

Gallium Nitrate Increases Type I Collagen and Fibronectin mRNA and Collagen Protein Levels in Bone and Fibroblast Cells

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Abstract Gallium is a Group IIIa transitional element with therapeutic efficacy in the treatment of metabolic bone disorders. Previously described antiresorptive effects of gallium on osteoclasts are not sufficient to account for the full range of effects of gallium on bone structure and metabolism. We have recently shown that gallium nitrate inhibits osteocalcin gene expression and the synthesis of osteocalcin protein, an osteoblast-specific bone matrix protein that is thought to serve as a signal to trigger osteoclastic resorption. Here we present evidence for an additional mechanism by which gallium may function to augment bone mass by altering matrix protein synthesis by osteoblastic and fibroblastic cells. Rat calvarial explants exposed to gallium nitrate for 48 h showed increased incorporation of ^3H -proline into hydroxyproline and collagenase digestible protein. In addition, gallium treatment increased steady-state mRNA levels for fibronectin and type I procollagen chains in primary rat calvarial osteoblast-enriched cultures, the ROS 17/2.8 osteoblastic osteosarcoma line, and nontransformed human dermal fibroblasts. These findings suggest that the exposure of mesenchymally-derived cells to gallium results in an altered pattern of matrix protein synthesis that would favor increased bone formation. © 1993 Wiley-Liss, Inc

Key words: calvarial cells, rat, fibroblasts, bone, metabolic bone disorders

Continuous remodeling of bone through resorption and formation enables the skeleton to maintain strength and to regenerate after injury. Small changes in modeling and remodeling activities by bone-forming osteoblasts and bone-resorbing osteoclasts can have significant functional consequences on skeletal integrity. Increased expression of selected bone matrix genes would be expected to favor bone formation.

Gallium nitrate is a recently described antiresorptive drug that is effective in the treatment of metabolic bone disorders characterized by accelerated bone resorption [Warrell and Bockman, 1989]. Clinical trials have led to the approval of gallium nitrate for the treatment of

cancer-related hypercalcemia, a life-threatening, metabolic disorder characterized by accelerated bone resorption [Warrell et al., 1984, 1987, 1988]. More recent clinical trials in patients with Paget's disease of bone demonstrated the efficacy of gallium nitrate to rapidly arrest disordered bone turnover [Matkovic et al., 1990; Warrell et al., 1990; Bockman et al., 1992]. Gallium nitrate has been shown to inhibit bone resorption in vitro and in vivo without cytotoxic effects on osteoblasts or osteoclasts [Bockman et al., 1986; Cournot-Witmer et al., 1987; Repo et al., 1988; Hall and Chambers, 1991; Schlesinger et al., 1991]. Gallium nitrate has also been shown to increase bone calcium and phosphorus incorporation and content in rats, suggesting that gallium can effect bone formation directly [Bockman et al., 1986; Repo et al., 1988]. Treatment of the osteoblastic osteosarcoma cell line ROS 17/2.8 with gallium nitrate decreased the synthesis of osteocalcin [Guidon et al., 1993], a noncollagenous bone matrix protein that may serve as a signal molecule for osteoclastic resorption [Malone et al., 1982; Mundy and Poser, 1983; Lian et al., 1984]. We previously reported preliminary results suggesting that treatment of ex-

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planted newborn rat calvariae with gallium increased collagen synthesis [Bockman et al., 1987]. In the present study, we describe several *in vitro* models of osteoblasts and one nonosteoblastic cell type, the dermal fibroblast in which gallium nitrate alters gene expression of type $\alpha 1(I)$ collagen and fibronectin in a manner consistent with new bone formation [Reynolds and Dingle, 1970; Kream et al., 1980; Lian et al., 1992].

