ABSTRACT

Prostaglandin A1 has a profound inhibitory effect on uridine incorporation into RNA of normal cartilage whereas N\(^\alpha\)-monobutyryladenosine 3',5'-cyclic monophosphate is either stimulatory or without an effect. Sera from intact and growth hormone-treated hypophysectomized rats stimulate RNA synthesis but serum from untreated hypophysectomized rats does not. The present study investigated the in vitro regulation of \([\text{\textsuperscript{3H}}]\)uridine incorporation into RNA of six human chondrosarcomas to determine if malignant human chondrocytes are under similar metabolic and hormonal regulation. Prostaglandin A\(_1\) (25 \(\mu\)g/ml) markedly inhibited uridine incorporation in all six tumors (56 to 80%). N\(^\alpha\)-Monobutyryladenosine 3',5'-cyclic monophosphate (1 \(\mu\)M) inhibited uridine incorporation in five tumors (20 to 50%). Uridine incorporation was stimulated by growth hormone-dependent serum factors in one tumor and by growth hormone-independent serum factors in two tumors. Two tumors were more responsive to serum from growth hormone-treated hypophysectomized rats than to serum from intact rats, and one tumor was unresponsive to serum stimulation. The data indicate that: (a) prostaglandin A\(_1\) is a very potent inhibitor of RNA synthesis in human chondrosarcomas; (b) N\(^\alpha\)-monobutyryladenosine 3',5'-cyclic monophosphate affects human chondrosarcomas differently than it does normal cartilage; and (c) responses of human chondrosarcomas to serum growth factors vary among individual tumors.

INTRODUCTION

The growth and metabolism of immature cartilage is regulated in vivo by many different hormones. Factors such as growth hormone, somatomedins, and insulin stimulate growth but not maturation. Thyroid hormones and sex steroids facilitate both maturation and growth. Since it is not possible to delineate the specific effects of a hormone on cartilage metabolism in vivo, a number of in vitro organ culture or cell culture techniques have been developed for this purpose. In such systems, growth hormone-dependent serum factors (somatomedins) stimulate DNA, RNA, total protein, and proteoglycan synthesis (11, 17, 18). In contrast, prostaglandin A\(_1\) inhibits in vitro synthesis of DNA, RNA, total protein, and proteoglycan (6, 10). Cyclic AMP\(^4\) has been shown to stimulate cartilage growth processes in some in vitro systems and inhibit it in others (1-5, 15).

The degree to which malignant transformation affects hormone responsiveness of chondrocytes might be an important factor in determining the growth characteristics of specific chondrosarcomas and might provide a basis for some therapeutic interventions to modify the growth of these tumors. To explore such possibilities, we carried out extensive studies assessing the metabolic and hormonal factors which influence the growth and metabolism of a well-differentiated transplantable rat chondrosarcoma. These studies showed that prostaglandin A\(_1\), N\(^\alpha\)-monobutyryl cyclic AMP (15), and serum somatomedins (14) have effects on the rat chondrosarcoma which are similar to those on normal cartilage. The present study was done to determine to what degree human chondrosarcoma growth might be regulated by such hormonal and metabolic factors. The in vitro responsiveness of 6 human chondrosarcomas has been studied, and the results are the subject of this report.

MATERIALS AND METHODS

The human chondrosarcomas used in this study were obtained at surgery from 6 patients at Duke University Medical Center. Chondrosarcomas A, B, C, and D were in intramedullary femoral tumors. Chondrosarcomas E and F were lung metastases which were obtained from patients who had had their primary tumors removed several years earlier. The tumors were graded histologically as to their malignant character using a scale of 0 to 4, with 0 being benign and 4 highly malignant. Tumors A and B were Grade 1, Tumors C, E, and F were Grade 2, and Tumor D was Grade 4. A benign enchondroma (Tumor D); Grade 0) was associated with chondrosarcoma D, and parallel studies were performed with it for comparative purposes.

