Vol. 175, No. 2, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1991 Pages 625-630

ANTITUMOR ACTIVITY OF NALTREXONE AND CORRELATION WITH STEROID HORMONE RECEPTORS

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Received February 4, 1991

Summary: We have evaluated the opiate peptide antagonist, naltrexone, for its effectiveness as an antitumor agent. For this evaluation, we tested the effect of naltrexone given daily in the diet on the growth of established 7,12-dimethylbenz(a)anthracene-induced mammary tumors. Tumors continued to grow actively in rats fed chow diet only (control group). In contrast, the naltrexone-supplemented diet (75 mg/kg diet) significantly decreased the size of the established mammary tumors in rats over the 25 day observation period, resulting in an average decrease in tumor volume by approximately 23% compared with their sizes at the beginning of the treatment. Tumor regression occurred in 70% of the rats.Tumors that respond to naltrexone showed appreciable amounts of estrogen and progesterone receptors while unresponsive tumors were negative for estrogen and progesterone receptors. For the first time, we report that naltrexone can regress established mammary tumors and that the inhibitory effect of naltrexone appears to be restricted to the hormonally responsive mammary tumors.

Endogenous opioid peptides (EOP) are best known for their analgesic and behavioral effects (1,2). In addition, EOP have been shown to participate in the control of anterior pituitary hormone secretion. EOP increase prolactin and growth hormone secretion (3-6) and decrease gonadotropin and thyrotropin secretion (3). Naloxone and NTX are competitors for the specific receptors of EOP in the brain, and hence can counteract the actions of EOP on hormone secretion (7). Recently, it was reported that naloxone or NTX can decrease prolactin and growth hormone levels in rats (7).

Recent studies have implicated EOP in cancer development and growth. Evidence that opioid peptides play a role in tumorigenicity derives from pharmacological studies

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Abbreviations: EOP, endogenous opioid peptides; NTX, naltrexone; DMBA, 7,12-dimethylbenz(a)anthracene.

0006-291X/91 $1.50

showing that opiate antagonists inhibit the growth of rodent tumors (8,9) and prolong survival time of mice implanted subcutaneously with neuroblastoma cells (10). We have recently shown that NTX inhibited the induction of rat mammary tumors by DMBA and stress (11,12). The purpose of the present study was to determine whether NTX had any effect on the size of the preestablished mammary tumors in rats and correlate it with presence of steroid hormone receptors in tumors.

MATERIALS AND METHODS

Mammary tumors were induced by treating 50-day old female Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) with single dose of 15 mg DMBA (Sigma Chemical Co., St. Louis, MO) in 1.0 ml sesame oil per rat by intragastric intubation (13). The rats were maintained on a chow diet, and allowed food and water ad libitum. When approximately 75% of the rats had at least one tumor/rat of 1.0-2.0 cm in diameter, the tumor-bearing rats were randomly assigned to 2 groups of 10 rats/group. The first group of rats were fed a chow diet only and thus represented the control group. The second group of rats were fed the chow diet supplemented with 75 mg of NTX/kg diet. Naltrexone (E.I. Dupont de Nemours & Co., Wilmington, DE) was blended into the chow diet using a Hobart food mixer. The diets were fed in stainless steel feeders designed with food hoppers. The food was replaced with freshly-prepared diets twice weekly.

All tumors which developed during the 3-4 month duration of this experiment were adenocarcinomas. Tumor measurements were done twice weekly beginning at the time the rats were placed on the supplemented diets. Tumor volume was calculated using the formula $4/3\ \pi\ r^3$ (14) where r is one-half the mean of the sum of the largest diameter and the axis at right angles to the latter diameter as determined with a micrometer caliper. The size of each tumor was compared with its initial size on day zero to obtain the percentage change in tumor volume. Weights of the rats in each group were recorded weekly. Tumor measurements were analyzed statistically by Analysis of Variance (ANOVA). Values are considered significant when $p<0.05$.

Measurement of Steroid Hormone Receptors

Estrogen and progesterone receptors were measured in the Hormone Receptor Laboratory of the Ohio State University by the multipoint dextran-coated charcoal method as previously described (15). The data on specific binding were analyzed by Scatchard plots (16) to determine the equilibrium dissociation constant and binding capacity, expressed as fmol of estradiol or progesterone (R.5020) specifically bound per mg of cytosol protein for estrogen and progesterone receptors respectively. Cytosol protein concentrations were determined by the procedure of Lowry et al (17).

RESULTS

Shown in Figure 1 is the change in tumor volume (± S.E.) in each group as a function of feeding the control or the NTX-supplemented diet. Tumors continued to grow actively in rats fed a chow diet only (control group). In contrast, the NTX-supplemented diet significantly inhibited the growth of mammary tumors in rats over the 25-day observation period. In this instance, tumors decreased in size by approximately 23% compared with

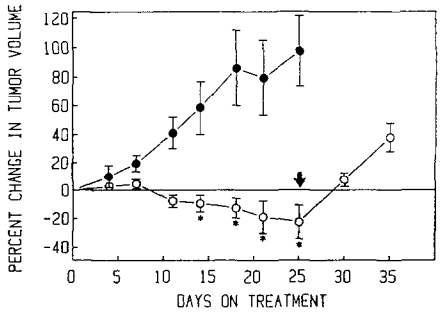

Figure 1. Time course change in volume of mammary tumors as a function of days on the various diets. Group I was fed a chow diet (●)-Group II was fed the chow supplemented with 75 mg naltrexone/kg diet (○). Bars represent standard error of the mean. The arrow shows the time where NTX treatment was stopped.* $p<0.05$.

their sizes at the beginning of the treatment. Tumor regression occurred in 70% of the rats. This growth suppression was rapidly reversed upon transfer of the rats from the NTX treatment to the control diet.

