Regional Infusion of Vinblastine and Hydrogen Peroxide in Tumor-bearing Rats

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SUMMARY

The present investigation was undertaken to evaluate the effectiveness of regional arterial infusion of vinblastine on a predictable experimental tumor and to determine whether the response to treatment might be potentiated by concomitant regional hyperoxygenation with hydrogen peroxide. Sprague-Dawley rats received single intra-aortic infusions of vinblastine (0.5 mg/kg) and hydrogen peroxide (3 mg/animal) and a combination of both compounds 1 week and 1 day after inoculation of a suspension of Walker 256 carcinosarcoma in the thigh muscles. Vinblastine partially inhibited subsequent growth of the established solid tumors. However, treatment 1 week after implantation did not prove therapeutic since the animals died from metastatic disease without prolongation of survival. When regional arterial infusion of vinblastine was performed 1 day after inoculation of the tumor, 40% of the animals became free of the tumor and lived indefinitely. This salutary effect was reduced by half when vinblastine was given in conjunction with hydrogen peroxide. In the animals that ultimately died from extension of the tumor after intra-arterial vinblastine injections, survival was significantly increased, with inhibition of local tumor growth and the rapid metastatic spread of this neoplasm. Hydrogen peroxide alone was not effective against this tumor, and rather than enhancing the therapeutic action of vinblastine, adjunctive use of hydrogen peroxide made treatment more lethal (the toxic mortality was 21–30%).

When vinblastine was injected into the internal carotid artery of individuals with intracranial neoplasms, it usually caused a prompt oculocutaneous toxicity. Though less severe, the direct local effects simulated the histotoxicity of the alkylating agents (6, 7, 9, 12, 13). Intrararterial infusion of hydrogen peroxide was reported to increase selectively the uptake of several radioactive compounds in cerebral tumors (3). When vinblastine was oxidized in vitro by the addition of hydrogen peroxide in excess, the anoxide products in 1 experiment had a biologic activity comparable to the parent alkaloid base (M. Gorman, Lilly Research Laboratories, personal communication). These observations prompted the following empirical inquiry into the possible usefulness of hydrogen peroxide as a potentiator of the response to regional arterial infusion of vinblastine. Underlying mechanisms are unknown. There is no evidence that the action of this alkaloid is oxygen dependent and therefore might be favorably enhanced by regional hyperoxygenation with hydrogen peroxide, as reported in rats with triethylene thiophosphoramide (11). In general, radiomimetic drug therapy utilizing other methods to vary oxygen concentration in the treated tissues has yielded unpredictable and conflicting results. Altering the ambient oxygen tension of rats with the Walker 256 carcinosarcoma by placing the animals in a high pressure chamber failed to change the therapeutic effect noted after i.p. administration of nitrogen mustard (5). Similar experiments in tumor-bearing mice exposed to increased oxygen tension failed to show a more therapeutic effect of nitrogen mustard in 1 report (2), whereas in another, the tumoricidal action of this alkylating agent seemed potentiated (4). Isolated perfusion studies in the canine hind limb, however, suggested that reduced oxygen saturation as well as

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other local hemodynamic factors favored regional retention of nitrogen mustard (1).

Previously it was shown that a single intraarterial infusion of vinblastine inhibited the Walker 256 carcinosarcoma in Wistar rats (8). However, because of poor compatibility between the tumor and host, the over-all therapeutic efficacy could not be evaluated. Hydrogen peroxide in the concentrations used caused no alteration in vitro in the indoledihydroimole moiety of the alkaloid. The present paper is an extended investigation of the response to intraarterial infusion of vinblastine and hydrogen peroxide in a more satisfactory experimental tumor system.

METHODS AND MATERIALS

The original Sprague-Dawley donor rats carrying the Walker 256 carcinosarcoma in the thighs were obtained from I. Wodinsky, Arthur D. Little, Inc. A stock tumor colony was maintained by periodic transfer in female rats of the same strain. For these experiments 3-ml aliquots of surgically excised tumor were homogenized in 12 ml of isotonic saline. The sterile suspension was agitated with a magnetic stirrer to prevent layering of the cells. In groups of approximately 25, unanesthetized rats were inoculated in the right posterior thigh muscles with 0.3 ml of this mixture through a 20-gauge needle. In 6 of 7 groups of inoculated animals observed for 1 week, tumor takes were 100%. In the remaining group takes occurred in only 16 of 26 animals, none of which was used.