The tumors were removed, immediately dissected free of any other tissues, minced, and forced through a wire screen having a mesh size of 2 x 2 mm into 0.9% NaCl. The resultant pieces, which weighed 2 to 4 mg each, were rinsed with 0.9% NaCl, thoroughly mixed, and recovered by filtration. Tumor pieces (100 mg/flask) were incubated as described previously for the Swarm chondrosarcoma (14). Because of the limited amount of each tumor available, tumor pieces could only be incubated with a single concentration of the various substances being studied. The concentration of each substance was chosen on the basis of previous dose-response studies with the Swarm rat chondrosarcoma (6, 14, 15). Tumor pieces were incubated in a Tris-buffered (pH 7.4) medium supplemented with electrolytes, penicillin, glucose, and essential amino acids (3) at 37 \(^\circ\)C for 8 hr in a gyrorotatory shaker bath. They were pulsed with [5-\text{\textsuperscript{3H}}]uridine (1 \(\mu\)Ci/flask) during the last 4 hr of incubation. In one experiment, [\text{\textsuperscript{3H}}]uridine (1 \(\mu\)Ci/flask) and L-[\text{\textsuperscript{14C}}]leucine (0.2 \(\mu\)Ci/flask) were added simultaneously. In the same experiment, the effect of prostaglandin A\(_1\) on [\text{\textsuperscript{35S}}] incorporation into proteoglycan was studied by pulsing tumor pieces with H\(_2\)35SO\(_4\) (1.25 \(\mu\)Ci/flask) during the last 4 hr of incubation. Following incubation, tumor pieces were separated from the medium by centrifugation, rinsed with 0.9% NaCl, and homogenized in 5% TCA. The TCA precipitate was recovered by centrifugation (15,000 \(\times\) g for 10 min), rinsed with 5% TCA, digested in 0.7 ml Protosol, and counted in 7.5 ml Omniflour. In several studies, an aliquot of the TCA supernatant was counted so that estimates of changes in uridine transport and alterations in uridine nucleotide pool-specific activities could be obtained.

For a given tumor, statistical significance of the difference between means was determined by Student's t test. The Student-Newman-Keuls test was used to make comparisons among the means for the different tumors for a given treatment (19).
Prostaglandin A. was kindly provided by the Upjohn Company (Kalamazoo, Mich.). Bovine growth hormone (Lot NIH GH-BB) was supplied by the Hormone Distribution Program of the National Institutes of Arthritis, Metabolism, Digestive Diseases and Diabetes. N6-Monobutyryl cyclic AMP was obtained from the Sigma Chemical Company (St. Louis, Mo.). Carrier-free H235SO4 (27 Ci/mmol) and [5-3H]Uridine (26 Ci/mmol) were purchased from Schwartz-Mann (Orangeburg, N. Y.). L-[U-14C]Leucine (300 mCi/mmol) was obtained from New England Nuclear (Boston, Mass.).

Serum was obtained from normal, hypophysectomized, and growth hormone-treated hypophyseomized rats after decapitation. Male Sprague-Dawley rats weighing 150 to 200 g were used. Growth hormone treatment consisted of 3 i.m. 0.5-mg injections of bovine growth hormone at 36, 24, and 8 hr before the rats were decapitated.

RESULTS

Effects of Prostaglandin A1 on Chondrosarcoma Metabolism in Vitro. Chart 1 shows the dramatic inhibition of [3H]uridine incorporation into RNA in vitro by Prostaglandin A1 (25 µg/ml) in the chondrosarcomas studied. All of the chondrosarcomas and the benign enchondroma showed prostaglandin A1 inhibition of [3H]uridine incorporation into RNA in vitro of 75% or greater. One of the metastatic tumors (Tumor E) showed a significantly (p < 0.01) lesser degree of inhibition, but it was still of the order of magnitude of 50%.

The prostaglandin A1-mediated decrease in [3H]uridine incorporation into RNA could be due to changes in uridine transport, alterations in the specific activity of the UTP pool, inhibition of RNA synthesis, or accelerated RNA degradation. The data shown in Table 1 indicate that prostaglandin A1 inhibits [3H]uridine incorporation into RNA significantly more than it inhibits [3H]uridine transport or entry into soluble precursors. These data are compatible with, but do not prove, a primary effect of prostaglandin A1 in inhibiting RNA synthesis.

The effects of prostaglandin A1 on 35SO4 incorporation into proteoglycan and L-[14C]leucine into total protein were studied in chondrosarcoma A (Table 2). Although the rate of incorporation of these radiolabeled precursors into the tumors was quite low in medium alone, the addition of prostaglandin A1 to the incubation medium significantly lessened the incorporation of both labeled compounds into macromolecules.

The inhibitory effect of prostaglandin A1 on [3H]uridine incorporation into RNA in vitro can occur at concentrations less than 25 µg/ml as illustrated in the dose-response curve for chondrosarcoma B shown in Chart 2. Significant inhibition was observed with as little as 2.5 µg prostaglandin A1 per ml. Effect of N6-Monobutyryl Cyclic AMP on Chondrosarcoma Metabolism in Vitro. Cyclic AMP effects on [3H]uridine incorporation into chondrosarcoma RNA were studied by incubating tumor pieces in vitro with the cyclic AMP analogue, N6-monobutyryl cyclic AMP. Results of these experiments are shown in Chart 3. One of the tumors, chondrosarcoma E, was unresponsive to N6-monobutyryl cyclic AMP. In the other 5 chondrosarcomas and in the benign enchondroma, N6-monobutyryl cyclic AMP significantly inhibited [3H]uridine incorporation into RNA. The degree of inhibition was somewhat variable. In Tumors B and C, the decrease in TCA-soluble counts in response to N6-monobutyryl cyclic AMP was at least as great as the decrease in TCA precipitable counts (Table 1). This suggests that the inhibition of uridine incorporation into RNA in these tumors might be due to an inhibition of uridine transport or conversion to nucleotide precursors rather than an inhibition of RNA synthesis. The data from Tumor F show much more inhibition of [3H]uridine incorporation into precipitable as compared to soluble counts, suggesting that N6-monobutyryl cyclic AMP may have different effects on different tumors.