MATERIALS AND METHODS

Calvarial Explant Cultures

Calvaria consisting of paired frontal and parietal bones were dissected from newborn Sprague-Dawley rats (Charles River, NY), cleansed of adhering membranes, and split along the sagittal midline. Each hemicalvarium was cultured on a stainless steel grid at the air-liquid interface in a 35-mm diameter dish containing 2 ml of medium. The culture medium consisted of 4 parts Dulbecco's Modified Eagle's Medium (DMEM) and 1 part RPMI-1640 (Gibco Laboratories, Gaithersburg, MD, #3201885 and #3201870, respectively) which was supplemented with non-essential amino acids (Gibco #3201140), 100 $\mu\text{g}/\text{ml}$ ascorbic acid and 10% fetal calf serum. After overnight equilibration, the medium was exchanged and test agents gallium nitrate or tumor necrosis factor- α (TNF α), a generous gift from Genentech (South San Francisco, CA), were added. Gallium nitrate (25 mg/ml) in 0.1 M sodium citrate [US 15001] was received from the Division of Cancer Treatment, National Cancer Institutes (Bethesda, MD), and diluted to 5 mM in sterile distilled water. A second calvarial culture system designed to maintain bone cell viability [Gronowicz et al., 1989] was utilized. In these experiments, 21 day fetal rat calvaria were exposed to 0 or 150 μM gallium in serum free medium supplemented with BSA, insulin, transferrin, selenium, and linoleic acid (BSA/ITSLA) [Gronowicz et al., 1989].

Cell Cultures

Primary osteoblast-enriched cell cultures were established from perinatal rat calvaria which were isolated as described for the explant cultures. Pooled bones were washed with phosphate-buffered saline (PBS), then subjected to sequential digestion with 2 mg/ml collagenase A (Boehringer-Mannheim #1088 785, Indianapolis,

IN) plus 2.5 mg/ml trypsin (Gibco #610-5090AG) in a 1:1 mixture of Minimal Essential Medium (MEM) and Dulbecco's phosphate buffered saline (PBS, Gibco #320-2561 and 310-4190, respectively) at 37°C on a rotary shaker at 150 rpm. Typically, 30–40 calvaria were digested using a volume of 10 ml. Cells released during the first 10–15 min of digestion were discarded; cells liberated during a second digest lasting 20–30 min were saved. The remnants of bone were minced with scissors, then subjected to a third digestion for 60 min. Cells released during the second and third digests were pooled and filtered through a sterile 70- μm mesh and washed to remove residual proteases prior to plating. Typically, $1\text{--}2 \times 10^6$ cells/calvarium were obtained, of which greater than 50% were histochemically positive for alkaline phosphatase when stained with Naphthol AS-MX phosphate plus Fast Red TR at pH 8.4 within 24 h of isolation. These cells were plated at a density of $0.6\text{--}1 \times 10^4/\text{cm}^2$ and grown to confluence in MEM with 10% FBS. Cultures were re-fed on the third day after plating and generally reached confluence by the fifth or sixth day. At this point, the cultures were fed with a differentiation-promoting medium consisting of BGJb (Fiton-Jackson modification, Sigma Chemical Company, St. Louis, MO) supplemented to a final concentration of 3 mM inorganic phosphate, 75 $\mu\text{g}/\text{ml}$ ascorbic acid, and 10% fetal calf serum (FCS).

The rat osteosarcoma cell line ROS 17/2.8 [Majeska et al. 1980] synthesizes osteoblast-specific proteins such as osteocalcin and responds to a variety of growth factors and calcitropic hormones such as transforming growth factor $\beta 1$ (TGF $\beta 1$), parathyroid hormone, and vitamin D $_3$ in an osteoblast-like manner. The ROS 17/2.8 cells were generously provided by Dr. R. Majeska (Mt. Sinai Medical College, New York, NY) and cultured as previously described [Guidon et al., 1993]. Transforming growth factor $\beta 1$ was obtained from the Sigma Chemical Company (St. Louis, MO).

Skin Fibroblasts

Human dermal fibroblasts derived from adult cadavers were generously provided by Dr. L. Staiano-Coico (Cornell University Medical College) and maintained in MEM supplemented with 10% FBS. Dermal fibroblasts and osteosarcoma cells were plated at a concentration of 30–40,000 cells/cm 2 and reached confluence

within 48 to 72 h. Cultures of fibroblasts and osteosarcoma cells were treated with test agents approximately 24 h after reaching confluence.

