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Effects of sodium cyanate in mice bearing B16 melanoma*

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Summary. Sodium cyanate injected IP at a dose level of 200 or 250 mg/kg caused a 90% or greater inhibition of the incorporation of [^{3}H]thymidine into DNA of B16 melanoma transplanted SC in mice. Despite the inhibitory effect of sodium cyanate on precursor incorporation into DNA, no significant effect on host survival was observed when sodium cyanate was administered as a single agent in the diet, in drinking water, or by IP injection to mice that had received IP transplants of B16 melanoma. The action of melphalan and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) in prolonging the survival time of melanoma-bearing mice was not enhanced by combined treatment with sodium cyanate. However, combined injections of sodium cyanate and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) increased the survival of tumor-bearing mice significantly more than injections of BCNU alone at a lower dose than the maximum tolerated one. These data and other studies suggest that B16 melanoma may be less responsive to the action of sodium cyanate than are murine leukemic cells or rat hepatomas.

Introduction

Inhibition of growth was one of the early observations made in studies on the pharmacology of cyanate [2]. The antimitotic action of cyanate was reported by Dustin in 1947 [6], but it was 19 years before there was a further report on this effect of cyanate [8]. An inhibitory action of cyanate on thymidine incorporation into DNA was observed with several proliferating tissues in rats and in leukemic L1210 cells of mice [12]. We found that sodium cyanate could cause a large inhibition of amino acid incorporation into protein of rat hepatomas under conditions in which there was no significant inhibitory effect in host liver [13]. Selective inhibition of protein synthesis has also been seen in colon tumors [1, 10] and transformed cell lines [3]. Inhibition of tumor formation has been reported when sodium cyanate has been administered subsequent to carcinogen exposure [17]. A phase 1 clinical trail of sodium cyanate has been conducted in patients with colorectal carcinoma [7].

In a study with P388 tumor-bearing mice no therapeutic effect on lifespan was seen with sodium cyanate but a synergistic effect with melphalan was recorded [5]. In the present work we sought to test the chemotherapeutic potential of sodium cyanate in mice bearing B16 melanoma. Different modes of administration were examined with sodium cyanate as a single agent, and combination chemotherapy studies were performed with the chemotherapeutic agents 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), and melphalan. A preliminary report of this work has been presented [14].

Materials and methods

Animals. Except where stated otherwise, female C57BL mice received either SC or IP transplants of 10^5 B16 melanoma cells. The number of viable cells was determined by trypan blue exclusion.

Incorporation of [^{3}H]thymidine. Either sodium cyanate or saline (control) was injected IP into mice bearing SC transplants of B16 melanoma. At 60 min after this injection the mice received an IP injection of [^{3}H]thymidine (2.5 μCi per 10 g body weight), and they were killed by cervical dislocation after a further 30 min. Dissected tumor was homogenized in water and adjusted to a final concentration of 1.0 *N* with respect to perchloric acid. The homogenate was centrifuged at 800 *g* for 10 min, and an aliquot of the acid-soluble supernatant was used for liquid scintillation counting. The acid-insoluble fraction was washed three times with 10% trichloroacetic acid, 5% sodium pyrophosphate, and RNA was hydrolyzed with 0.33 *N* KOH at 37 °C for 60 min. DNA and protein were precipitated by adding an equal volume of 20% trichloroacetic, 10% sodium pyrophosphate and was then washed once at 0 °C with 10% trichloroacetic acid, 5% sodium pyrophosphate. DNA was extracted with 5% trichloroacetic acid at 90 °C for 20 min. DNA was assayed by the procedure of Burton [4], and radioactivity was measured by liquid scintillation counting.

Treatment of mice with cyanate and other drugs. Sodium cyanate was obtained from ICN Pharmaceuticals, Plainview, NY. Solutions for IP injection were freshly prepared in distilled water. When sodium cyanate was included in powdered diet, the food was changed daily and control an-

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imals also received powdered diet. When included in the drinking water, sodium cyanate was dissolved in distilled water and changed every 2 days. BCNU was dissolved in ethanol and then diluted with 9 volumes saline. CCNU was dissolved in DMSO and melphalan was dissolved in DMSO and acidic saline as described by Dufour et al. [5]. Control mice received injections of the appropriate solvent vehicle.

