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Lack of Prevention of Large Intestinal Cancer by VPS, an Extract of *Coriolus Versicolor* Mushroom*

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Abstract. *Cancer prevention studies were conducted with VPS, a hot water extract of the Coriolus versicolor (CV) mushroom, in female Swiss mice. The extract was administered in the diet for life to the animals. Three groups of mice received the following treatments: a). 1,2-dimethylhydrazine dihydrochloride (1,2-DMH) was administered as 10 weekly subcutaneous injections of 20 μg/g body weight, starting at 9 weeks of age; b). VPS was given at a 2% dose level starting at 7 weeks of age followed by 1,2-DMH, as described in group a; c). 1,2-DMH was administered as described in group a followed by VPS at a 2% dose level starting at 21 weeks of age. The number of animals with large intestinal tumors and the total number of these tumors were: a). 30,321; b). 29,359; and c). 28,415. These differences are not statistically significant. Because extracts of the CV mushroom are used by cancer patients as nutritional supplements in the U.S., and particularly in the Orient, the present negative result should caution its users.*

The mushroom *Coriolus versicolor* (CV) was recognized during the Ming Dynasty in China as being beneficial to health and longevity, if consumed regularly (1). Its therapeutic activity was not established until the 1970s, when Japanese researchers isolated its active ingredient proteoglucans (polysaccharide peptides) (2, 3). Several commercially available products from the CV mushroom are on the market. In general, they contain the β-glucan as part of the proteoglucan. Even though no proper estimates are available concerning the annual use of CV mushroom extracts in the United States, one source estimates that a few thousand people annually use VPS, a hot water extract. It is further stated by the JHS Natural Products Company that 10,000-12,000 people annually use various CV supplements in the United States. These products are primarily used as nutritional supplements by cancer patients, mainly in the Orient.

A significant number of studies have been published in the medical literature concerning the anti-carcinogenic effects of CV mushroom extracts. These investigations were conducted with PSK extract, which is derived from the CM-101 strain of CV. These studies and trials were conducted in rodents and humans and, indeed, the large majority concluded that the PSK extract resulted in better survival times and decreased incidences of cancer (4-16).

The aim of the present investigation was to reveal the possible anti-carcinogenic effect of VPS, an unstudied extract of CV, in Swiss mice. In this undertaking, in combination with VPS, a highly effective large intestinal cancer-inducing agent, 1,2-dimethylhydrazine dihydrochloride (1,2-DMH), was used.

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 in 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 64 and 79˚F, the humidity between 30 and 70%, while the lighting was rotated in a 12 hours on and 12 hours off cycle.

The carcinogen used was 1,2-dimethylhydrazine dihydrochloride, symmetrical (1,2-DMH), (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. The mice were given subcutaneous 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, Figure 1. 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

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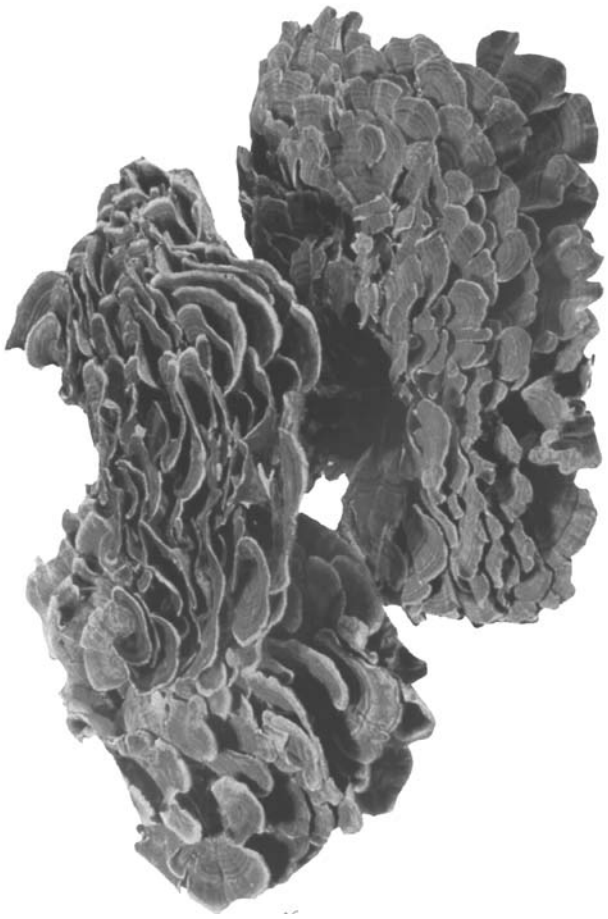

Figure 1. *Gross photograph of the Coriolus versicolor mushroom.*

constituent. VPS was obtained from JHS Natural Products (P.O. Box 50398, Eugene, OR, USA) 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 10 weekly injections of 20 μg/g body weight in 0.01 ml physiological saline to 30 mice, which were 9 weeks old at the beginning of the experiment.

Group 2. VPS was administered at 2% w/w basis in the powdered diet for life to 30 mice, which were 7 weeks old at the beginning of the experiment. VPS treatment was followed by 1,2-DMH, as described in group 1.