Collagen Synthesis

Collagen synthesis was measured in two ways; by [³H]-proline incorporation into collagenase digestible protein (CDP), and by following the conversion of the [³H]-proline to hydroxyproline. After a 12 h preincubation, the test substances gallium nitrate (50–100 μM) or TNFα (0.3 μM) were added. Twenty-four hours later, 10 μCi of [³H]-proline (112 Ci/mmol, NET #483, New England Nuclear Co., Boston, MA) was added to each culture plate containing a hemicalvarium. Twenty-four hours later (i.e., 48 h after the addition of test agents), the explants were washed extensively with cold phosphate-buffered saline (PBS), dehydrated with 100% ethanol followed by diethyl ether, then dried overnight at 70°C. Each hemicalvarium was weighed, rehydrated, and digested with 27 μg of highly purified collagenase (EC3.4.24.3, Type VII collagenase containing less than 0.005 units of tryptic activity, Sigma Chemical Co., St Louis, MO) to yield soluble collagen-derived peptides containing ([³H]-prolyl and [³H]-hydroxyprolyl residues derived from the incorporation of [³H]-proline. The soluble radioactivity in an aliquot of the digest was counted in a Packard Model 3255 Tri-Carb liquid scintillation counter and expressed as counts per minute (cpm) per mg dry weight for each bone. Most of the remaining solubilized material was hydrolyzed in 6N HCL at 120°C for 18 h. The acid hydrolysate containing free amino acids was loaded onto a Dowex-50 column in order to separate hydroxyproline from proline [Firschein and Alcock, 1969], and radioactivity that eluted in the hydroxyproline fraction was determined. Proline incorporation into CDP for the calvaria that were cultured in the BSA/ITSLA media was measured after a shorter incubation time. After 48 h the medium in these cultures was replaced with medium containing 0.2 mM proline and 4 mCi ³H-proline for 4 h. The calvaria were subsequently digested with purified collagenase for 3 h and the ³H-proline released was counted in a Packard Tri-Carb β-scintillation counter as described above.

RNA Isolation and Analysis

Total RNA was isolated from ROS 17/2.8 and human fibroblasts by a modification of the Pep-

pel/Baglioni procedure [Peppel and Baglioni, 1990] developed in our laboratory [Salvatori et al., 1992]. Due to the extensive collagenous matrix produced by calvarial cell cultures, isolation of RNA required lysis in 4 M guanidinium thiocyanate followed by acid-phenol extraction and isopropanol precipitation [Chomczynski and Sacchi, 1987]. This RNA-containing pellet was further purified using the modified Peppel/Baglioni method. Equivalent amounts of total RNA were size-separated on 1% agarose/MOPS gels containing 2.2 M formaldehyde, transferred to nylon membranes (Duralon-UV, Stratagene) by capillary blotting in 10× SSPE (1× SSPE = 0.15 M sodium chloride, 10 mM sodium phosphate, 1 mM EDTA, pH = 7.4), and UV-crosslinked. Ethidium bromide (50 μg/ml) was added to each sample prior to loading and gels were photographed using a 254-nm UV light source so that relative levels of RNA in each lane could be estimated by densitometric analysis of ribosomal RNA bands on the negative image. Blots were prehybridized for at least 4 h at 42°C in 50% formamide, 5× SSPE, 5× Denhardt's solution, 0.1–1% SDS, 0.1 mg/ml denatured, sheared salmon sperm DNA, then hybridized with random-primed ³²P-labeled probe (10⁶ cpm/ml) under the same conditions for at least 14 h. Following hybridization, blots were washed with 2× SSPE/0.5% SDS for 15 min at room temperature followed by 0.1–1× SSPE/0.1% SDS for 30–60 min at 60°C, then exposed to Kodak XAR-5 film with intensifying screens at –70°C. All densitometric analyses were carried out using a Molecular Dynamics 300 Series computing laser densitometer running Imagequant, version 3.0 software.

cDNA Probes

The following restriction fragments were labeled by incorporation of ³²P-dCTP using the random primer method [Feinberg and Vogelstein, 1983]: the 1.3-kb Pst 1-BamH 1 fragment derived from rat α1(I) procollagen cDNA clone pα1R1 [Genovese et al., 1984], the 0.5-kb EcoR 1 fragment from rat osteocalcin cDNA clone pR22-11 [Celeste et al., 1986], the 0.55-kb EcoR1 insert from rat fibronectin cDNA clone p-rlf-1 [Schwartzbauer et al., 1983], and the 0.5-kb EcoR1 insert from human skeletal actin cDNA subclone pHMαA-PX [Gunning et al., 1983] which recognizes all vertebrate actin transcripts.

