Effects of Mithramycin on Bone Resorption in Vitro

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SUMMARY

Embryonic rat bones labeled with $^{45}$CaCl$_2$ are stimulated in vitro by parathyroid extract to release $^{45}$Ca. Mithramycin prevents this active release over a broad range of dose levels that are nonlethal to the bones, as evidenced by their subsequent responsiveness to additional parathyroid extract. The similarity of dose levels used to concentrations (based on calculated dilution) after clinical administration supports the interpretation that clinical effects of mithramycin in hypercalcemic patients are due to a direct effect on bone.

INTRODUCTION

MTM$^2$ is a cytotoxic antibiotic derived from a culture of Streptomyces tanashiensis (19). It has been of clinical value in the treatment of certain advanced carcinomas of the testis (3, 7, 8, 10) and glioblastoma multiforme (8). MTM was noted to lower the serum calcium of normocalcemic patients (3, 18, 21) as well as that of hypercalcemic patients with cancer of various types (1, 5, 9, 10, 13) or with hyperparathyroidism (5). In patients with Paget's disease (20, 21), there was a striking symptomatic relief of bone pain after MTM administration. MTM was also found to lower the serum acid phosphate of patients with carcinoma of the prostate with bony involvement (11). MTM may produce hypocalcemia by one of the following possible mechanisms: (a) it may stimulate the thyroid gland to secrete thyrocalcitonin; (b) it may affect the tumor directly; (c) it may render the patient vitamin D resistant, since the hypocalcemia it produced could not be reversed by a high p.o. dose of vitamin D (12); or (d) it may inhibit bone resorption, for it not only lowers the serum calcium but also decreases the urinary excretion of calcium and hydroxyproline (12, 13, 21, 23).

The first possibilities were cast in doubt by Singer et al. (23). They reported a patient with functioning parathyroid carcinoma who failed to sustain normocalcemia despite large doses of exogenous salmon thyrocalcitonin but who responded to repeated administration of MTM. During the fall of plasma calcium and the decreased urinary excretion of calcium and hydroxyproline, concentrations of immunoassayable circulating PTH remained elevated. It was therefore postulated that MTM caused inhibition of bone resorption, independent of PTH activity.

Because of the variable factors found in vivo, an in vitro system of bone tissue culture was adopted to study the effects of MTM on bone resorption.

MATERIALS AND METHODS

A tissue culture system utilizing embryonic rat bone labeled with $^{45}$CaCl$_2$, following the method of Raiz (14), was used. Pregnant 18-day-old Charles River rats (Sprague-Dawley strain) were given s.c. injections of 500 μCi $^{45}$CaCl$_2$ (Nuclear Science and Engineering, Pittsburgh, Pa.). On the next day, embryos were sacrificed, and the paired embryonic radii and ulnae were dissected out, washed, and cultured individually in 0.5 ml of modified Biggers, Gwatkin, Judah (BGJ) medium (16) (Grand Island Biological Co., Grand Island, N. Y.), containing bovine serum albumin, 1 mg/ml, in an atmosphere of 5% CO$_2$, 20% O$_2$, and 75% N$_2$. One of the pair was treated with either PTE (Parathyroid Injection; Eli Lilly and Co., Indianapolis, Ind.), 1 U.S.P. unit/ml; MTM (Mithracin, Pfizer, Inc., New York, N. Y.) in a range of doses; or both PTE, 1 unit/ml, and MTM, for 48 hr. At the end of 48 hr, treated bones were washed 3 times with fresh BGJ medium, with the use of a Pasteur pipet. The treated bones were then gently transferred to fresh medium and recultured for another 48 hr with PTE, 1 unit/ml. Control bones were washed at 48 hr and returned to control medium for another 48 hr. Aliquots of medium were taken at the end of the 48th hr from each 1st medium and at the end of the 96th hr from each 2nd medium for scintillation counting. The ratio of release of previously incorporated $^{45}$Ca from treated bones to that from paired untreated controls served as a measure of bone resorption.

Two groups of experiments were performed, as follows:

Group 1. In the 1st 48-hr culture, each set of paired bones received no treatment or was treated with PTE or PTE plus MTM at doses of 0.0075, 0.015, 0.0225, 0.03, and 0.045 μg/ml. In the 2nd 48 hr, after washing, PTE was given to all the cultures other than the control bones.