Group 3. 1,2-DMH was given to 30 mice, as described in group 1, followed by VPS, as described in group 2. However, the VPS treatment was started at 21 weeks of age.

During the study, the animals were allowed to die or were killed with CO_2 when found to be in poor condition. Complete necropsies were preformed on all animals. All 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

Table I summarizes the survival rates of the treated mice at 10-week intervals. It is apparent from these data that the treatments had no significant effects on the survival rates.

Table II presents the number, percentages of mice with tumors and their ages at death. The most important neoplasms occurred in the large intestines, lungs and lymphoreticular tissue, which are described in detail.

Tumors of the large intestine. In group 1 in which the animals were treated with 1,2-DMH, 30 mice (100%) developed 321 tumors of the large intestine. Their average age at death was 41 weeks. The first tumor was observed at the 17th week and the last at the 67th week of age. Of these, 12 mice had 13 adenocarcinomas of the cecum, 28 had 194 adenocarcinomas of the colon, 1 developed a polypoid adenoma and 7 adenocarcinomas of the colon and 29 mice had 106 adenocarcinomas of the rectum.

In group 2, in which the animals were treated with VPS starting at 7 weeks of age followed by 1,2-DMH, 29 mice (96%) developed 359 tumors of the large intestine. Their average age at death was 40 weeks. The first tumor was observed at the 32nd week and the last at the 61st week. Of these, 4 mice had 4 adenocarcinomas of the cecum, 29 had 219 adenocarcinomas of the colon, 27 mice had 127 adenocarcinomas of the rectum, and 1 mouse developed a polypoid adenoma and 8 adenocarcinomas of the rectum.

In group 3, in which the animals were treated with 1,2-DMH followed by VPS starting at 21 weeks of age, 28 mice (93%) developed 415 tumors of the large intestine. Their average age at death was 43 weeks. The first tumor was observed at the 29th week and the last at the 56th week. Of these, 3 mice had 3 adenocarcinomas of the cecum, 24 mice developed 1 fibroma and 271 adenocarcinomas of the colon and 28 mice had 140 adenocarcinomas of the rectum.

The number of large intestinal tumors per mouse was compared between groups 1, 2 and 3 using the Kruskal-Wallis test (17). Group 1 had a median of 8 tumors per mouse, group 2 had a median of 12 tumors per mouse and group 3 had a median of 14 tumors per mouse. There was no statistically significant difference between the 3 groups ($p=0.27$).

The large intestinal tumors regarding location, distribution, macroscopic and microscopic descriptions were similar to those published earlier in this laboratory (18).

Lung tumors. In group 1, in which the animals were treated with 1,2-DMH, 8 mice (26%) developed 10 adenomas of this organ. Their average age at death was 49 weeks. The first tumor was observed at the 37th week and the last at the 67th week of age.

In group 2, in which the animals were treated with VPS starting at 7 weeks of age followed by 1,2-DMH, 7 mice

Table I. *Treatment and survival rates of 1,2-dimethylhydrazine dihydrochloride (1,2-DMH)- and VPS*-treated Swiss mice.*

Group	Treatment	Initial no. of mice	No. of survivors (age in weeks) 10	20	30	40	50	60	70
1	Ten *s.c.* injections of 1,2-DMH, 20 μg/g starting at 9 weeks	30 ♀	30	29	27	18	6	2	-
2	VPS, 2% in diet starting at 7 weeks followed by 1,2-DMH as in group 1	30 ♀	30	30	29	11	3	1	-
3	1,2-DMH as in group 1 followed by VPS as in group 2, starting at 21 weeks	30 ♀	30	28	27	16	3	-	

*Extract of *Coriolus versicolor* mushroom
s.c.: subcutaneous

Table II. *Treatment and tumor incidences in 1,2-dimethylhydrazine dihydrochloride (1,2-DMH)- and VPS*-treated Swiss mice.*

Group	Treatment	Initial no. of mice	Animals with tumors of: Large intestine			Lungs			Malignant lymphoma			Other organs***
			No.	%	Age at death**	No.	%	Age at death**	No.	%	Age at death**	
1	Ten *s.c.* injections of 1,2-DMH, 20 μg/g starting at 9 weeks	30 ♀	30	100	41 (17-67)	8	26	49 (37-67)	10	33	42 (17-67)	2 adenocarcinomas of duodenum (40, 41); 1 adenocarcinoma of jejunum (30); 1 fibromyxosarcoma of uterus (63); 1 adenocarcinoma of anal gland (41).
2	VPS, 2% in diet starting at 7 weeks followed by 1,2-DMH as in group 1	30 ♀	29	96	40 (32-61)	7	23	39 (36-49)	7	23	40 (20-61)	4 adenocarcinomas of duodenum (34, 38, 43, 50); 4 adenocarcinomas of ileum (34, 36, 41, 49); 3 squamous cell carcinomas of anus (38, 38, 50); 1 adenocarcinoma of jejunum (34).
3	1,2-DMH as in group 1 followed by VPS as in group 2, starting at 21 weeks	30 ♀	28	93	43 (29-56)	3	10	51 (40-55)	5	16	32 (17-44)	3 adenocarcinomas of duodenum (41, 42, 49); 2 squamous cell carcinomas of anus (40, 48); 2 hepatomas (55, 56).

