CHEMOTHERAPY IN EXPERIMENTAL CANCER

I. ATTEMPTS TO PRODUCE INTRATUMORAL CLOTTING BY THE COMBINED ACTION OF LEAD AND THROMBOGENIC AGENTS

W. A. SELLE, FELIX PAQUIN AND PAUL BRINDLEY

(From the Departments of Physiology, Biochemistry and Pathology, University of Texas Medical School, Galveston, Texas)

Chemical agents being tested for their action on tumor growth are usually injected into animals with the hope that they will have a specific action on the malignant cell. While some chemicals have produced necrosis and regression of tumors, the effects often appear to be due to ischemia and cellular starvation resulting from vascular injury and thrombosis, rather than to a selective action of the chemical on the cell itself.

Although evidence for the therapeutic value of heavy metals, particularly lead, is contradictory, it may be said that some metals given in sufficiently large doses produce vascular impairment, thrombosis, and necrosis, as was shown by Izar and Basile (1912) for colloidal sulphur, Weil (1913) for copper, and Wood (1926) for lead. Wood states that thrombi are abundantly present in tumor capillaries twenty-four hours after the injection of colloidal lead into rats bearing sarcoma 39. In order to produce extensive injury of the larger vessels and marked destruction of the tumor, the dosage must, however, approach the lethal. The great toxicity of the heavy metals in effective doses seems to be the chief obstacle to their more extensive use.

Since the inhibitory effect of certain heavy metals would seem ascribable to the results of thrombosis (Cruickshank, 1931), attempts to enhance thrombus formation by the addition of clot-forming agents are warranted. In combination with active thrombogenic agents, such as thrombin, thromboplastin, or cephalin, the dose of the toxic metal might be reduced to a level effective and safe. As rapidly growing endothelial cells of tumor vessels are more susceptible to traumatization than are cells of mature vessels, the site of thrombus formation might be localized to areas of rapid growth.

Accordingly, attempts were made to establish intratumoral clots by first traumatizing the tumoral vessels with a heavy metal and later injecting thrombogenic agents. It should be pointed out that attempts to devise methods by which the coagulability of blood can be controlled have a practical bearing in application to pathological conditions other than cancer in which the blood balance is disturbed in the direction of an increased or decreased coagulability.

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2 Deceased.

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EXPERIMENTAL MATERIALS

Of the various heavy metals used in therapeutic experiments, lead appears to have given the most encouraging results. Compounds of this metal were, therefore, selected as the most promising traumatizing agents. Four relatively non-toxic preparations found by the Liverpool Medical Research Organization (1936) to have favorable carcinotropic properties were tested. These compounds, lead benzene sulphonylglycinate, lead p-iodobenzene sulphonylglycinate, lead p-toluene-2:4-disulphonylglycinate, and lead calcium phosphate, were prepared by methods described by members of the Liverpool group. Trials were also made with lead glycinate, prepared by the method of Sanders et al (1932), lead oleate and lead carbonate, prepared by the methods of Woodhouse (1936), and Bischoff’s tri-lead phosphate, obtained from the Santa Barbara Cottage Hospital. All preparations contained 5 mg. lead per c.c. with exception of tri-lead phosphate, which contained 4 mg.

Stable preparations of the thrombogens were obtained by methods yielding products of high potency. Thrombin was prepared from calf serum by the method of Roberts (1935), thromboplastin and cephalin from calf brain by the methods of Quick (1936) and Maltaner (1931) respectively. These preparations were standardized for intravenous use in rats and mice. The potency of the stock solution of thrombin was adjusted so that 0.2 c.c. clotted 1 c.c. of citrated rat plasma in fifteen seconds at 38°C; thromboplastin and cephalin were adjusted so that 0.2 c.c. of the suspension clotted 1 c.c. of citrated plasma in twenty to twenty-five seconds when recalcified. Injected intravenously in 200 gram rats, 0.5 c.c. of either the stock thrombin or thromboplastin produced extensive systemic clotting and death; in mice 0.15 c.c. was usually fatal. Much larger doses of cephalin (1 c.c. for rats and 0.6 c.c. for mice) were necessary to produce similar results. For routine intravenous use the stock solutions were diluted 1:2 with physiological saline and given in doses of 1 c.c. per 100 grams of body weight.

