to health and longevity, if consumed regularly (1). When, in the 1970s, Japanese investigators, used randomized trials, they isolated polysaccharides from the mushroom which suggested antitumor activity in association with an immunomodulatory effect. The biologically active proteoglucans isolated from CV mushrooms are polypeptide chains or small proteins to which polysaccharide β-glucan chains are stably attached (2, 3). One of the preparations designated as PSK (polysaccharide-K) is from the CM-101 strain of CV, which is obtained by hot water extraction, ammonium sulfate preparation, desalting and drying.

There are additional commercially-available products obtained from CV mushrooms which are sold as nutritional supplements for use by cancer patients. One of these is designated as VPS, which is a hot water extract from the fruit body of the fungus. This extract contains no less than 36% polysaccharide, which is considered to be the active ingredient.

According to the JHS Natural Product Company (Eugene, OR, USA), 10,000-12,000 people use various CV supplements in the U.S. each year. The company further states that 1,000-1,500 people annually use VPS extract in the United States. Considering the extensive preclinical pharmacology and clinical studies undertaken with PSK, and since PSK has proven clinical benefits, it is of obvious interest to study extracts such as VPS for alternative therapeutic activity. Unlike PSK, which has been extensively studied in both animals and humans, hardly any investigations have been undertaken with the VPS extract of the CV mushroom.

In a recent study, the lifelong administration of a 2% VPS extract in the diet in combination with the large intestinal cancer-inducing agent 1,2-dimethylhydrazine dihydrochloride (1,2-DMH) in Swiss mice was investigated. Contrary to our expectations, no anticarcinogenic effect was demonstrated under these conditions (4). In the present series of experiments, a similar protocol was used, however, the mice were subjected to serial sacrifice conditions in order to detect a difference in tumor inhibition early in their lives.

Materials and Methods

Female Swiss albino Webster CFW outbred mice from Charles River Laboratories (Wilmington, MA, USA) were used. They were housed in plastic cages with granular cellulose bedding, separated

*Presented, in part, at the Annual Meeting of the American Society for Investigative Pathology, April 1-5, 2006, San Francisco, California, U.S.A.

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Key Words: Prevention, cancer, intestine, VPS, mushroom.

Table I. *Treatments and survival rates in 1,2-dimethylhydrazine dihydrochloride (1,2-DMH)-, VPS*- and physiological saline (PS)-treated Swiss mice sacrificed at 26 weeks after the start of 1,2-DMH or PS treatment.*

Group	Treatment	Initial no. and sex of mice	No. of survivors (age in weeks)							
			5	10	15	20	25	30	35	40
1	1,2-DMH, 10 weekly *s.c.* injections at 20 μg/g	30 ♀	30	30	30	29	29	27	21	-
2	VPS, 2% in diet + 1,2-DMH as in Group 1	30 ♀	30	30	30	30	29	28	27	-
3	VPS as in Group 2 + PS, 10 weekly *s.c.* injections at 0.01 ml/g	30 ♀	30	30	29	29	27	27	26	-
4	PS as in group 3	30 ♀	30	30	30	30	30	29	27	-

*VPS: An extract of the *Coriolus versicolor* mushroom.

into groups of five. They were given Harlan Teklad Rodent powdered diet and tap water *ad libitum*. The mice were kept in a modified barrier facility, housed in micro-isolator cages on ventilated racks. The temperature was kept between 17.8 and 26˚C, the humidity between 30 and 70%, while the lighting was rotated in a 12-hour on and 12-hour off cycle.

The carcinogen used was 1,2-DMH, symmetrical, (molecular weight, 133.02, melting point, 168˚C), which was obtained from Aldrich Chemical Company, Inc., Milwaukee, WI, USA. The chemical was dissolved in sterile physiological saline (PS). The mice were given subcutaneous *(s.c.)* injections in the interscapular region with a tuberculin syringe using 24-gauge needles.

The other agent used was VPS, an extract of the *Coriolus versicolor* mushroom. VPS is a hot water extract from the fruit body of the fungus. It is a brown powder which contains no less than 36% polysaccharide, which is considered to be the active constituent. VPS was obtained from JHS Natural Products and was mixed with the diet and given to the animals orally.