*Extract of *Coriolus versicolor* mushroom.
s.c.: subcutaneous
**Average and range in weeks.
***Age at death given in weeks in parentheses.

(23%) developed 14 adenomas of the lungs. Their average age at death was 39 weeks. The first tumor was observed at the 36th week and the last at the 49th week of age.

In group 3, in which the animals were treated with 1,2-DMH followed by VPS starting at 21 weeks of age, 3 mice (10%) developed 7 adenomas of the lungs. Their average age at death was 51 weeks. The first tumor was observed at the 40th week and the last at the 55th week of age.

Macroscopically and histopathologically, these lung tumors were similar to those published earlier by us (19, 20).

Malignant lymphomas. In group 1, in which the animals were treated with 1,2-DMH, 10 mice (33%) developed lymphoreticular tissue tumors. Of these, 7 were lymphocytic types, 1 was a stem cell type, 1 was a mixed cell type and the last 1 was a histocytic type. Their average age at death was 43 weeks. The first tumor was observed at the 17th week and the last at the 67th week of age.

In group 2, in which the animals were treated with VPS starting at 7 weeks of age followed by 1,2-DMH, 7 mice (23%) developed malignant lymphomas of the lymphocytic type. Their average age at death was 40 weeks. The first lesion was observed at the 20th week and the last at the 61st week of age.

In group 3, in which the animals were treated with 1,2-DMH followed by VPS starting at 21 weeks of age, 5 mice (16%) developed malignant lymphomas of the lymphocytic type. Their average age at death was 32 weeks. The first tumor was observed at the 17th week and the last at the 44th week of age.

Grossly and histopathologically, these lymphoreticular tissue tumors were similar to those described in detail in this laboratory (21).

Other tumors. In a number of cases, tumors of other tissues were also found and they are described in Table II.

Discussion

The current findings demonstrate that VPS, when given in conjunction with 1,2-DMH, had no apparent inhibitory effect on the development of large intestinal cancers in Swiss mice. In one of the groups, VPS was administered at 7 weeks of age followed by 1,2-DMH starting at 9 weeks of age. In this method of administration, we hoped to use VPS preventatively before the development of large intestinal cancer. In the other group, we started to give the carcinogen 1,2-DMH at 9 weeks of age followed by VPS at 21 weeks of age and onwards. In this particular set-up, our aim was to use VPS therapeutically to treat the already developed large intestinal cancer. Neither schedule was succesful.

As far as the published literature is concerned, there was only one study in which 1,2-DMH was used in conjunction with PSK, another extract of the CV mushroom, in rats. In this investigation, PSK was given at a 2% dose level in the feed and the animals received 16 weekly injections of 1,2-DMH at 15 μg/g. Subsequently, the rats were serially sacrificed at 15, 25, 35 and 45 weeks later. As a result of the treatment, a significant reduction in the incidence and distribution of 1,2-DMH-induced gastrointestinal cancer was observed at weeks 25 and 35 (4). There have been two additional studies with PSK in rodents. In the first study in mice, the mammary tumor-inducing 3- methylcholanthrene was used in combination with PSK. Resection of the primary tumor (mammary) accompanied the treatments. It was concluded that PSK inhibited the growth of recurrent and metastatic tumor cells (6). In the following study in rats, the hepatoma-inducing carcinogen 3'-methyl-4-dimethylamino-azobenzene was used in combination with PSK. The treatment with PSK significantly decreased the survival time of the rats (5).

With regard to human cancer patients receiving extracts of CV, over two dozen investigations have been reported in the literature. Of these, ten have assessed survival and disease response (7-16). In these ten clinical investigations, eight were randomized clinical trials. Of the randomized studies, only one compared PSK with a placebo for patients who had colorectal cancer. In this trial, treatment with PSK resulted in significantly longer disease-free intervals and survival than the placebo group (10). In the other studies using patients with different types of neoplasms, including lung, breast, esophagus, nasopharynx, *etc.*, most observed beneficial effects of PSK (7-9, 11, 13-16).

In contrast to the aforementioned investigations, our findings did not provide any evidence of preventative or therapeutic effects of VPS on large intestinal cancer development. This is somewhat surprising because, in a similar experimental model using 1,2-DMH in rats, the 2% dose level of PSK did result in a reduction of intestinal cancer (4). However, the PSK extract is different from the VPS extract of CV, since VPS is less pure than PSK. In addition to the purity of the products and to the species difference, in our study we did not terminate the life of the experimental animals as was done in the rat study. Our animals were allowed to complete their normal lifespan. From this point of view, therefore, our study is more relevant to the human experience. In addition, to the intestinal cancer model, the PSK extract prevented the growth of breast and liver tumors in earlier investigations. Finally, in a substantial number of clinical trials in humans, the PSK extracts had beneficial effects in various cancer models including the colorectal system.

Therefore, additional experimentation is recommended to further elucidate these issues.

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