TABLE I. Effect of Gallium or TNF α on Collagen Synthesis in Explanted Rat Calvariae*

Treatment	CDP	OH-Pro
Control	40,457 \pm 12,680	5,498 \pm 1,924
TNF	29,466 \pm 11,852	3,177 \pm 1,630
Ga(NO ₃) ₃		
50 μ M	61,845 \pm 26,076	10,373 \pm 2,916
100 μ M	76,450 \pm 33,210	12,763 \pm 5,250

*After a 12-h preincubation, explanted hemicalvaria from newborn rats were treated with 50 or 100 μ M gallium nitrate or 0.3 μ M TNF α for 24 h, then [³H]-proline was added. After an additional 24 h (i.e., 48 h total incubation with test agents), the incorporation of [³H]-proline into collagenase digestible protein and its conversion to OH-Pro were determined. Values are the mean cpm/mg dry calvaria weight \pm standard deviation for N = 4 individual hemicalvaria per treatment group. Only the OH-Pro value for the 100 μ M gallium group differed significantly compared to controls, $P < 0.05$ by the Student's two-tailed t -test or by a one way analysis of variance.

RESULTS

Collagen Synthesis

The uptake of ³H-proline into collagenase digestible protein (CDP) and the conversion of ³H-proline to ³H-hydroxyproline (OH-Pro) in newborn rat calvaria was measured. As seen in Table I, treatment with gallium nitrate at both doses tested caused a 1.5–2.3-fold increase of proline incorporation and conversion to hydroxyproline, results consistent with increased collagen formation. Treatment with TNF α reduced the level of proline incorporation into newly formed collagen as well as the conversion to hydroxyproline as expected and served as a negative control to demonstrate the hormone-responsiveness of the cultures [Bertolini et al., 1986; Centrella et al., 1988; Solis-Herruzo et al., 1988]. In separate experiments calvaria cultured in defined media and pulse-labeled with ³H-proline for 4 h, gallium treatment also resulted in an increase of ³H-proline incorporation into CDP in the absence (857 \pm 310 vs. 1,114 \pm 594 CPM/mg calvaria) or presence of BSA/ITSLA (1,120 \pm 478 vs. 1,492 \pm 742 CPM/mg).

Effect of Gallium on Osteoblastic Cells

Increased collagen synthesis by gallium-treated rat calvaria suggested that gallium might act directly on osteoblasts at the level of gene expression. Calvarial cell cultures, enriched for osteoblasts by sequential enzymatic digestion,

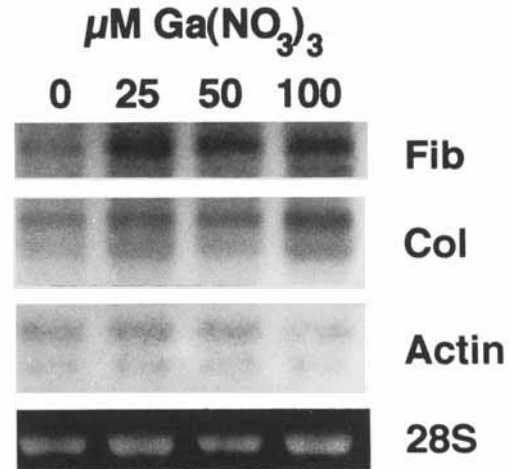


Fig. 1. Effect of gallium nitrate on matrix protein mRNA levels in 10-day postconfluent osteoblast-enriched primary cell cultures. Cell cultures were exposed to 0–100 μ M gallium nitrate for 48 h immediately prior to being harvested. A Northern blot with 5 μ g total RNA per lane was hybridized sequentially to α 1(I) procollagen, fibronectin and actin cDNA probes. The sizes of transcripts specifically hybridized by each probe were as follows: approximately 6.5 kb by fibronectin, 4.7 kb and 5.7 kb by α 1(I) procollagen [Genovese et al., 1984], 2.0 kb (β and γ isoforms) and 1.7 kb (α -like isoforms) by actin [Ponte et al., 1983]. Ethidium bromide fluorescence of the 28S ribosomal RNA is shown in the bottom panel. The relative sizes of the hybridization bands were 28, 18, and 18S, respectively.