The infusions were done under i.p. pentobarbital anesthesia (32 mg/kg). The aorta was exposed and cannulated with a 30-gauge needle between the renal and iliac arteries. The animals all received the same volume of fluid, 0.83 ml of isotonic saline, infused intraarterially in 10–20 sec. The dose of vinblastine was 0.5 mg/kg and that of hydrogen peroxide was uniformly 3 mg/animal, an amount calculated to yield 1 ml of molecular oxygen. No ischemic or other hind limb complications occurred.

Animals that died within 24 hr of infusion from any cause were excluded from the study. The rats were caged in pairs and provided with a standard laboratory diet and water. At weekly intervals or less the animals were weighed and the tumors measured. The maximum transverse diameters of the normal and the tumor-involved thighs were determined by measurements with calipers. The difference in mm between the 2 hind limbs was taken as the relative value for tumor diameter.

The effects of intraaortic infusion were studied 1st in 127 rats with established tumors. One week after inoculation of the tumor suspension, 91 of these animals were infused arbitrarily in 4 groups with vinblastine, a combination of vinblastine and hydrogen peroxide, hydrogen peroxide, or isotonic saline solution. Five to 10 rats from each group (with different generations of tumor) were used cumulatively as untreated controls (36 rats). Average weight (±1 S.D.) of the animals receiving vinblastine was 137 ± 18 gm and ranged from 151 ± 8 gm to 159 ± 7 gm in the other groups. Postmortem studies were performed on 75% of the animals, including all rats that died within 2–3 weeks after tumor inoculation. Microscopic sections were done where necessary to confirm the gross pathologic observations and to study the histology of the thigh tumors.

Intraaortic infusions were done 24 hr after inoculation of the tumors in a 2nd series of 76 rats. In groups of 20 the animals received vinblastine, vinblastine and hydrogen peroxide, or hydrogen peroxide. Five or 6 animals from each group were used as untreated controls. Average weight in this series ranged from 135 ± 6 gm to 153 ± 4 gm. Autopsies were carried out in 51 of the 63 deaths and included all but 4 animals that died within 2–3 weeks of tumor inoculation. A selective histologic survey was also made.

RESULTS

One-week series.—One animal infused with vinblastine survived over 120 days with complete tumor regression. All others treated at 1 week succumbed, as did all the controls. Mean survival in the 3 treated groups varied from 30 to 35 days after inoculation compared with 35 and 32 days, respectively, in the operated and untreated control groups.

Infusions of vinblastine and vinblastine and hydrogen peroxide were obviously toxic in some animals. Symptoms were commonly noted within 1 week but were seen occasionally up to the 3rd week. The animals were hypoactive and had diarrhea. Two of the 8 deaths within a week after infusion of vinblastine were toxic in origin and occurred on the 3rd and 4th days. Both animals had hemorrhagic pulmonary consolidations, small livers, and ileus. Toxic manifestations were more severe in the animals receiving the combined infusions. These rats lost an average of 10% of body weight in the 1st week following treatment, in contrast to a moderate over-all gain in the other animals. Five of 7 early deaths were toxic in nature with no evidence of significant spread or growth of the implanted tumors. In addition, 2 of 4 animals that died within 24 hr after the combined infusions, and therefore were excluded from this study arbitrarily, also had unequivocal evidence of acute toxicity. The terminal mean tumor diameter with S.E. was 9 ± 0.8 mm in the 5 toxic deaths compared with a mean diameter of 23 ± 1.9 mm in the 19 animals that also had both drugs and died from progressive tumors. Only 1 animal in the group receiving hydrogen peroxide infusions alone had toxic signs, but autopsy also revealed extensive pulmonary metastases.