The experimental groups were as follows:

Group 1. 1,2-DMH was administered as ten weekly *s.c.* injections of 20 μg/g body weight in 0.01 ml PS to 30 mice, which were nine weeks old at the beginning of the experiment.

Group 2. VPS was administered at 2% w/w basis in the powered diet to 30 mice, which were seven weeks old at the beginning of the experiment. VPS treatment was followed by 1,2-DMH, as described in Group 1.

Group 3. VPS was administered as in Group 2. It was followed by PS which was given as ten weekly *s.c*. injections of 0.01 ml/g body weight to 30 mice which were nine weeks old at the beginning of the experiment.

Group 4. PS was administered as in Group 3 to 30 mice.

The animals in Groups 1-4 were sacrificed at 26 weeks after the first injection of 1,2-DMH or PS.

Group 5. The animals received the same treatment as Group 1.

Group 6. The animals were given the same treatment as Group 2.

Group 7. The mice received identical treatment to Group 3.

Group 8. They received the same treatment as Group 4.

The mice in Groups 5-8 were sacrificed at 35 weeks after the first injection of 1,2-DMH or PS.

During the study, the animals were allowed to die or were killed with CO_2 when found to be in poor condition. All the organs were examined macroscopically and were fixed in 10% buffered formalin. Histological studies were done routinely on the intestines (large and small) as well as on those organs showing gross pathological changes. Sections from these tissues were stained with hematoxylin and eosin and studied by light microscopy.

Results

Experiments in which the animals were sacrificed at 26 weeks after the first injection of 1,2-DMH or PS

Tumors of the large intestine. Table I summarizes the survival rates of the treated animals at 5-week intervals. Of the animals treated with 1,2-DMH, 29 mice (96%) developed 438 tumors of the large intestine. Their average age at death was 33.8 weeks. The first tumor was observed in the 28th week and the last in the 35th week of age. Of these, seven mice had seven adenocarcinomas of the cecum, 27 mice developed 270 adenocarcinomas of the colon, 28 mice had 160 adenocarcinomas of the rectum and one mouse developed a squamous cell carcinoma of the anus.

Of the animals treated with VPS plus 1,2-DMH, 29 mice (96%) developed 344 tumors of the large intestine. Their average age at death was 34.2 weeks. The first tumor was observed at 28 weeks and the last at 35 weeks of age. Of these, one mouse developed an adenocarcinoma of the cecum, 26 mice had 201 adenocarcinomas of the colon, 27 mice developed 141 adenocarcinomas of the rectum and one mouse had a squamous cell carcinoma of the anus.

Of the animals treated with VPS plus PS, none developed tumors of the large intestine.

Of the animals treated with PS, only one mouse (3%) developed a single adenocarcinoma of the cecum at 35 weeks of age.

Table II presents the number and percentages of mice with tumors and their ages at death.

Table II. *Treatments and tumor incidences in 1,2-dimethylhydrazine dihydrochloride (1,2-DMH)-, VPS*- and physiological saline (PS)-treated Swiss mice sacrificed at 26 weeks after the start of 1,2-DMH or PS treatment.*

Group	Treatment	No. and sex of mice	Animals with tumors of:									Other tumors***
			Large intestine			Malignant lymphomas			Lungs			
			No.	%	Latent period**	No.	%	Latent period**	No.	%	Latent period**	
1	1,2-DMH, 10 weekly *s.c.* injections at 20 μg/g	30♀	29	96	33 (28-35)	3	10	34 (34-35)	3	10	35 (35-35)	1 Adeno-carcinoma duodenum (32)
2	VPS, 2% in diet + 1,2-DMH as in Group 1	30♀	29	96	34 (28-35)	6	20	31 (21-35)	6	20	35 (35-35)	-
3	VPS as in Group 2 + PS, 10 weekly *s.c.* injections at 0.01 ml/g	30♀	-	-	-	5	16	30 (14-35)	-	-	-	-
4	PS as in Group 3	30♀	1	3	35	4	13	32 (28-35)	-	-	-	-

*VPS: An extract of the *Coriolus versicolor* mushroom.
** Average and range in weeks.
*** Age at death given in weeks in parentheses.