The test animals employed were young Wistar rats (80–200 grams) bearing transplantable sarcoma 39, and mice of the C3H strain bearing spontaneous breast tumors.

TECHNIC OF ANIMAL TESTS

Injections of the lead compounds, given intravenously in moderate doses (0.8–1.5 mgm. per 100 gm.), which did not usually impair seriously the nutritive condition of the animals, were begun in rats seven to ten days after inoculation of the tumor fragments, and in mice when the spontaneous tumors were 7 to 10 mm. in diameter. Following a series of three to six injections over a period of ten to twenty days, three or more injections of the thrombogenic agent were given. Although made under rigid asepsis, the injections were frequently limited or brought to an end by inflammation or necrosis and sloughing off of part or all of the tail.

At termination of the experimental period, usually after the fourth week of treatment, the animals were sacrificed, the tumors exposed and studied under low-power dissecting binoculars for the presence of clots in the larger vessels supplying the growth. Preparatory to this an anticoagulant (heparin, peptone,
or sodium citrate) was injected in sufficient amount to prevent post-mortem clotting. Histologic studies were made for most groups.

**Results**

In four preliminary experiments the effect of the thrombogenic agents given alone was tested on groups of 10 to 20 mice receiving five or six injections at intervals of three or four days. One group received thrombin, another thromboplastin, a third a mixture of thrombin and thromboplastin, and the fourth cephalin. On comparison with an approximately equal number of untreated animals, no inhibitory action was evident.

Combined treatment with the lead and thrombogenic preparations was likewise without significant effect. With the 7 preparations of lead tried in 17 experiments on 556 rats and in 7 experiments on 330 mice, the only tumors suggesting inhibition of growth were the transplantable rat tumors treated by lead oleate and lead glycinate. These preparations greatly impaired the nutritive condition of the animals, making difficult an evaluation of the treatment itself. The experiments were repeated on mice, and while the treated tumors were slightly smaller than the controls, due probably to the poorer nutritive condition of the treated hosts, the difference was not statistically significant.

In two groups, one consisting of 19 rats treated with colloidal lead phosphate, the other of 21 rats treated with lead-calcium phosphate, anesthesia was induced with nembutal following three injections of lead and one injection of a mixture of thrombin and thromboplastin. Under aseptic conditions, the skin of the tumor was reflected to expose the vessels on the surface of the growth. In 6 animals of the first group and 4 of the second, clots were found in the superficial vessels of the capsule. Some of the thrombi extended as far as the larger tributaries. The skin was replaced with silk sutures and the animals allowed to recover. When the experiment ended, two weeks later, a slight difference, statistically insignificant, was noted between these tumors and those of the corresponding controls. The nutritive condition of the operated animals, however, was poor. A repetition of the experiments on mice, omitting the exploratory operation, showed the differences to be statistically insignificant.

Histologic examination of the tumors revealed severe regressive degeneration with many thrombi and extensive necrosis. The relation of the degenerative changes to the therapeutic procedures, however, is questioned in view of the fact that the control tumors often displayed areas of necrosis as extensive as the treated. Even relatively large vessels in the periphery of the control tumors were thrombosed. There was no evidence that the various lead preparations in the dosage employed had a damaging effect on the intima of either the peripheral or deep vessels.

**Summary and Conclusions**

Attempts were made to affect the growth of the transplantable rat sarcoma 39 and the spontaneous breast tumor of the mouse by procedures designed to produce clots in the tumor vessels.
Three thrombogenic agents (thrombin, thromboplastin and cephalin) given alone and in combination with seven preparations of lead, some of which were stated to have therapeutic value, were given intravenously in relatively non-toxic doses.

The results of twenty-eight experiments on approximately 900 animals were negative. Several experiments suggesting positive results for the transplantable rat sarcoma were found misleading when the experiments were repeated on the spontaneous tumor of the mouse.

The gross effect produced by the combined treatment of lead and the thrombogens appears to be similar, at least superficially, to that of treatment with lead alone. None of the combinations tried resulted in retardation of tumor growth unless the nutritive condition of the host became badly impaired.

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BIBLIOGRAPHY