Inhibition of Ehrlich Mouse Ascites Tumor Growth by Cordycepin*

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SUMMARY

Cordycepin, a nucleoside isolated from cultures of the mold *Cordyceps militaris*, has been demonstrated to increase the survival time of mice bearing the Ehrlich mouse ascites tumor. In all these studies the drug was administered daily for a 7-day period. When drug inoculations were started the same day as tumor inoculations, inhibition of tumor growth was noted at levels of from 15 to 200 mg/kg body weight. When the initial administration of drug was begun on animals in which the tumor was 5 days old, a dosage of 150 mg/kg body weight resulted in a significantly increased survival time.

In 1951 Cunningham *et al.* (2) described the isolation and characterization of cordycepin, a metabolic product produced from cultures of the mold *Cordyceps militaris*. In a second paper by Bentley *et al.* (1) the structure of this compound was established as that of a nucleoside, containing adenine and a 3-deoxypentose with a branched carbon chain.

Our interest in this problem was twofold: first, to determine the metabolic sequence by which the mold produces this substance, and, second, to test this substance as an antitumor agent. It is the purpose of this paper to present our results on the effectiveness of cordycepin as an inhibitor of Ehrlich mouse ascites tumor growth.

MATERIALS AND METHODS

White Swiss mice were used in these experiments and were purchased from Mr. L. H. Stout of Plymouth, Michigan. The Ehrlich mouse ascites tumor was from a stock maintained in this laboratory by repeated transplantation every 7 days. Cordycepin was isolated from liquid cultures of *Cordyceps militaris* by a published procedure (5). In essence, it involved growing the mold over a 30-day period in broths containing glucose and casein hydrolysate. At the end of this period of time mycelia were removed by filtration, and the cordycepin was extracted from the medium by processes of low temperature concentration and ion-exchange chromatography. The final product was recrystallized 3 times from H_2O and dissolved in 0.9 per cent NaCl for injection.

Adenosine and deoxyadenosine were purchased from California Corporation for Biochemical Research and were used without further purification. The stock culture of *Cordyceps militaris* (Linn) Link was obtained from Centraalbureau voor Schimmelculture, Baarn, Holland. The culture was maintained by repeated transplantation on oatmeal agar every 21 days. Mice were maintained on Rockland Rat Diet and water.

Recipient mice of approximately 20 gm. weight were given injections of 0.2 ml. of ascites fluid from donor mice that had carried the tumor for 7 days. In any given experiment fluid from donor mice was pooled and thoroughly mixed so that all recipient mice in the experiment received the same material. All injections of ascitic cell suspension, cordycepin, or saline were made intraperitoneally under light ether anesthesia. Survival time, weight measurements, and subjective examination for degree of abdominal distention were all used as indices of antitumor activity. In each set of experiments, tumor-bearing animals were divided into groups of five, and all animals of a group received identical treatment. Control mice in groups of five simultaneously received injections of 0.9 per cent

* A preliminary report of this work was presented before the American Society of Biological Chemists, April, 1960 (6). This work was supported in part from grants from the National Science Foundation, NSF G-6423; and the American Cancer Society Institutional Research Grant 44-F.

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Received for publication August 8, 1960.

NaCl. The volumes of injected solutions were less than 1.0 ml. in early experiments and less than 0.5 ml. in later ones. Control animals in all cases received a corresponding volume of saline.

RESULTS

Owing to a limited supply of cordycepin we have not carried out stepwise toxicity studies with the drug. We have found, however, that single injections of cordycepin into mice at dosages up to 900 mg/kg of body weight have no outward harmful effects. For our tumor studies we chose to inject cordycepin daily over a 7-day period. Under these conditions we found doses of up to 300 mg/kg of body weight to be uniformly tolerated. With the double insult of both tumor and cordycepin there seems to be a lowering of this tolerance, so that daily dosages of 200 mg/kg of body weight led to toxic effects. In the studies to be reported here on the delayed drug response (Chart 2) a dose of 150 mg cordycepin/kg body weight daily for 7 days was tolerated by the tumor-bearing mice without any deaths that could be attributed to the drug.