Group 2. In the 1st 48-hr culture, each set of bones was either untreated or treated with PTE alone or MTM alone at concentrations of 0.015, 0.03, and 0.06 μg/ml. In the 2nd 48 hr, the treated bones were washed and recultured with PTE. Results are expressed as cpm/ml medium and as the ratio of $^{45}$Ca released to the culture fluid by the 2 members of the bone pair. Radioactivity was counted in a Packard Model 3375 liquid scintillation spectrometer with liquid scintillation fluid (POPOP and PPO, Packard Instrument Co., Downers Grove, Ill.) diluted with toluene solution. Appropriate corrections were made for quenching and background.

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2 The abbreviations used are: MTM, mithramycin; PTH, parathyroid hormone; PTE, parathyroid extract.

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RESULTS

Chart 1 illustrates the effects of PTE on bone resorption and the effects of MTM on PTE-induced bone resorption, as indicated by the lesser release of $^{45}$Ca from bone to the medium in treated cultures. When MTM was added simultaneously with PTE, the effect of PTE was significantly inhibited. Viability of the cultured bones was demonstrated in the 2nd 48 hr by increased $^{45}$Ca release, when treated by PTE. In bones previously treated with MTM, 0.045 μg/ml, no effect of subsequent PTE was found, indicating residual toxicity of MTM or bone cell death. A repeat experiment is shown in Table 1.

DISCUSSION

Stimulation of bone resorption involves a cell transformation consisting of osteoclastic and fibroblastic proliferation and disappearance of osteoblasts. This transformation can lead to complete resorption of embryonic bone after a brief exposure to PTH (23) or hydroxycholecalciferol (24). Stimulation of resorption by either PTH or hydroxycholecalciferol can be inhibited by dactinomycin (15), an inhibitor of DNA-dependent RNA synthesis (6).

MTM is an antibiotic related to dactinomycin. It has been found to inhibit DNA-dependent RNA synthesis (22, 25). The studies reported here show that MTM can inhibit PTE-induced bone resorption by a direct effect on bone. The $^{45}$Ca release from 1 unit of PTE was abolished by simultaneous treatment with MTM at 0.0075, 0.015, 0.0225, and 0.03 μg/ml. After being rewashed and recultured, these bone cultures were normally responsive to PTE in the 2nd 48 hr. This indicates their viability and the transient nature and reversibility of MTM effect. MTM used alone, even at toxic levels, does not inhibit spontaneous $^{45}$Ca release from embryonic bone in this system, which inhibition is probably due to physical solution. Clinically effective doses of MTM for hypercalcemia are 25 μg/kg (1, 21); our results are thus of the proper order of magnitude for relevance in vivo. The reversible effects correlate with the transient duration of action of MTM in the treatment of hypercalcemia.

Parathyroid extract can produce bone resorption by stimulating DNA-dependent RNA synthesis in existing osteoclasts without increasing their numbers (2). Available evidence suggests that the action of PTH on bone also involves the activation of the adenyl cyclase system (4, 17). The effects of MTM are probably due to the temporary inhibition of DNA-dependent RNA synthesis or to some metabolic process in the bone, rather than to a specific interaction with PTE. The present data provide evidence that MTM inhibits bone resorption in vitro.

Table 1

<table>
<thead>
<tr>
<th>Treatment during 1st 48 hr</th>
<th>Treated/control ratio of $^{45}$Ca release</th>
<th>Treatment during 2nd 48 hr</th>
<th>Treated/control ratio of $^{45}$Ca release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>MTM (μg/ml)</td>
<td>PTE (1 unit/ml)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1.02 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1.00 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>+</td>
<td>2.54 ± 0.04</td>
</tr>
<tr>
<td>4</td>
<td>0.0075</td>
<td>+</td>
<td>1.35 ± 0.09</td>
</tr>
<tr>
<td>5</td>
<td>0.015</td>
<td>+</td>
<td>1.24 ± 0.05</td>
</tr>
<tr>
<td>6</td>
<td>0.0225</td>
<td>+</td>
<td>0.96 ± 0.05</td>
</tr>
<tr>
<td>7</td>
<td>0.03</td>
<td>+</td>
<td>0.99 ± 0.04</td>
</tr>
<tr>
<td>8</td>
<td>0.045</td>
<td>+</td>
<td>0.97 ± 0.03</td>
</tr>
</tbody>
</table>
resorption in vitro and offer a basis for an understanding of the clinical efficacy of the drug in the treatment of hypercalcemia.

ACKNOWLEDGMENTS

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REFERENCES

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