Table III. *Treatments and survival rates in 1,2-dimethylhydrazine dihydrochloride (1,2-DMH)-, VPS*- and physiological saline (PS)-treated Swiss mice sacrificed at 35 weeks after the start of 1,2-DMH or PS treatment.*

Group	Treatment	Initial no. + sex of mice	No. of survivors (age in weeks)								
			5	10	15	20	25	30	35	40	45
1	1,2-DMH, 10 weekly *s.c.* injections at 20 μg/g	30 ♀	30	30	30	30	30	29	25	16	-
2	VPS, 2% in diet + 1,2-DMH as in Group 1	30 ♀	30	30	30	30	30	28	27	23	-
3	VPS as in Group 2 + PS, 10 weekly *s.c.* injections at 0.01 ml/g	30 ♀	30	30	30	30	30	30	28	27	-
4	PS as in Group 3	30 ♀	30	30	30	29	28	26	25	25	-

*VPS: An extract of the *Coriolus versicolor* mushroom.

Experiments in which the animals were sacrificed at 35 weeks after the first injection of 1,2-DMH or PS

Tumors of the large intestine. In Table III, the survival rates of the treated mice at 5-week intervals are summarized. Of the animals treated with 1,2-DMH, 30 mice (100%) developed 323 tumors of the large intestine. Their average age at death was 38.6 weeks. The first tumor was observed at 27 weeks and the last at 44 weeks of age. Of these, four mice had four adenocarcinomas of the cecum, 25 mice developed 213 adenocarcinomas of the colon, one mouse had a polypoid adenoma and four adenocarcinomas of the colon, 25 mice had 100 adenocarcinomas of the rectum and two mice developed two squamous cell carcinomas of the anus.

Of the animals treated with VPS plus 1,2-DMH, 29 mice (96%) developed 521 tumors of the large intestine. Their average age at death was 40.8 weeks. The first tumor was observed at 28 weeks and the last at 44 weeks of age. Of these, 16 mice had 16 adenocarcinomas of the cecum, 28 mice developed 334 adenocarcinomas of the colon, 25 mice had 167 adenocarcinomas of the rectum, one mouse developed a polypoid adenoma and two adenocarcinomas of the rectum and one mouse developed a squamous cell carcinoma of the anus.

Table IV. *Treatments and tumor incidences in 1,2-dimethylhydrazine dihydrochloride (1,2-DMH)-, VPS*- and physiological saline (PS)-treated Swiss mice sacrificed at 35 weeks after the start of 1,2-DMH or PS treatment.*

Group	Treatment	No. and sex of mice	Animals with tumors of:									Other tumors***
			Large intestine			Malignant lymphomas			Lungs			
			No.	%	Latent period**	No.	%	Latent period**	No.	%	Latent period**	
1	1,2-DMH, 10 weekly *s.c.* injections at 20 μg/g	30♀	30	100	38 (27-44)	4	13	38 (37-43)	3	10	40 (38-44)	1 Adenocarcinoma of ileum (35) 1 Granulosa cell tumor (41)
2	VPS, 2% in diet + 1,2-DMH as in Group 1	30♀	29	96	40 (28-44)	3	10	36 (28-44)	3	10	38 (36-44)	3 Adenocarcinomas of duodenum (44,44,44) 1 Adenocarcinoma of ileum (36) 1 Hemangiosarcoma in liver (43) 1 Hepatoma (44)
3	VPS as in Group 2 + PS, 10 weekly *s.c.* injections at 0.01 ml/g	30♀	1	3	44	4	13	40 (31-44)	-	-	-	1 Fibroma of tail (44)
4	PS as in Group 3	30♀	-	-	-	4	13	32 (17-44)	-	-	-	-

*VPS: An extract of *Coriolus versicolor* mushroom.
** Average and range in weeks.
*** Age at death given in weeks in parentheses.

Of the animals treated with VPS plus PS, one mouse developed two adenocarcinomas of the cecum at 44 weeks old.

Of the mice treated with PS, none developed intestinal tumors.

In addition to the incidences of large intestinal tumors, the mice developed other types of neoplasms listed in Table IV. It is apparent that the appearance of these other lesions was not related to the treatments.

The large intestinal tumors were similar in terms of their location, distribution, macroscopic and microscopic appearances to those published earlier (5).

