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Inhibition of Experimental Tumors by Orthophenylenediamine (OPDA). (24557)

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The presence in animals of certain tumors has been observed to provide either biochemical protection or liability in terms of survival time for the host when challenged with a lethal dose of specific chemical agents such as the phenylenediamines(1,2). The Cloudman S91 mouse melanoma protects its host against the acute toxicity of paraphenylenediamine (PPDA) whereas the same tumor is a disadvantage to the host when the challenging compound is orthophenylenediamine (OPDA). With the above experimental design this phenomenon extended to other combinations of tumor and compound variously to the advantage, disadvantage, or neither for the host. In possible correlation, it was also observed that PPDA combines with certain pigmented tumor constitutents *in vitro* as determined by oxygen-consuming reactions in the Warburg apparatus when added to melanoma tissue, urine, blood plasma, or pure dihydroxyphenylalanine (DOPA)(3,4,5). Further pursuit of these phenomena led to chemotherapeutic testing of phenylenediamines against melanoma and other tumors. The results with the ortho isomer, which is the least toxic and most effective, are reported here with a variety of transplanted mouse and hamster experimental tumors.

Materials and methods. Several inbred and hybrid strains of mice were employed for carrying the tumor types studied. Most melanoma experiments utilized the Cloudman S91 (6) although the Harding-Passey(7), the Fortner hamster melanoma(8), and several non-melanoma tumors were used for comparative purposes. The transplanted hamster melanoma is designated as Melanotic Melanoma No. 2 (H.M.M. #2) and originated "spontaneously" in a Syrian golden hamster. The histological and biological characteristics have been described(8,9). The Cloudman S91 mouse melanoma was carried in both male and female DBA/2 hybrid mice. The Harding-Passey melanoma and the Ehrlich car-

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cinoma(10) were grown in male Hauschka ICR Swiss albino mice. The Fortner hamster melanoma was carried in Syrian golden hamsters obtained from commercial stock. Tumors were transplanted by subcutaneous inoculation of 0.1 ml of 10 or 20% tissue suspension into the right hip. All measurements of tumors were with calipers in 3 dimensions. Volumes were determined by multiplying length by width times thickness, which gave acceptable correlation with tumor weights. Weights of individual animals were obtained with a Mettler single pan balance with precision of ± 0.03 g and are expressed in the Figures as calculated group averages. Orthophenylenediamine (OPDA) was used as the free base. Most commercial preparations originally obtained were impure or contained oxidized products and, although they possessed antitumor activity, efforts were made to further purify such products to reduce nonspecific toxicity. A satisfactory commercial preparation of OPDA (free base) is now available.† Solutions for animal injections were prepared fresh daily in distilled water or 0.5% CMC (sodium carboxymethylcellulose) solution. Unless otherwise indicated, all animals were injected intraperitoneally once a day, 5 days/week.

Results. Fig. 1 illustrates the effect of OPDA on tumor growth and total mouse weight when administered to animals with well-established transplanted melanomas. The tumor in this experiment was the Cloudman S91 pigmented melanoma which had been implanted 17 days prior to start of treatment. The initial intraperitoneal dose was 100 mg kg/day for 5 successive days with an increase the second week to 150 mg/kg and finally to 200 mg/kg maintained for over 70 days. The injection schedule is shown in Fig. 1. It may be seen that treated tumors continued to grow slowly under this dose schedule for approximately 30 days with a drug-induced inhibition of about 75%. At this point when untreated control animals were dying from their extensive tumors a significant decrease in size of treated tumors began to occur. This slow growth and regression of the tumors is also reflected in total weights of animals. Each point on the graph is the average of the surviving animals with tumors, in the case of tumor volume curves and of total number of animals in each group in the weight curves. There were 10 mice in each group at beginning of experiment with a gradual decrease to zero survivals at 30 days in saline-injected controls and to 2 in the treated group at 70 days.

