A Study of the Tumor Necrotizing Effects with S. Marcenscens Polysaccharide in Mice *

Transplanted Sarcomas (S-37)

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A considerable literature has accumulated in recent years on the subject of a group of bacterial products which produce necrosis in tumor tissues. Much has been written on the clinical effects of certain bacterial infections upon the course of some types of tumors in patients—notably with Coley's so called "mixed toxins". Research workers had repeatedly shown in the past that certain bacterial products had a destructive effect in tumor tissue, but their margin of safety in destroying the tumor was so small, that they had to be abandoned for the time being. Recently, by chemical fractionation it has been possible to separate the active agent from the other toxic but inactive components present in the bacterial preparation and thereby reducing the toxicity, wich permits a further contribution of these bacterial preparation to the field of chemotherapy in cancer.

Shear and his co-workers has shown that intraperitoneal or intraveinous injections of the specific polysaccharide from B. prodigiosus induces hemorrhage in a few hours in both the transplanted and primary sarcomas in mice. Hemorrhage is not seen when given in the normal tissue. With increasing dosage, to 0.01 mlgm, which is the ninimal lethal dose, the extent of damage to the tumor by the polysaccharide increases. Necrosis parallels or rapidly follows the induced hemorrhage.

Investigation has been going on at the Montreal Cancer Institute, on the method of reducing the primary shock which follows the injection of the polysaccharide which may be related to the substance itself, or to the secondary shock, which follows the reabsorption of toxins liberted by cytological damage of tumor cells.

MATERIALS AND METHODS

Laboratory strain white mice, 8 to 10 weeks old, furnished by a local dealer, received in the right flank implants of S.37 which was obtained from the National Cancer Institute at Washington. Implants were carried out under strict aseptic condition. The tissue ground and normal saline solution aspirated into a seringe, to which is attached a nº 14 trocar is injected into the right flank. Implants have shown a 100% take, when carried out under strict aspect condition. The size of the

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tumor, at ten days, was the size of a small grape. Animals were sacrificed for further transfer at 10 days interval. After 10 days, mice show sign of cachexia and die at about 20 days following the sarcoma implant.

The polysaccharide was supplied by D. Shear bearing the stock number P.10. Mice received 1 cc. injection intraperitoneally and the dose was standarized to 50 micrograms per 1 cc.

Mice bearing one week old implants of sarcoma 37 received 50 micrograms of polysaccharide (nº P. 10) were sacrificed at 8,12 and 42 hres. after injection for study of necrotizing and hemorrhagic effects on the tumor. Gross autopsy findings are recorded and tumor tissue is fixed in Brouin for further investigation.

Tumor bearing mice studied in four sets of experiments. Group one made no 4.9 sarcoma bearing mice, received pyridoxine hydrochloride (10 mlgms.) with or without the polysaccharide.

A second group of 19 mice in an effort to prevent primary and secondary shock caused by the polysaccharide received variable doses of antihistamine like substances (Antistine Ciba) previous to or in association with the polysaccharide.

In a third group made up of 15 mice, in which the hemorrhagic action of the polysaccharide may be due to a capillary deficiency, Rutin was given in association with the saccharide.

Finally, in a fourth group of mice bearing implants of sarcoma 37, natural polysaccharide obtained from the Department of Agriculture (Dominion of Canada, Ottawa) was given by oral route (in drinking water). We are herewith including our results in this paper.

EXPERIMENTAL RESULTS

Results on sarcoma implanted mice with polysaccharide in association with pyridoxine hydrochloride are shown in table 1.

Pyridoxine hydrochloride has no effect on normal mice, whereas it appears to have a highly toxic action in sarcoma bearing mice.

There is a 50% increase in mortality following the use of pyridoxine hydrochloride with the polysaccharide.

Pyridoxine hydrochloride itself seems to have a somewhat necrotizing and hemorrhagic action in the sarcoma cells.

An antihistamine like substance (Antistine Ciba) had no effect on the mortality rate within the 24 hrs. following the administration of polysaccharide. Antistine proved highly toxic for normal mice when high dosage.

Sarcoma bearing mice, given antihistamine like substance previous to polysaccharide on the tumor bearing mice, as compared to normal animals.

It does not seem probable as revealed by these results with antihistamine like substance that an anaphylactic like reaction may be the cause of the tumor cell necrosis.

In the group of tumor-bearing animals which received Rutin, with the polysaccharide it will he noted that the Rutin shows a marked effect reducing mortality rate (after 24 hrs.), but nevertheless without effect on the hemorrhagic like action of the polysaccharide on the tumor.

Natural polysaccharide given by oral route seem quite effective and appear non toxic. These occurs a marked suppression of tumor growth and the survival period of tumor bearing

mice for this group appears to be markedly increased by some weeks.

The tumor mass, grossly, takes on a bluish hue. There occurs frequently in animals auto amputation of the limb bearing the sarcoma transplant, on section the tumor mass appears partially necrotic and gelatinous like.

There has not been noted complete regression of the tumor, but rather a standstill or inhibition of group of the tumor.

Further investigation being carried out, before definite conclusions as to the value of the new chemotherapy can be drawn.

DISCUSSION

The above preliminary experiment demonstrate that Serrata Marcenscens polysaccharide have a definite cytotoxic action on sarcoma bearing mice. Definite proof is still to be submitted which may incriminate the reabsorption of toxins as liberated by a cytological damage to the tumor cells as being the cause of the primary shock Which fol- the injection of the polysaccharide.

The data in table 1 reveals that pyridoxine hydrochloride, while inhancing the cytotoxic like action of polysaccharide, increases the mortality rate quite appreciably. For the present it seems difficult to understand why pyridoxine hydrochloride should become lethal, when associated with polysaccharide.

The use of antihistamine like substance to eliminate primary and secondary shock and which has shown to be without effect, as well as the effort to diminish capillary fragility by using various doses of Rutin, are quite in favor of postulating that the necrotizing like action, as the polysaccharide, is not brought about by an anaphylactic like action, nor that the diminished fragility with Rutin has a definite bearing on the hemorrhagic factor, which seems to occur following the injection of polysaccharide.

The encouraging results obtained with natural polysaccharide i.e. inhibition of growth and the prolongued survival period of the tumor bearing mice and its less toxic effects, warrant further investigation. Further biochemical studies are now being undertaken.

SUMARY

Mice bearing sarcoma implants (S.37) obtained from the National Cancer Institute received sublethal dosage of Serrata Marcenscens polysaccharide (as obtained from Dr. Shear).

The cytotoxic like action characterized by hemorrhage and necrosis in the tumor mass has been investigated, as to its relationship to an anaphylactic action, or as due to an increased capillary fragility.

Results are herewith reported.