Effects of Growth Hormone-dependent Serum Factors (Somatomedins) on Chondrosarcoma Metabolism in Vitro. To determine to what extent the growth of human chondrosarcomas might be modulated by serum growth factors, we examined the effects of the addition of serum from normal, hypophyseomized, and growth hormone-treated hypophyseomized rats to the incubation medium on the in vitro incorporation of [3H]uridine into RNA in the various chondrosarcomas. Chart 4 shows that human chondrosarcomas differ remarkably in their responsiveness to serum factors. In chondrosarcoma A, sera from normal and growth hormone-treated hypophyseomized rats stimulated [3H]uridine incorporation into RNA whereas

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Table 1

<table>
<thead>
<tr>
<th>Additions to incubation medium</th>
<th>Tumor B</th>
<th>Tumor C</th>
<th>Tumor F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soluble [%H] (µCi/µg)</td>
<td>Precipitable [%H] (µCi/µg)</td>
<td>Soluble [%H] (µCi/µg)</td>
</tr>
<tr>
<td>5% normal rat serum</td>
<td>18.2 ± 6.7</td>
<td>17.6 ± 7.1</td>
<td>23.5 ± 11.6</td>
</tr>
<tr>
<td>5% serum from hypophysectomized rats</td>
<td>22.9 ± 6.9</td>
<td>14.6 ± 12.5</td>
<td>23.5 ± 5.1</td>
</tr>
<tr>
<td>5% serum from growth hormone-treated hypophysectomized rats</td>
<td>34.5 ± 6.4</td>
<td>34.3 ± 3.1</td>
<td>10.6 ± 10.7</td>
</tr>
<tr>
<td>N6-Monobutyryl cyclic AMP (1 µM)</td>
<td>-40.5 ± 4.5</td>
<td>-28.9 ± 7.7</td>
<td>-30.3 ± 3.9</td>
</tr>
<tr>
<td>Prostaglandin A1 (25 µg/ml)</td>
<td>-70.6 ± 0.8</td>
<td>-83.3 ± 0.6</td>
<td>-44.0 ± 5.5</td>
</tr>
</tbody>
</table>

a Mean ± S.E. of 5 samples containing 100 mg of tumor pieces per sample.
b p < 0.01 soluble versus precipitable counts.
serum from untreated hypophysectomized rats had no effect. These data suggest that this chondrosarcoma is responsive to a somatomedin. Serum added to the medium also stimulated [3H]uridine incorporation into RNA in 4 of the other human chondrosarcomas. The stimulatory effect of serum on chondrosarcoma E did not appear to be due to growth hormone-dependent factors because serum from hypophysectomized rats which lacks growth hormone-dependent serum factors stimulated [3H]uridine incorporation into RNA with an effectiveness equal to serum from intact and growth hormone-treated hypophysectomized rats. In chondrosarcoma D, the stimulatory effect of growth hormone-treated hypophysectomized rat serum was significantly greater (p < 0.05) than that of serum from intact and hypophysectomized rats. Chondrosarcoma B was responsive only to serum from growth hormone-treated hypophysectomized rats whereas chondrosarcoma F and the enchondroma were unresponsive to serum stimulation.

An analysis of the effects of the various types of serum on TCA-soluble counts versus TCA-precipitable counts from Tumors B and C (Table 1) suggest that the serum additions may increase the incorporation of [3H]uridine into RNA through an increase in [3H]uridine transport rather than through an increase in RNA synthesis in these tumors.