Statistical analysis. The median number of large intestinal tumors was compared between the 1,2-DMH and VPS plus 1,2-DMH-treated groups in animals sacrificed at 35 weeks after the first injection of the carcinogen, using the Wilcoxon rank-sum test (6). The difference was statistically significant ($p=0.03$).

Discussion

The current experimental set-up is similar to our earlier study in which a hot water extract, designated as VPS, of the *Coriolus versicolor* mushroom was given at a 2% dose level in the diet of female Swiss Webster CFW outbred mice for life. The animals also received either 1,2-DMH as ten weekly *s.c.* injections at 20 μg/g body weight or PS as ten weekly subcutaneous injections at 0.01 ml/g body weight. Contrary to the earlier study, however, in the present series of experiments the mice were sacrificed at 26 weeks or 35 weeks after the first injection of 1,2-DMH or PS. It was hoped that early sacrifice would enable us to observe the time sequence of tumor inhibition, which could not have been detected in lifelong experiments. In spite of our expectations, no statistically significant tumor inhibition was observed in the large intestine in terms of the number of mice with such tumors and the total number of these tumors. As a matter of fact, the VPS treatment enhanced the number of large intestinal tumors induced by 1,2-DMH in mice sacrificed at 35 weeks after the first administration of the carcinogen. Currently, no satisfactory explanation can be provided for this unexpected finding.

Prior to our studies, there had been a number of investigations using the PSK extract of the *Coriolus versicolor*. In one of them, 2% PSK was administered *via* the feed of rats, beginning on the first day of the 16 weekly injections of

1,2-DMH. PSK was given for 45 weeks and the rats necropsied 15, 25, 35 and 45 weeks later. As a result of the treatment, a significant reduction in the incidence of 1,2-DMH-induced gastrointestinal cancer was observed at weeks 25 and 35 (7). In other studies, PSK was given to rats for 12 weeks prior to the administration of 3-methyl-4-dimethylaminobenzene (MDAB), a hepatoma-inducing agent. The treatment resulted in a significant increase in survival (8). Additional support for the therapeutic activity of PSK in autochthonous tumors was provided by 3-methylcholanthrene-induced mammary tumor models in C57BL/6 mice. In this particular study, the PSK therapy was combined with surgery. As a result of the treatment, in some groups the median survival time was prolonged, together with a reduction in the frequency of tumor relapse (9).

More than two dozen studies have been reported in the medical literature concerning the use of the PSK extract in humans. It is of interest to note that no human study with the VPS extract has been published to date. Ten investigations assessed the survival and disease response after treatment with the PSK extract (10-19). Another ten studies reported survival without disease response (20-28), while five dealt with the immune regulatory effects of this agent (29-33). In the ten clinical investigations with PSK which measured both survival and disease response, eight were randomized trials. Of the randomized studies, only one dealt with colorectal cancer, reporting positive results as far as survival and disease-free interval are concerned (17). The remaining seven randomized investigations reported patients with various other types of cancer after PSK therapy in conjunction with chemotherapy and radiotherapy. In all of these trials, longer disease-free intervals were observed. Survival studies without disease response included three randomized studies. In two of these studies, better survival times for esophageal cancer patients were reported. In one of these studies no significant increase in survival was noted. Finally, immune augmentation in randomized clinical studies has been reported concerning cancer of the colon, stomach, head and neck of patients treated with PSK.

In our present serial sacrifice experiments, in which the mice were sacrificed at 26 and 35 weeks after the first injection of the carcinogen or the solvent, the continuous administration of VPS failed to inhibit the development of large intestinal cancer. Since an earlier study in which the VPS extract was given for life also yielded negative results, it is safe to conclude that this *Coriolus versicolor* extract has no large intestinal cancer inhibitory effect in Swiss mice. This finding is in sharp contrast to those studies in which a different type of extract of the *Coriolus versicolor* mushroom, designated as PSK, exhibited inhibition of gastrointestinal, breast and liver tumors. It is relevant to note that the PSK extract was substantially more pure than the VPS extract.

Acknowledgements

This work was supported by grant 1R21 AT001739 from the National Institutes of Health, USA.

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Received March 7, 2006
Accepted March 14, 2006