EFFECT OF GALLIUM NITRATE ON TAMOXIFEN INDUCED HYPERCALCEMIA IN RATS BEARING MAMMARY TUMOR

G. Arumugam1, *, P. Shanthi2, P. Sachdanandam1
1Department of Medical Biochemistry, Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, India
2Department of Pathology, Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, India

Aim: To study the effect of gallium nitrate in the treatment of flare hypercalcemia in rats, bearing mammary tumor with bone metastasis. Materials and Methods: Female Sprague-Dawley albino rats were used in the study. Animals were divided into 5 groups: normal control; hypercalcemic rats bearing DMBA-induced mammary tumors; flare hypercalcemic animals bearing DMBA-induced mammary tumors (hypercalcemic rats, treated with tamoxifen at the dose of 10 mg/kg); flare hypercalcemic rat bearing DMBA-induced mammary tumors, treated with gallium nitrate at the dose of 2.5 mg/kg; control rats, treated with gallium nitrate at the dose of 2.5 mg/kg. Eligibility criteria — serum calcium levels were 11.0 mg% or above. Biochemical parameters were measured, using standard methods. Urinary excretion of calcium, creatinine ratio, urinary bone marker were also evaluated by using standard method. Results: All flare hypercalcemic rats were treated with gallium nitrate and developed normocalcemia. Biochemical parameters were measured in hypercalcemic and flare hypercalcemic animals. Calcium level in blood serum, alkaline phosphatase were significantly higher in flare hypercalcemia than in hypercalcemic rats. Urinary pyridinoline, deoxypyridinoline and hydroxyproline were also elevated in flare hypercalcemic rats. In contrast, intact parathyroid hormone and albumin levels were lowered in flare as well as hypercalcemic groups when compared with normal control groups. After gallium nitrate treatment all the above parameters returned to normal level. Conclusions: Administration of gallium nitrate in vivo is highly effective in treatment of flare hypercalcemia.

Key Words: tamoxifen, gallium nitrate, DMBA, hydroxyproline, β-glucuronidase, pyridinoline, deoxypyridinoline.

Received: April 17, 2005.

*Correspondence: Fax: 91-44-24926709
E-mail: psachdanandam2000@yahoo.co.in

Abbreviations used: ALP — alkaline phosphatase; cAMP — cyclic adenosine monophosphate; DMBA — 7,12 dimethyl benzathracene; HPLC — high pressure liquid chromatography; IL-1 — interleukin-1; IL-6 — interleukin-6; iPTH — intact parathyroid hormone; NSCLC — non-squamous lung cancer; PTH — parathyroid hormone; Pyr — pyridinoline; D-Pyr — deoxypyridinoline; TAM — tamoxifen; TNF — tumor necrosis factor; TiH — tumor induced hypercalcemia; VIG — vinblastine, ifosamide, Gallium nitrate.
ously for 60–90 days. Blood was taken for monitoring of calcium level. Hypercalcemic rats were selected from tumor-bearing animals after confirmation that serum or plasma calcium levels reached 11.0 mg/dl or more.

Gallium nitrate was given to the rats with confirmed hypercalcemia at the dose of 2.5 mg/kg body weight by intravenous infusion for 7 days. During experiment, animals body weight was periodically recorded. At the end of the study, animals were sacrificed and tumors, liver and kidney tissues were collected, weighed and homogenized. Blood serum or plasma samples were collected and analyzed.

There are 5 groups of animals (6 rats per group): 1) group 1 — control; 2) group 2 — tumor-bearing animals selected by presence of hypercalcemia (hypercalcemic group); 3) group 3: hypercalcemic bearing mammary tumor rats treated with tamoxifen at the dose of 10 mg/kg body weight by subcutaneous injections for 30 days (“Flare” hypercalcemic group); 4) group 4 — flare hypercalcemic animals at the dose of 2.5 mg/kg body weight by intravenous infusions for 7 days; 5) group 5 — control rats, treated with gallium nitrate at the dose of 2.5 mg/kg body weight by intravenous infusion for 7 days by intravenous infusion.