The effect of OPDA on growth of the Harding-Passey melanoma, when administered at constant dose level of 200 mg/kg/day 5 times a week, is illustrated in Fig. 2. In this experiment the compound was administered subcutaneously rather than intraperitoneally, which seemed to inhibit as effectively or slightly better than i.p. injections at this dose level with the Harding-Passey melanoma. Slightly more than 80% tumor growth inhibition was obtained after 40 days treatment. As also illustrated in Fig. 2, there was a steady weight gain in the treated animals, which suggested that this dose level was not the maximum that could be tolerated.

Fig. 3 summarizes the results of OPDA treatment of a rapidly growing, metastasizing hamster melanoma. Tumor growth inhibition was approximately 85% after 40 days of treatment with 14 doses of 200 mg/kg and 15 doses of 250 mg/kg. It may also be seen that there was a steady weight gain in treated animals during this dosage regime. The added increase in weight of the untreated controls was due largely to tumor growth which eventually affected the animals' weight adversely as the tumors increased in size and metastatic development took place.

Fig. 4 illustrates the results of OPDA treatment at higher dose levels on the Ehrlich solid carcinoma. Treatment was started on one-day-old tumor transplants at a dose of 100 mg/kg. The second day it was increased to 150 mg/kg and, following a 2-day intermission, treatment was continued for 5 consecutive doses at 200 mg/kg followed again by a 2-day recess. On the fourteenth day the dose was raised to 300 mg/kg/day. This dose was then maintained 5 days a week until the thirty-fourth day at which time treatment was

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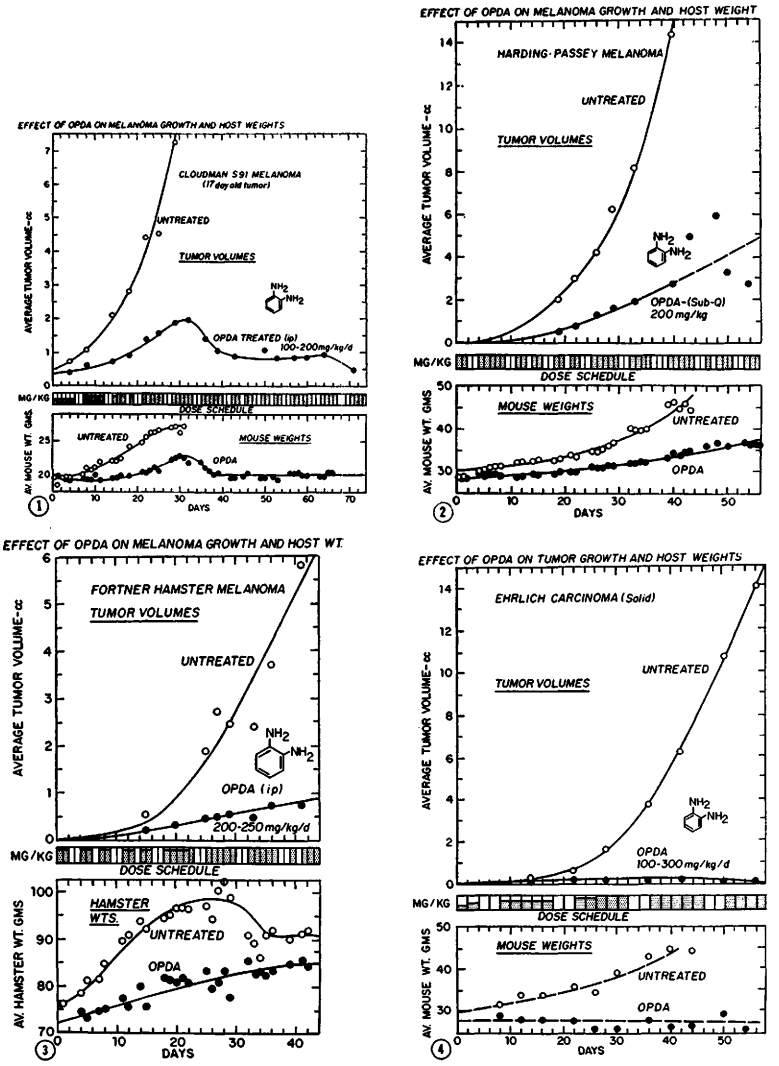