Presentation and analysis of data. Results are presented as means ± standard deviation. Differences between means were evaluated by Student's *t*-test, and probabilities less than 0.05 were considered to be significant.

Results

Initial studies were performed on the uptake of [^{3}H]thymidine and incorporation into DNA to determine whether the response to cyanate in the mouse melanoma resembled that which we have seen in other neoplastic cells or tissues [12]. The data in Fig. 1 indicate that there was no significant effect of cyanate on uptake into the acid-soluble fraction but a large inhibition of [^{3}H]thymidine incorporation into DNA was observed with sodium cyanate 200 or 250 mg per kg body weight IP.

Sodium cyanate is known to have a cumulative effect on growth. We observed that inclusion of 0.025 mmol sodium cyanate per gram of food did not significantly affect the body weights of mice which received IP transplants of 10^5 B16 melanoma cells. Sodium cyanate was included in the diet from day 4 to day 15 after transplantation of the tumor cells. With 0.05 mmol or 0.10 mmol sodium cyanate per gram of food the mean body weights at 15 days after transplantation of the tumor were decreased to 90% and 84% of control weight, respectively. As shown in Fig. 2, this dietary treatment did not have a significant effect on host survival, nor did an IP injection of BCNU (10 mg per kg body weight) given on day 7. However, there was a significant (27%) increase in survival time ($P<0.01$) when the mice received a combined treatment of 0.05 mmol sodium cyanate per gram of food and an injection of BCNU.

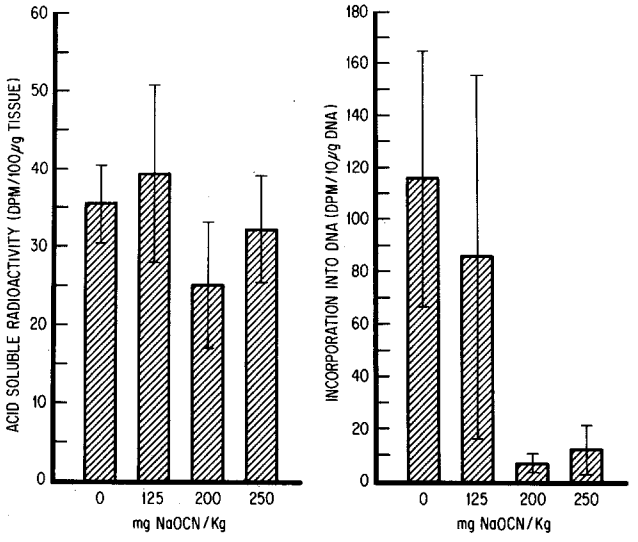

Fig. 1. Effect of sodium cyanate on the uptake of [^{3}H]thymidine in B16 melanoma. Sodium cyanate was injected IP into C57BL mice bearing SC tumor transplants. The mice were killed 30 min after IP injection of [^{3}H]thymidine (250 μCi/kg), which was administered 60 min after the cyanate. The data are means for 4–15 mice ± standard deviation

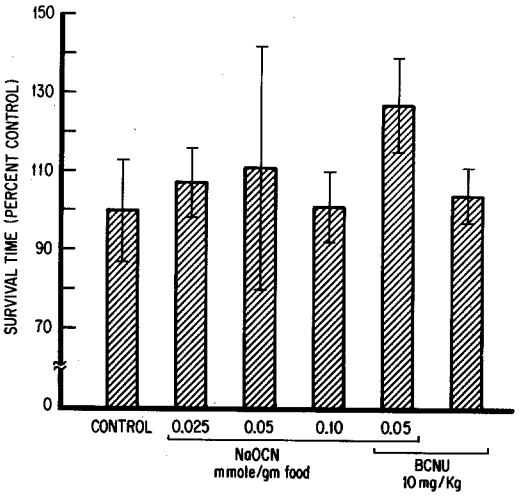

Fig. 2. Survival of mice bearing B16 melanoma after treatment with sodium cyanate and BCNU. Sodium cyanate was included in the diet at the stated concentration from day 4 to day 15 after IP transplantation of 10^5 tumor cells. BCNU (10 mg/kg) was injected IP on day 7 after transplantation. Means ± standard deviation are given for 5–9 mice