In our first series of experiments (dose-response study, Chart 1) we were interested in determining what level of cordycepin was necessary to produce a significant increase in survival time in mice bearing the tumor. In these studies the injections of cordycepin began the same day as tumor inoculation and continued at the dosage indicated for a 7-day period. Two groups of control mice simultaneously received 0.9 per cent NaCl. The results of these experiments indicate that a dosage as low as 15 mg/kg body weight is effective in increasing the survival time of the mice. A dosage of 5 mg/kg body weight was ineffective.

In a second series of experiments (delayed dose-response study) it seemed of interest to test the capacity of cordycepin to inhibit the further growth of tumor in animals who have carried the tumor for several days. A total of twelve groups was used in which there were 0, 1, 2, 3, 5, and 7 days between inoculation of ascitic fluid and the start of drug or saline administration. Cordycepin was given daily at a level of 150 mg/kg body weight for a total of 7 days. The results of this experiment are shown in Chart 2. As can be seen in this chart, even when the tumor was given a 5-day start a significant increase in survival time was observed. The mice who had carried tumor 7 days prior to the start of drug inoculation were not helped by the administration of cordycepin.

In a separate experiment (Chart 3) weight changes are shown over the first 10 days after injection of cell suspension in the same type of study. From this experiment it appears that a significant weight reduction occurred in animals which had borne the tumor 4 days prior to the start of drug inoculation. In this experiment the dosage was as in the delayed dose response, at a level of 150 mg/kg body weight.

In two other experiments, adenosine and deoxyadenosine were administered at a level of 150 mg/kg body weight to mice which had borne the

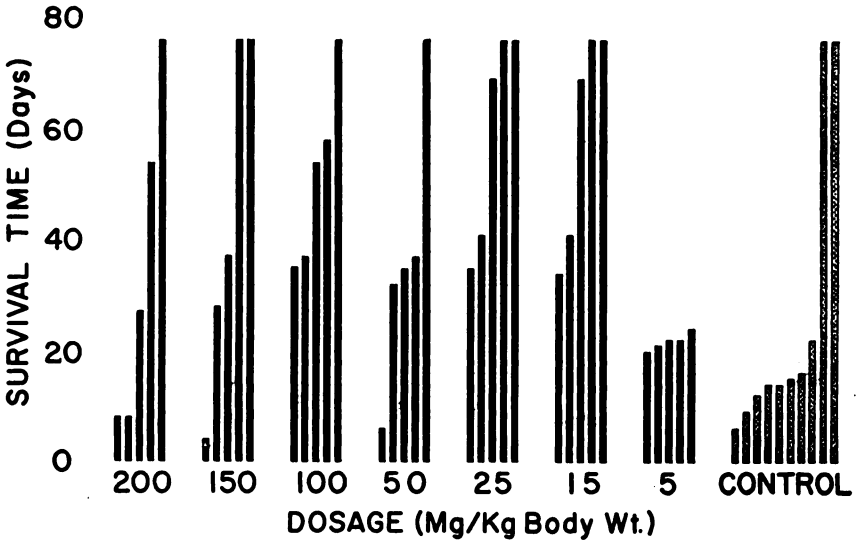

CHART 1.—Dose-response study. The method of administration of cordycepin, as well as the grouping of animals, is described in the text. A shaded bar represents the life span of one control animal. A solid bar represents the life span of one cordycepin-treated animal. At the end of 76 days all surviving animals were sacrificed. The two surviving control mice were found to contain tumor in a solid rather than ascitic form. Under these conditions a longer survival period would be expected (4). The nine surviving experimental animals were found to be without detectable ascitic or solid tumor.