Following inoculation the tumors grew rapidly and metastasized early. Average diameters measured at 1 week ranged from 10 to 14 mm. At this time the involved leg was invariably paretic. Metastatic para-aortic nodes were observed in the majority of animals operated on at 1 week. These reached diameters of 10 mm or more in some of the animals. This early metastatic spread was noted in 100% of the 15 animals infused with saline. Intrathoracic metastases (lungs, pleura, mediastinum) were verified in 82% of the autopsies; abdominal metastases were present in 63% of the total examined. No differences in metastatic distribution or histology were noted between the control and the treated groups. No distinctive histopathologic or specific cytomorphologic changes attributable to the infusions were recognized in the treated thigh tumors. This effect was specifically looked for in the early deaths during the 1st week after treatment with
vinblastine and vinblastine and hydrogen peroxide. However, in the 2nd week of growth, both the control and treated tumors showed similar areas of hemorrhage, necrosis, and serous transudation.

At death in the untreated and saline controls mean tumor diameters were 31 mm and reached 34 mm in the animals infused with hydrogen peroxide. In the animals infused with vinblastine the tumors were smaller at death, with an average diameter of 27 mm. This difference approached statistical significance (P < 0.1). This effect was greater when vinblastine was infused in combination with hydrogen peroxide. The final mean tumor diameter in these rats was 20 mm, or 23 mm when the 5 early toxic deaths were omitted. When compared with the controls this difference was significant, with P < 0.001 (P < 0.005 with the 5 toxic deaths excluded). The over-all effect of vinblastine treatment remained highly significant (P < 0.001) when the data were analyzed statistically after adjustments for the differences in initial tumor size.

Chart 1 shows the average tumor diameter in the surviving animals of each group over successive weekly intervals. The growth rate was comparable in the untreated and operated controls—about 5 mm/week from the 2nd to the 6th week. The tumors appeared to increase in size at slightly greater rates after treatment with hydrogen peroxide. This apparent increase over the growth rate in the controls did not result in any significant difference in mean tumor diameter at death, however. During the 1st week after infusion with vinblastine and vinblastine and hydrogen peroxide, there was complete inhibition of tumor growth. Thereafter, compared with the controls the tumors increased at slightly slower rates of approximately 4.2 mm weekly. The inhibitory effects shown in this manner were similar and were approximately 15% less than the growth rate observed in the controls.

One-day series.—Control saline infusions were not done in these experiments because in the 1st series these results were no different from those in the untreated animals. There were 8 survivors in the group infused with vinblastine. Four survived following the combined infusions, and 1 lived after hydrogen peroxide. All lived beyond 120 days and were free of tumors. All control animals died from spread of the tumors. The favorable results with vinblastine proved significant (P < 0.02).

In the animals that died, mean survival increased to 69 days after infusion of vinblastine and to 64 days after vinblastine with hydrogen peroxide, compared with 31 days in the controls. Infusion with hydrogen peroxide alone did not prolong the life-span (mean survival, 29 days). The longevity noted after vinblastine was not statistically different than that following vinblastine plus hydrogen peroxide. The prolongation of life following both types of infusions was highly significant (P < 0.001).

Toxic effects like those reported in the 1-week series were noted only after infusions of vinblastine plus hydrogen peroxide in the animals treated at 24 hr. In this group there was an average weight loss of 20% in the 1st week with no change or a corresponding increase in body weight in the other groups. Six of the 16 deaths observed after treatment with both drugs were toxic in origin, occurring at an average of 6 ± 1 days after infusion, and without evidence at autopsy of tumor locally in the thigh or metastatic spread. This toxic mortality was not statistically different from that observed in the 1st series of animals.

The biologic behavior of the tumor paralleled that observed earlier. At 24 hr there was seldom an appreciable difference between the diameters of the tumor and control thighs. Metastatic tumor deposits were noted in the para-aortic nodes in fewer than 10% of the animals operated upon within 24 hr of tumor inoculation. In animals that died from the effects of tumor there were no differences in the metastatic patterns between the control and the treated groups. Intrathoracic metastases occurred in 85% and abdominal nodes in 65%.

Mean tumor diameters at death were 25 mm in the controls and in those infused with hydrogen peroxide. In the animals that died following treatment with vinblastine and in the tumor deaths after infusion of vinblastine and hydrogen peroxide, mean tumor diameters were 32 mm and 29 mm, respectively. These differences approached statistical significance (P < 0.1).