FIG. 1. Effect of OPDA (orthophenylenediamine) on melanoma growth and host wt. (Tumor: Cloudman S91 mouse melanoma, 17 days post implant in DBA/2 inbred mice. OPDA aqueous solution inj. i.p. at 100-200 mg/kg/day, controls inj. with physiological saline.)

FIG. 2. Effect of OPDA on Harding-Passey melanoma and host wt at constant dose. (Tumor 1 day old at start of treatment. OPDA-water solution, subcut. injections of 200 mg/kg/day.)

FIG. 3. Effect of OPDA on Fortner hamster melanoma and host wt. (Tumor: HMM-2, 1 day old at start of treatment. OPDA-water solution, 200-250 mg/kg/day, intraper. inj.)

FIG. 4. Effect of OPDA on the solid Ehrlich carcinoma and host wt. (Tumor 1 day old at start of treatment in Swiss ICR male mice. OPDA-CMC solution, 100-300 mg/kg/day, intraper. inj.)

discontinued. As illustrated by the plot of treated tumor volumes, over 95% tumor growth inhibition was obtained. The points plotted represent average tumor volume of tumor-bearing animals and do not include mice in the treated group in which the tumor failed to reach a measurable size. About half of the treated animals failed to develop tumors to the point where they could be measured. By the thirty-third day the tumors under treatment with OPDA (in CMC) had regressed to a point where they could no longer be measured. Animals in similar experiments have remained free of tumors for several

months and when rechallenged with a similar tumor tissue suspension on the opposite hip were resistant to the second implant.

OPDA has been submitted to the SKI tumor spectrum(11) where it was tested against a variety of 24 mouse, rat, and hamster tumors. A number of tumors showed inhibition within this test system and the results will be detailed elsewhere.

Discussion. The phenylenediamines are physiologically active compounds(12,13,14), and it is therefore a complex experimental problem to establish the mechanism of action of their antitumor properties. Their ability to combine in oxygen consuming reactions with certain catechol amines such as dopa and related products present in some pigmented tumors, is provocative but inconclusive evidence of their mode of action. This class of compounds has also been reported to have inhibitory effects against DPN-dependent enzyme systems(14). In testing the effect of ortho-, meta-, and paraphenylenediamine on respiration and glycolysis of Ehrlich ascites cells in manometric experiments, inhibition by all 3 isomers was observed in respiration but not in glycolysis under conditions of testing (unpublished). This suggests that DPN enzymes are not critically involved in the inhibition of Ehrlich carcinoma cells. It also indicates that aerobic respiration of the cell is more sensitive to these compounds than the anaerobic pathways. From the chemotherapeutic standpoint the structural simplicity of OPDA is of special interest and lends itself to simple chemical substitution and radioactive labeling for a systematic study of its action.

Summary. The antitumor effects of OPDA (orthophenylenediamine) on mouse and hamster melanomas and the Ehrlich solid carcinoma are reported. Complete inhibition of growth of the Ehrlich solid carcinoma was obtained as well as complete regression of some established and measurable tumors.

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Effect of Growth Hormone and Oxytocin Upon Milk Yield in the Lactating Rat.* (24558)

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It has been demonstrated on basis of milk yields that growth hormone (GH) causes an increase in milk secretion in cows(1,2), sheep, (3), and goats(4); while litter growth data suggested that it may be ineffective in rats (5). It is well known, however, that amount of milk removed during nursing or milking is related to quantity of oxytocin discharged from the neurohypophysis(6,7). It seemed of interest, therefore, to reinvestigate the role of

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