As an alternative approach to the administration of sodium cyanate this compound was given in the drinking water. Sodium cyanate is subject to hydrolysis in aqueous solution in a manner which is influenced by pH, temperature, and concentration. To assess the stability of sodium cyanate dissolved in distilled water under our conditions, we assayed cyanate colorimetrically [9] and the formation of ammonia was determined by reaction with Nessler's reagent. By both criteria we observed less than 2% hydrolysis of sodium cyanate at room temperature in 48 h, which was the period we used before preparation of a fresh solution. Groups of nine mice which received SC transplants of 1.7×10^5 B16 melanoma cells were given either distilled water (controls) or a drinking solution containing sodium cyanate 60 mg per 100 ml. Preliminary studies had revealed that lower concentrations of sodium cyanate (7.5, 15, and 30 mg per 100 ml drinking water) were well tolerated by the mice. There was a loss of body weight in mice receiving 60 mg sodium cyanate per 100 ml drinking water. For example, at 22 days after transplantation of the tumor cells there was a 19% loss in mean body weight in the cyanate-treated mice but less than a 1% change in mean body weight in the controls. The mean survival time of the cyanate-treated mice was 38 ± 7 days and was not significantly different from that of the control animals bearing SC tumors (33 ± 8 days).

In an attempt to avoid the loss of cyanate by hydrolysis in the acid environment of the stomach the next study was performed with daily IP injections of sodium cyanate. As illustrated in Fig. 3, the daily IP injection of sodium cyanate (50 mg per kg body weight) caused a loss of body weight in mice which had received IP transplants of 10^5 B16 melanoma cells. This was accompanied by a progressive decrease in food consumption. There was no signifi-

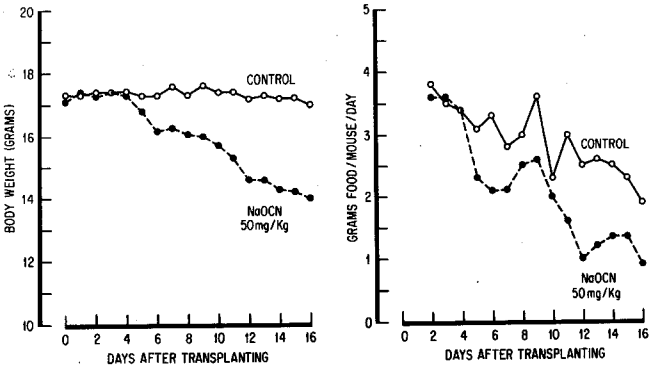

Fig. 3. Effects of daily IP injections of sodium cyanate (50 mg/kg) on body weight and food consumption of mice bearing B16 melanoma. The data are means for 10 control and 12 cyanate-treated mice. Treatment with sodium cyanate was started 24 h after IP transplantation of 10^5 tumor cells

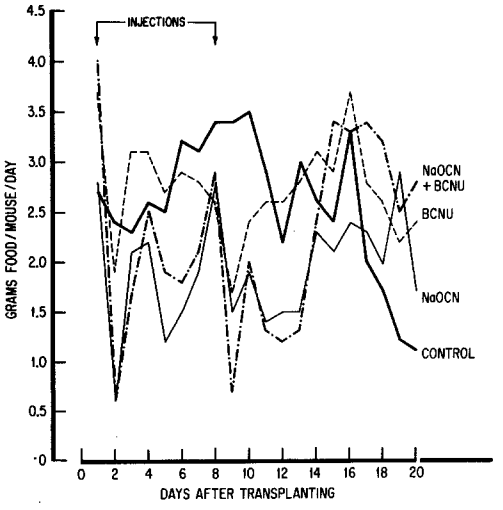

Fig. 4. Effects of sodium cyanate (200 mg/kg) and BCNU (10 mg/kg) on food consumption of mice bearing B16 melanoma. The IP injections were given 1 day and 8 days after IP transplantation of 10^5 tumor cells. The data represent means for 10–12 tumor-bearing mice