Weight gain and food intake may affect tumor growth (18). Therefore, rats in both groups were weighed weekly, and their initial and final weights during the 25-day period of this experiment are shown in Table 1. Food intake and weight gain were essentially identical in the two groups.

In this study, attempts have been made to correlate tumor regression in response to NTX treatment with tumor estrogen and progesterone receptor levels. Although estrogen and progesterone receptor amounts did not change after NTX treatment, the inhibitory effect of NTX appears to be restricted to the hormonally responsive mammary tumors. Shown in Table 2 are the estrogen and progesterone receptor amounts in the tumors that regressed in response to NTX treatment. The other tumors that failed to regress and continued to grow were in fact estrogen and progesterone receptor negative.

DISCUSSION

We have earlier reported (19) that tumor-bearing rats had higher contents of opioid peptides compared to animals with no tumors, suggesting that an alteration in the level of

TABLE 1
Average Weight of Rats Just Before Treatment and at the End of the Experiment

Group	Weight (g)	
	Initial	Final
Control (Chow)	418 ± 28	437 ± 20
Naltrexone-Treated	411 ± 13	423 ± 14

* Values = Means ± S.E.

TABLE 2
Estrogen and Progesterone Receptors of Established Mammary Tumors

Group	Estrogen Receptors	Progesterone Receptors
	fmol/mg of protein*	
Control (Chow diet)	46.7 ± 8.9	96.6 ± 13.9
Naltrexone-treated (Responsive)	52.6 ± 6.0	91.3 ± 11.0
Naltrexone-treated (Unresponsive)	< 3.0	< 5.0

* Values = Means ± S.E.

these neuropeptides occurs during the process of chemical carcinogenesis. Further studies (12) showed that the opiate antagonist, NTX, inhibited the induction and development of rat mammary tumors by DMBA and stress. These studies were extended to determine whether NTX has an effect on the size of the established mammary tumors. The results of this study demonstrate, for the first time, that NTX significantly inhibited the growth of the preestablished DMBA-induced rat mammary tumors, and caused tumors to regress in 70% of the rats. These data may be relevant to humans in whom a drug is considered active in any particular tumor histiotype if it gives positive results in at least 70% of the patients (20).

Several reports indicated that NTX can either inhibit (8,9,11,12) or enhance (10) carcinogenesis depending upon the particular dose used and the duration of blockage of opioid receptors. Thus investigators (8) who used low doses of NTX were unable to observe mammary tumor regression. The rapid elimination of the administered NTX and its relatively short duration of action (21) offers a possible explanation of its low biological activity. Thus, it appears that for NTX to be effective, it has to be given at consistent doses to maintain a stable level of NTX in plasma. In this study, the growth inhibitory effect was more pronounced since the rats were allowed to consume NTX continuously from the diet in contrast to the one time daily injection that has been used by those investigators who failed to show any response.

There are several possible mechanisms by which NTX might influence the development and growth of mammary tumors. Naltrexone is a potent inhibitor of prolactin secretion by the pituitary (5-7), and this hormone has been implicated in growth and maintenance of human breast cancer (21,22). In fact, a significant decrease in plasma prolactin levels was associated with the NTX-induced inhibition of rat mammary tumor induction by DMBA (11,12). A similar mechanism of action appears more likely to explain the NTX-induced regression in the same mammary tumor model. These results are in agreement with the effects of prolactin release-inhibiting drugs, such as l-dopa and ergocryptine (23,24) which not only prevent growth but also induce regression of mammary

tumors. The observation that tumor growth resumed after cessation of treatment with NTX suggests that EOP may stimulate mammary tumor growth via their ability to promote prolactin and growth hormone secretion.

Additional mechanism(s) of action cannot be ruled out especially in virtue of recent studies that suggested an important role for NTX in the modulation of the host immune system. An increase in T-cell proliferation was observed in association with the NTX induced inhibition of mammary tumor induction by DMBA (11,12). These studies, beside NTX's lack of effect alone in vitro (data not shown), indicate an indirect mode of action of the opioid antagonist in controlling tumor growth.

In conclusion, this is the first report on the regression of established mammary tumors in vivo by NTX. The growth inhibitory effect of NTX appears to be restricted to the hormonally responsive tumors. Since the majority of human breast cancers are estrogen/progesterone receptor positive and thus believed to be hormonally responsive, this observation may be of importance clinically. However, it will be necessary to determine the effects of NTX on a number of estrogen and progesterone receptor-positive (MTW9), as well as estrogen and progesterone receptor-negative (MTW9D) tumor model systems in vivo before a more generalized conclusion can be drawn.

ACKNOWLEDGMENTS

This study was supported by OSU Development Fund 537420. The shared services of the OSU Comprehensive Cancer Center were supported by NCI Grant P-30-CA-16058.

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