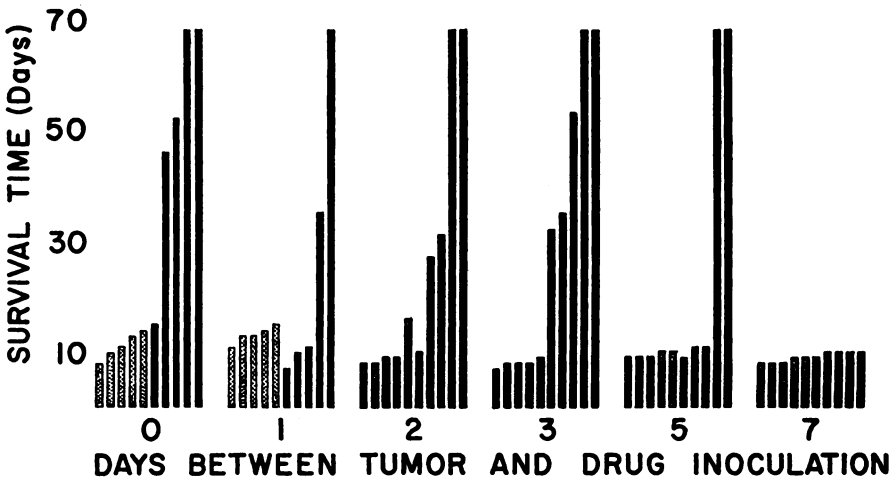

CHART 2.—Delayed dose-response. The method and level of administration of cordycepin are described in the text.

A shaded bar represents the life span of one control animal. A solid bar represents the life span of one cordycepin-treated animal.

At the end of 68 days all surviving animals were sacrificed.

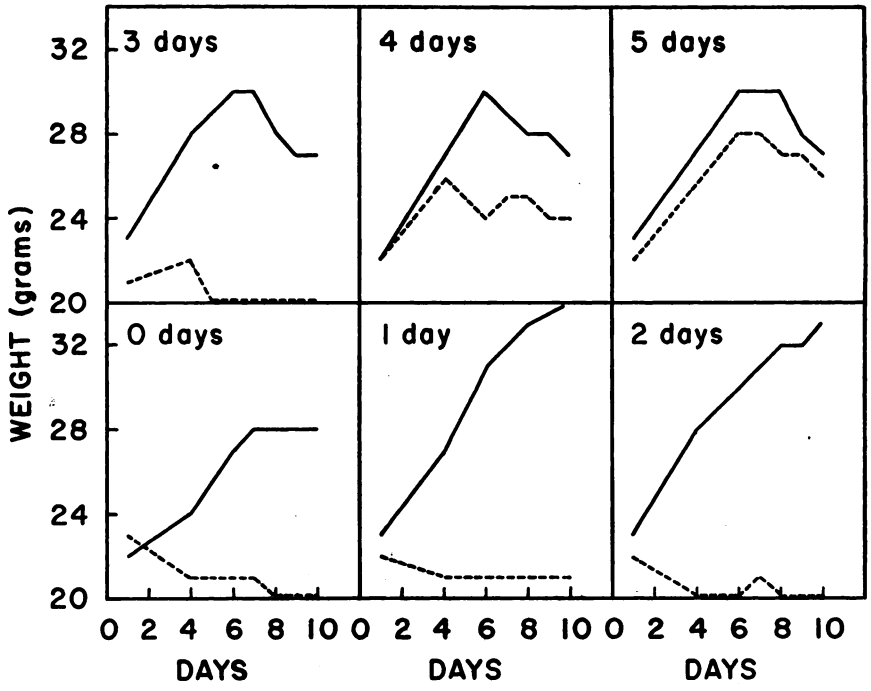

CHART 3.—Weight changes of tumor-bearing animals. The method and level of administration of cordycepin are given in the text. The solid lines represent the weights of tumor-bearing animals receiving 0.9 per cent NaCl, the broken lines represent the weights of animals receiving cordycepin. In each experiment there were five animals in both the control and experimental group. The weight given is the weight of the group divided by the number of surviving animals in that group. The number of days given in the upper left-hand corner of each graph is the number of days between tumor inoculation and the start of drug or saline administration.

tumor for 3 days. No increase in survival time was noted in these animals over comparable controls receiving NaCl.

From the results of our dose-response study it is apparent that cordycepin was able to increase the survival time of mice bearing the Ehrlich mouse ascites tumor at levels of from 15 to 200 mg/kg body weight. At a level of 5 mg/kg body weight no appreciable increase in survival time was affected. In one control group of mice in this study it was observed that two mice survived as long as experimental animals. On close inspection it was noted that these animals were carrying the tumor in a solid rather than ascitic form. Under these conditions a longer survival period would be expected (4). At the end of 76 days there were nine surviving experimental animals. They were sacrificed and found to be without detectable solid or ascitic tumor.