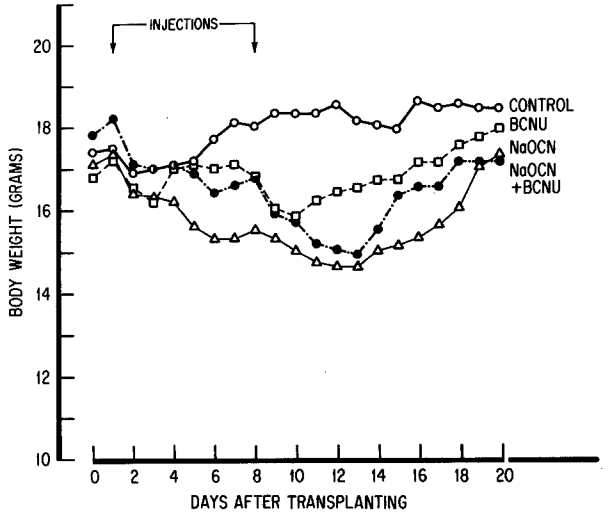

Fig. 5. Effects of sodium cyanate (200 mg/kg) and BCNU (10 mg/kg) on body weights of mice bearing B16 melanoma. See legend to Fig. 4 for further details

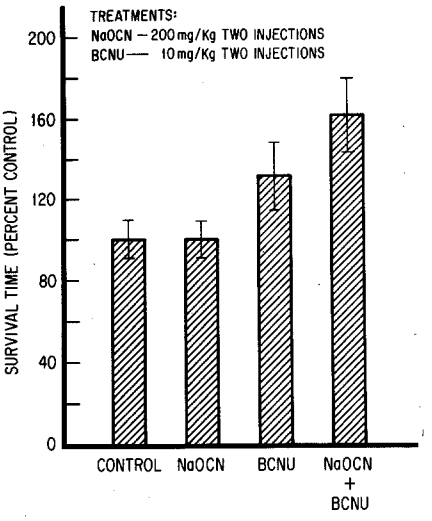

Fig. 6. Effects of sodium cyanate (200 mg/kg) and BCNU (10 mg/kg) or the survival of mice bearing B16 melanoma. Survival is expressed relative to control animals as 100%. The IP injections were given 1 day and 8 days after IP transplantation of 10^5 tumor cells. The data represent means of 10–12 tumor-bearing mice

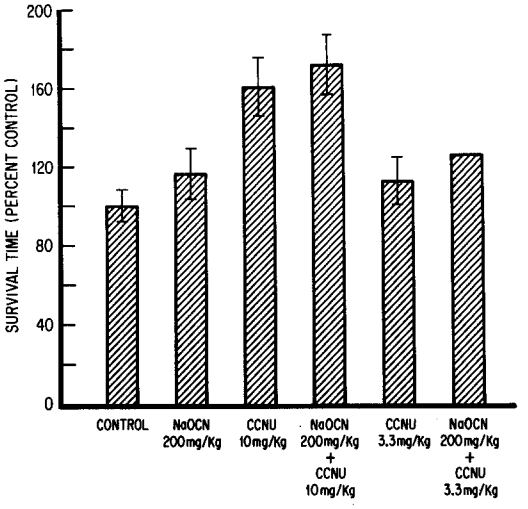

Fig. 7. Effects of sodium cyanate (200 mg/kg) and CCNU (10 mg or 3.3 mg/kg) on the survival of mice bearing B16 melanoma. The IP injections were given 1 day and 8 days after IP transplantation of 10^5 tumor cells. The data represent means ± standard deviation for 3–15 tumor-bearing mice and are expressed relative to mean survival of control animals, which was 17 ± 1 days

cant effect of the cyanate treatment on host survival, which was a mean of 22±3 days for the controls and 20±2 days for the treated mice. When the cyanate dose level was decreased to 35 mg per kg body weight per day there was a less marked effect on body weight and food consumption, but again there was no significant effect on host survival (data not shown).

Owing to the cumulative toxicity of sodium cyanate, high dose levels can only be repeated after a period for recovery. A sharp decrease in food consumption was observed when sodium cyanate (200 mg per kg body weight) was injected IP into mice which had received IP transplants of 10^5 B16 melanoma cells (Fig. 4). There was a re-

covery after 7 days, and a second injection then caused another drop in food consumption. The data in Fig. 5 show the decrease in body weight and subsequent increase which follows this treatment. The mean survivals of mice in this experiment are presented in Fig. 6 relative to survival of control tumor-bearing mice. A signficant increase in host survival was not observed with the two injections of sodium cyanate, but was seen with two injections of BCNU (10 mg per kg body weight). The longest mean survival was found with combination treatment with sodium cyanate and BCNU. The protocol in a subsequent experiment was similar to that in those recorded in Fig. 4–6, except that CCNU was substituted for BCNU (Fig. 7). There was more prolonged host survival with CCNU that with BCNU when it was administered at a dose level of 10 mg per kg body weight. No further statistically significant enhancement of host survival was detected when the treatment included sodium cyanate in addition to CCNU.