**DISCUSSION**

Since hormones and metabolic regulators modulate the growth and maturation of normal cartilage and a transplantable rat chondrosarcoma, it is of considerable theoretical and potentially practical importance to know to what degree human chondrosarcomas are under similar control. Our data indicate that the in vitro metabolism of all 6 of the human chondrosarcomas we studied was inhibited by prostaglandin A1. N6-Monobutryl cyclic AMP inhibited uridine incorporation in 5 of the 6 tumors and serum factors, either growth hormone dependent or independent, stimulated uridine incorporation in 5 of the 6 chondrosarcomas. The inhibitory effect of prostaglandin A1 on DNA, RNA, protein, and proteoglycan synthesis in normal cartilage and in the Swarm rat chondrosarcoma in short-term organ cultures were initially reported by Eisenbarth et al. (6-8). Because prostaglandin A1 stimulated cyclic AMP production and did not inhibit oxygen consumption, they postulated that prostaglandin A1 was exerting a specific inhibitory effect on macromolecule synthesis rather than a nonspecific toxic action. Kirkpatrick (9, 10), on the other hand, studied the effects of prostaglandin A1 on growth and morphology of embryonic chick femoral and tibial rudiments during 8 days of in vitro organ culture and concluded that much of its effects were due to a general cellular toxicity. In all of the studies of prostaglandin A1 effects on cartilage in vitro, including the one presented in this paper, concentrations were used which must be considered pharmacological. While much work remains to be done in order to clarify the nature of the inhibitory effect of prostaglandin A1 on malignant chondrocytes, our data suggest that this substance may possess potential as a therapeutic agent in controlling the
growth of cartilage tumors.

The effects of cyclic AMP on $[^3H]$uridine incorporation into RNA vary in normal and neoplastic cartilage. In embryonic chick cartilage, N$\beta$-monobutyryl cyclic AMP stimulates RNA synthesis (4). In contrast, cyclic AMP does not appear to affect $[^3H]$uridine incorporation into RNA of rat costal cartilage (15). In the Swarm rat chondrosarcoma, N$\beta$-monobutyryl cyclic AMP stimulates uridine incorporation into RNA but does so by increasing uridine transport and UTP synthesis rather than by stimulating RNA synthesis (16). The striking in vitro inhibition of $[^3H]$uridine incorporation by N$\beta$-monobutyryl cyclic AMP in 6 of 7 human cartilage tumors was therefore unexpected. The data in Table 1 suggest that the inhibition in Tumor F might have been due to an interference with RNA synthesis; however, in Tumors B and C, an interference with uridine transport or UTP synthesis is a more likely explanation. It is unclear whether this inhibitory action of N$\beta$-monobutyryl cyclic AMP has any implication for the treatment of human chondrosarcomas.

Serum contains both growth hormone-dependent and -independent growth factors. Growth of normal cartilage and the Swarm rat chondrosarcoma are stimulated both in vivo and in vitro by growth hormone-dependent serum factors (somatomedins) (14, 16). The responses of the human chondrosarcomas indicate that there is considerable variation in their in vitro responses to growth hormone-dependent and -independent serum factors. This is in keeping with the observation of Mankin et al. (12, 13), who have demonstrated that human chondrosarcomas are markedly different with respect to histological and biochemical characteristics. Chondrosarcoma A exhibits the classic response to somatomedin in that $[^3H]$uridine incorporation is stimulated by the addition of serum from normal and growth hormone-treated hypophysectomized rats to the medium but not by the addition of serum from untreated hypophysectomized rats. Chondrosarcomas B and D show some increased stimulation by addition of serum from growth hormone-treated hypophysectomized rats. Chondrosarcoma E is stimulated by a growth hormone-independent serum factor. Chondrosarcoma F and enchondroma D' were unresponsive to serum factors. Thus, we can conclude that different tumors are responsive to a variable degree to somatomedins and other unknown serum growth factors. Further delineation of the nature of these serum effects could be very important in defining the spectrum of growth factors that can modulate human chondrosarcoma growth.

The present study undertook the task of characterizing the hormone and metabolic responses of individual human chondrosarcomas in order to determine if such information could generate potential approaches to treatment. A major limitation of our study is the modest amount of each tumor available for study. Thus, responses to different doses or to different incubation conditions could not be adequately done. Likewise, additional data on the mechanisms of the various effects were not possible. The information presented here should be considered as a basis for further inquiry into this area of investigation. Appropriate techniques to allow growth of human chondrosarcomas in culture or nude mice will make more extensive and complete studies of each individual tumor possible.

Human chondrosarcomas do not respond to radiation or chemotherapy and at present can only be treated by surgical extirpation. If the tumor is metastatic or unresectable, it is essentially untreatable. Thus, a nonresectable tumor that is growth hormone dependent might be treated by a hypophysectomy or a long-acting somatostatin derivative. Hypophysectomy has been shown to markedly alter the histology and growth characteristics of the growth hormone-dependent Swarm rat chondrosarcoma (16). Tumors that are responsive to other growth factors might be treated by appropriate agents to block production or action of those growth factors. Our initial data presented here suggest that extensive investigation into hormone receptors and hormone responsiveness of human chondrosarcoma may prove fruitful. Whether prostaglandin A1 will influence the growth of human chondrosarcomas in vivo remains to be determined, but in vitro data suggest that this should be investigated.

ACKNOWLEDGMENTS

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