Biological studies. Total serum calcium, inorganic phosphorus, creatinine, albumin, and alkaline phosphatase (ALP) activity were measured using an autoanalyzer 4010 Boehringer Mannheim Germany and Technicon 100 Germany) Serum calcium were levels corrected for albumin concentration according to the following formula: corrected serum calcium (mg/dl) = total calcium (mg/dl) — serum albumin (g/dl) + 4.0. Urine samples were collected in disposable plastic containers with boric acid (8 g/L) as preservative. Urinary hydroxyproline was measured, using modified method (Prockop and Underfriend [1960]), intact parathyroid hormone (iPTH) was determined by N-terminal radioimmunoassay. Lysosomal enzyme β-D-glucuronidase was measured by standard assays.

Pyridinoline (Pyr) and deoxypyridinoline (D-Pyr) were measured in urine samples from overnight fasting rats. Urine was stored at –20°C with 3% hydrochloric acid. After acid hydrolysis of urine at 110°C overnight, partial purification on CFI cellulose column was performed. Pyr and D-Pyr were separated by reverse phase high performance liquid chromatography (HPLC) and detected by spectrophotometry.

Statistical analysis. The values were expressed as mean ± standard deviation (M ± SD). The differences between the groups were evaluated using Student’s t-test and ANOVA.

RESULTS

Table 1 demonstrates the levels of calcium in blood plasma and in liver tissue in animals from control and experimental groups. In group 2, calcium level was increased and significantly elevated after tamoxifen administration. In group 4 calcium level returned to normal one after administration of gallium nitrate therapy. Table 2 shows the activity of ALP in liver, kidney and plasma in control and experimental animals. In group 2 and also in flare hypercalcemic rats after tamoxifen therapy, the activity of ALP was significantly increased. After gallium nitrate treatment, level of ALP was decreased to nearly normal one. Table 3 depicts the level and activity of lysosomal enzyme β-D-glucuronidase in plasma, liver and kidney of control and experimental animals. The levels were dramatically increased in flare group, comparing to hypercalcemic group. After treatment with gallium nitrate, levels were return to normal. Levels were significantly elevated in both hypercalcemic and tamoxifen flare hypercalcemic groups. Table 4 indicates the level of blood parameters in plasma of control and experimental animal. In group 2 contents of urea, creatinine and uric acid levels were increased and significantly elevated after tamoxifen initiation. In group 4 levels of blood parameters returned to normal after administration of gallium nitrate therapy.
Table 5. Biochemical analysis of concentration of various biochemical parameters (mean ± STD) before and after gallium nitrate therapy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before therapy</th>
<th>After therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>13.0 ± 1.20</td>
<td>9.0 ± 0.85*</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.60 ± 0.40</td>
<td>3.85 ± 0.35*</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>2.85 ± 0.21</td>
<td>3.80 ± 0.35*</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>2.09 ± 0.20</td>
<td>1.08 ± 0.10</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP) (IU/ml)</td>
<td>788.60 ± 60.99</td>
<td>210.85 ± 21.12</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>9.85 ± 0.85</td>
<td>12.0 ± 1.05**</td>
</tr>
<tr>
<td>PTH (ng/ml)</td>
<td>20 ± 8.6</td>
<td>44 ± 20.5*</td>
</tr>
<tr>
<td>Calcium/creatinine ratio (mmol/mmol)</td>
<td>1.50 ± 0.38</td>
<td>1.05 ± 0.14*</td>
</tr>
<tr>
<td>Hydroxyproline (nmol/mmol.cre)</td>
<td>127 ± 58</td>
<td>42.0 ± 21.5*</td>
</tr>
<tr>
<td>Pyr (nmol/mmol.cre)</td>
<td>224 ± 68</td>
<td>58 ± 22*</td>
</tr>
<tr>
<td>D-Pyr (nmol/mmol.cre)</td>
<td>42 ± 13</td>
<td>13.5 ± 5.2*</td>
</tr>
</tbody>
</table>

Notes: Values are expressed as mean ± SD for 6 animals.

**Pared tested: *p < 0.05; **p < 0.01; ***p < 0.001

**DISCUSSION**

The life threatening metabolic complication of tamoxifen induced hypercalcemia (TIH), generally occurs within the first few weeks of initiation of tamoxifen therapy and is a sign of the worst prognosis in metastatic breast cancer within the short survival of approximately 3–3 months [15–16]. TIH may be due to a crucial agonist effect followed by an antagonist effect and also due to increased production of parathyroid Hormone — related protein (PTHrP) by tumor tissue which may be responsible for the development of hypercalcemia due to its iatrogenic effect of tamoxifen therapy [17]. The drug, gallium nitrate is a very effective and safe for use in TIH [18] and also more effective than bisphosphonate and calcitonin because of its dual effect of both anti-neoplastic as well as antihypercalcemic effect whereas calcitonin and bisphosphonate possess only antihyper-
calcemic effect. The renal toxicity is also manageable by decreasing the dosage of gallium nitrate.