To determine whether sodium cyanate would increase the chemotherapeutic effect of melphalan in mice bearing B16 melanoma in a similar manner to that reported by Dufour et al. [5] for P388 tumor-bearing mice, the following protocol was adopted. Mice received IP transplants of 10^5 B16 melanoma cells, and after 24 h they were given IP injections of sodium cyanate (200 or 250 mg/kg) and melphalan (5 or 10 mg/kg), either as single agents or in combination. As in previous studies, sodium cyanate given as a single agent did not significantly increase host survival. The longer survival time seen with melphalan treatment was not increased further by combined administration of sodium cyanate (data not shown).

Discussion

Although our previous studies on the action of sodium cyanate on tumor metabolism had been performed chiefly with rats bearing hepatomas [10–13], we chose mice bearing B16 melanomas as a model for chemotherapy studies. This selection was influenced by the relative economics of studies with mice and rats and the challenge which melanomas present in their resistance to chemotherapy. Despite the marked inhibitory effect on the incorporation of thymidine into DNA seen after administration of sodium cyanate, we were unable to establish conditions in which treatment with sodium cyanate as a single agent caused a significant increase in the survival time of tumor-bearing mice. Earlier work had suggested reversibility in the effect of cyanate on DNA synthesis in rat hepatomas [12], and this may have been a factor in the mouse melanomas.

The route of administration is a subject for concern with sodium cyanate as with other potential chemotherapeutic agents [15]. The LD_{50} for cyanate is lower with oral than systemic administration, but this may be related to acid lability and hydrolysis in the stomach. In patients, sodium cyanate has been given orally together with an antacid in an effort to counteract this effect [7]. It was concern for this problem which led us in our later studies with tumor-bearing mice to examine the effects of IP injection of sodium cyanate. However, we did not see significant effects on host survival when sodium cyanate alone was administered by IP injection.

Some modest synergistic effects were recorded by Dufour et al. [5] when P388 tumor-bearing mice received a combination treatment of melphalan and sodium cyanate. Our studies with B16 melanoma-bearing mice did not reveal a significant effect of this type and may reflect tumor-specificity in the response. On the other hand, combned treatment with sodium cyanate was observed to enhance the effect of BCNU in prolonging the survival of mice bearing B16 melanoma. Some nitrosoureas, such as BCNU and CCNU, have both carbamoylating and alkylating activity. The latter activity is generally believed to be more important in cancer chemotherapy, but the carbamoylating activity may be significant in inhibiting DNA repair [16]. Such an action might be additional to an apparent effect of cyanate on circulation in tumors [11]. It has been suggested that the organic isocyanates liberated from nitrosoureas would be more effective than inorganic cyanate in entering cells [16], but this point does not appear to have been documented. At neutral pH, sodium cyanate is hydrolyzed much more slowly than 2-chloroethyl isocyanate, which is liberated from BCNU. Sodium cyanate could, therefore, provide a slowly acting carbamoylating agent, which may complement the action of other cancer chemotherapeutic agents. However, a simple relationship with carbamoylating activity was not apparent in our studies. Melphalan does not have carbamoylating activity, while the carbamoylating activity of CCNU is greater than that of BCNU, and it was only with the combination of BCNU and sodium cyanate that we detected an additive effect.

In contrast to the observations with sodium cyanate as a single agent in melanoma-bearing mice, an inhibitory effect on the growth of transplanted hepatomas in rats has been observed (M. A. Lea and M. R. Koch, unpublished observation). Two groups of six rats bearing SC transplants of Morris hepatoma $9618A_2$ were given either saline injections or 50 mg sodium cyanate per kg body weight IP at daily intervals, starting 24 h after the last injection. The mean body weights in the cyanate-treated rats were 12% lower than those in the saline-treated controls, but there was a 29% decrease in tumor weights ($P < 0.001$). We plan to extend these studies with hepatoma-bearing rats and mice. The objective will be to further characterize the responsiveness of different tumor types to the action of sodium cyanate either alone or in combination treatment.

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