In the delayed dose-response study, mice carrying the tumor for 7 days prior to the start of administration of cordycepin showed no increase in survival time, whereas the survival times of two mice which had borne the tumor 5 days prior to the start of drug administration were increased significantly. In this study experimental animals received 150 mg cordycepin/kg body weight daily for 7 days from the start of drug administration. At the end of 68 days there were nine experimental animals surviving. They were sacrificed and found to be without detectable solid or ascitic tumor. This experiment has since been repeated at a dose level of 125 mg/kg body weight. Although there was a significant increase in survival time of animals receiving cordycepin, the results were not as striking as those in which 150 mg. was used.

DISCUSSION

Several of the compounds in use today as antitumor agents are analogs of the purine and pyrimidine constituents of nucleic acids. More recently interest has evolved in the antitumor activity of nucleosides which contain analogs of the sugar moiety of nucleic acids. Psicofuranine, an adenine nucleoside isolated from cultures of the mold *Streptomyces hygroscopicus*, has been shown to inhibit certain animal tumors (3) as well as to inhibit the growth of *E. coli* (8). This compound is an adenine nucleoside in which the pentose portion of the molecule is replaced by the ketohexose, D-psicose (7). Cordycepin is also an adenine nucleoside in which the sugar moiety of the molecule is a branched pentose, cordycepose. The structural similarity of cordycepin to deoxyadenosine, a component of the DNA molecule, is indicated in Chart 4. In this representation of the structure we have assumed the nucleoside has the β-configuration, although this has not been proved.

At the present time we do not know the mechanism by which cordycepin inhibits the growth of the Ehrlich mouse ascites tumor. Inhibition might occur by one of several means. Because there is a free secondary and primary alcoholic function in cordycepin, the possibility exists that this substance is actually incorporated into the DNA of the tumor cell, thereby making a "fraudulent" molecule. Another possibility is that cordycepin is serving as an inhibitor of phosphorylation of nucleosides or nucleotides to their di- and triphosphate derivatives. We have observed in an entirely separate study that dimethylamino nucleoside, obtained on hydrolysis of puromycin, can serve as a competitive inhibitor in the phosphorylation of adenylic acid to adenosine diphosphate with purified rabbit muscle adenylate kinase.[1] It has also been observed by Wilson *et al.* (9) that this same nucleoside may be acting in a similar manner by blocking the phosphorylation of adenosine by yeast adenosine kinase. It is indeed possible that cordycepin may be acting by blocking the phosphorylation of either deoxyadenosine or deoxyadenylic acid to its di- or triphosphate derivative, thereby inhibiting nucleic acid synthesis of the tumor cell. A third interesting possibility is that cordycepin may be blocking the amination of xanthylic acid to guanylic acid as has recently been reported for psicofuranine in the inhibition of *E. coli* growth (8).

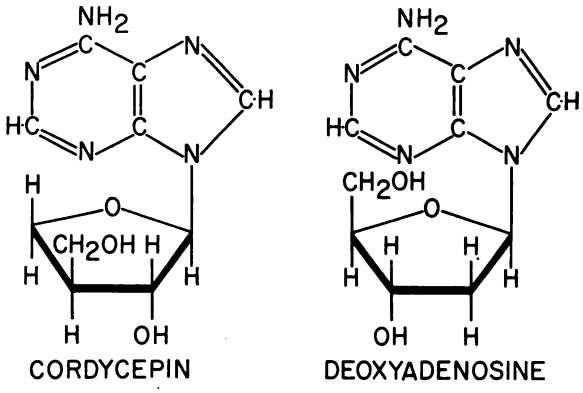

CHART 4.—Similarity of structure of cordycepin and deoxyadenosine.

At the present time we are attempting to determine the mechanism of the inhibition, as well as to test the effect of cordycepin on other animal tumors.

[1] Unpublished experiments of W. F. Oliver and A. J. Guarino.

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