The mechanism and action of gallium nitrate on bone remains unclear. Some of the mediators like TNFα, TGFβ, cytokines such as IL-1 and IL-6 have been identified as a stimulators which are released by tumor cells that may act to stimulate osteoclast mediated bone resorption resulting in hypercalcemia [19, 20]. Treatment with gallium nitrate enhances calcium phosphate content of bone without causing cytotoxic effect [21]. Some other data suggest that antiresorptive effect of gallium nitrate alter bone cells (osteoclast and osteoblast) function to promote or stabilize crystal structure yielding a matrix with more crystalline hydroxyapatite that has increased calcium phosphate content and reduced carbonate content [22].

Gallium nitrate preferentially accumulating in bone actively targets areas of bone and favourably alter mineral property to enhance hydroxyapatite crystallization and reduce mineral solubility. The mineral content, most likely can directly affect osteoclast function and possibly osteoblastic function. Short term treatment with gallium nitrate is associated with increased accumulation of new calcium into bone and an increase in bone calcium content and also increase the synthesis of bone collagen. Gallium replace calcium chemically in a large number of its insoluble from including hydroxyapatite. The presence of gallium within hydroxypatite can change the solubility of crystalloids, since devitalized bone power from gallium treated animals was found resistant to a monocyte cell line capable of resorbing bone [23–25]. Gallium nitrate acts on the cellular components of bone to reduce bone resorption by decreasing acid secretion by osteoclasts [26]. And also had markedly prolonged median survival in patients with hypercalcemia and suggests that gallium nitrate may have a positive, indirect on survival in patients by decreasing rate of bone resorption [27].

From the above study, gallium nitrate may be a valuable treatment for tamoxifen flare hypercalcemia. Gallium nitrate could make possible the safe readministration of tamoxifen after the flare effect, and is unique among the available antiresorptive or antihypercalcemic agent in mechanism of action. Result from these study shows that gallium nitrate is highly effective in the treatment of THM.

REFERENCES
ВЛИЯНИЕ НИТРАТА ГАЛЛИЯ НА ГИПЕРКАЛЬЦЕМИЮ, ИНДУЦИРОВАННУЮ ТАМОКСИФЕНОМ, У КРЫС С ОПУХОЛЬЮ МОЛОЧНОЙ ЖЕЛЕЗЫ

Цель: изучить влияние терапии нитратом галлия на острую гиперкальциемию у крыс с опухолью молочной железы и метастазами в кости скелета. Материалы и методы: в опытах использовали белых линейных крыс. Исследовано 5 групп животных: 1) контрольная; 2) животные-опухоленосители с гиперкальциемией; 3) животные-опухоленосители с острым гиперкальциемией (гиперкальциемические крысы, получавшие тамоксифен в дозе 10 мг/кг); 4) животные группы 3, получавшие нитрат галлия в дозе 2,5 мг/кг, 5) контрольная группа, получавшая нитрат галлия в дозе 2,5 мг/кг. Критериями оценки служил уровень кальция в сыворотке крови — 11,0 мг% и выше. Биохимические показатели измеряли стандартными методами. Содержание кальция в моче, показатель креатинина, показатель плотности костей по моче также оценивали стандартными методами. Результаты: после лечения нитратом галлия у животных с острым гиперкальциемией уровень кальция в крови возвращался к норме. У крыс с острый формой гиперкальциемии содержание кальция, алкилирующей фосфатазы, антигенов CA 15.3 и CEA в крови было значительно выше, чем таковые у животных с гиперкальциемией. Содержание пиридинолина, дезоксипиридинолина и гидроксипролина в моче у крыс с острым гиперкальциемией было также повышенным, в то время как содержание паратироидного гормона и альбумина было ниже у животных с гиперкальциемией и ее острым формой, чем таковые в контрольной группе. После лечения нитратом галлия все указанные параметры возвращались к норме. Выводы: применение нитрата галлия in vivo эффективно для лечения осторой гиперкальциемии. Ключевые слова: тамоксифен, нитрат галлия, ДМБА, гидроксипролин, β-глюкуронидаза, пиридинолин, дезоксипиридинолин.