The growth rates were plotted (Chart 2) and were identical in the controls and in those which received hydrogen peroxide. The estimated rate of slightly over 5 mm/week was equivalent to that seen in the previous series. In the animals treated with vinblastine or vinblastine and hydrogen peroxide, there was a closely parallel inhibition of tumor growth. Mean tumor diameters increased about 3.5 mm per week. Compared with the same treatment of the established tumors in the previous series, the inhibitory effect on tumor growth in these animals was approximately twice as great, or 30%.

Also shown in Chart 2 are corresponding observations of the regressive lesions in the 12 indefinite survivors from...
the 1st 2 groups. In 10 of these animals there were measurable differences in thigh diameters for several weeks. Relative size in both groups reached a plateau at 5 mm by the 2nd week after the inoculation and began to decrease after the 5th week.

COMMENT

Single intraaortic infusions of vinblastine and vinblastine plus hydrogen peroxide in Sprague-Dawley rats with an established Walker 256 carcinosarcoma caused similar mild but definite inhibition of tumor growth. Despite the appreciable systemic toxicity manifested by the doses used, there was no indication of a total regional oncolytic effect. Although an anti-tumor effect was demonstrated locally in the thighs, infusions of these agents were not therapeutic in rats treated 1 week after inoculation. With 1 exception, treatment did not alter the fatal outcome or prolong survival. Regional infusion of the primary neoplasm at 1 week was not effective, because in the majority of animals distant metastases, which eventually caused death, occurred before the time of treatment.

When the same dose of vinblastine (0.5 mg/kg) was infused intraarterially 24 hr after injection of the tumor cell suspension in the thighs, 40% of the animals became free of tumor and survived. This same tendency was noted after intraaortic infusion of vinblastine with hydrogen peroxide, although the attendant toxic mortality of 30% reduced the number of “cures” by half. Some rats failed to develop measurable evidence of tumor locally. A majority, however, had diffusely enlarged limbs for several weeks after inoculation. The involved thighs were indistinguishable, and the average growth observed (Chart 2) was the same for nearly 2 weeks, regardless of whether the animals ultimately died or survived free of tumor. Since the histologic nature of the regressive lesions was not determined, an exact interpretation of these observations is lacking. Although the mechanisms involved are obscure, regional infusion of vinblastine at 24 hr prevented takes and establishment of autonomous growth and spread of the Walker tumor in a significant number of animals.

Surgical observations showed that the abdominal lymph nodes in several animals were infiltrated by tumor as early as 24 hr; this accounted for some of the failures of infusion treatment at that time. However, intraarterial injections of vinblastine and vinblastine with hydrogen peroxide 1 day after tumor inoculation provided significant palliation in the rats that eventually died of their disease. Tumor growth was inhibited, and the treated animals lived over twice as long as the controls. Increased longevity was 3–4 times greater than local inhibition (30%) of tumor size. These results indicate that the palliative effects of regional infusion were determined by the degree of inhibition vinblastine had on local growth and the ultimately fatal metastatic dissemination of the neoplasm. These effects were not unlike observations reported previously with an alkylating agent (10).

Although intraaortic infusion of vinblastine or vinblastine and hydrogen peroxide gave a remarkably similar over-all response in the 2 series of animals, there were striking differences in the toxic manifestations. The combined infusion caused an acute toxic mortality of 21–30% and partially vitiated the practical results of treatment. Unlike earlier observations in Wistar rats (8), which disclosed a similar toxicity with intraaortic infusion of vinblastine or hydrogen peroxide, the present investigation failed to show a convincing toxicity with infusion of the latter compound. Intraarterial infusion of vinblastine alone caused few toxic complications, which were limited to the animals with established tumors. These variations may be attributed to different drug susceptibilities between host strains and to different sensitivities based on sex and maturity (I. S. Johnson, personal communication). Also, resistance to physiologic stress in general was very likely reduced in rats with more advanced, week-old tumors, as suggested by the higher anesthetic mortality observed in these animals.

The observations in this study indicate that infusion of hydrogen peroxide with vinblastine in intraarterial chemotherapy would not favorably influence the clinical response to this vinca alkaloid.

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Mealey—Infusion of Vinblastine and Hydrogen Peroxide


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