were used to examine the effect of gallium on gene expression in bone-forming cells. These cultures undergo a temporal sequence of phenotypic changes that mimics intramembranous bone formation [reviewed in Owen et al., 1990; Lian et al., 1992]. Cultures acquired a progressively more differentiated character which included the induction of osteocalcin and osteopontin mRNAs beginning approximately 7–10 days postconfluence. Morphological maturation was manifested by the development of multilayered, alkaline phosphatase-rich nodules containing an extensive collagenous matrix that eventually mineralized when provided with physiologic levels of inorganic phosphate.

The ability of calvaria-derived cells to respond to a 48 h treatment with gallium was restricted to a discrete temporal period in our culture system. This is illustrated by a representative Northern blot (Fig. 1) and the graph showing data compiled from three independent experiments (Fig. 2). Gallium had no effect on any of the mRNAs examined up to day 6 post-confluence. Cultures exposed to gallium beginning on day 8 and harvested on day 10 showed a statistically significant increase in both 4.7 and 5.7-kb

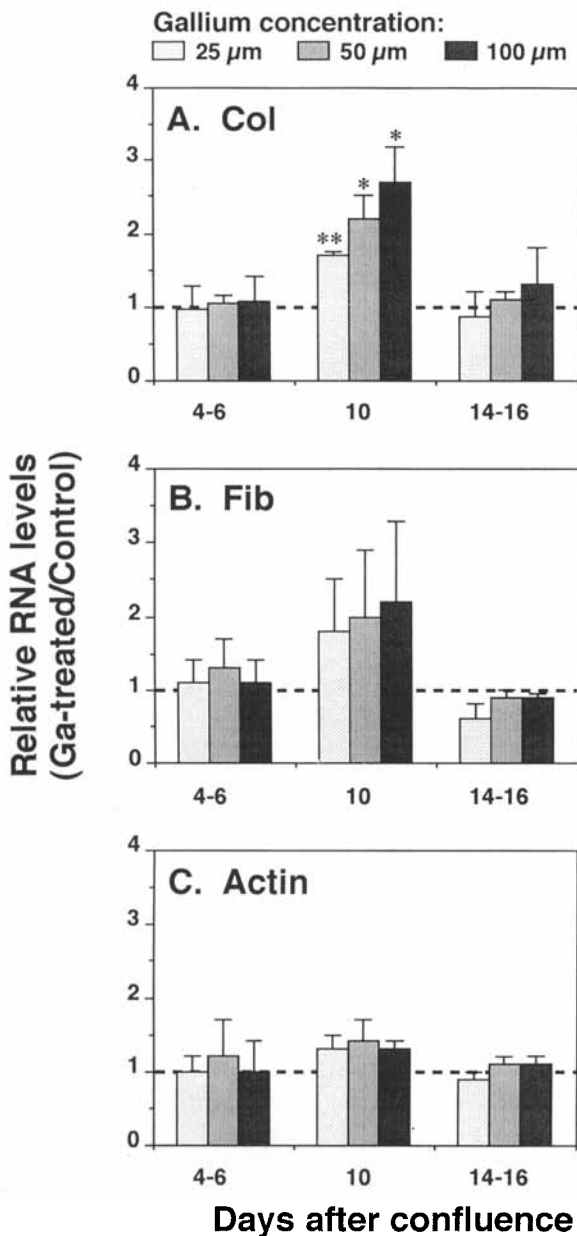


Fig. 2. Response of calvarial cells to gallium nitrate treatment at different stages in culture. Cultures were treated with 0, 25, 50, or 100 μM gallium nitrate for 48 h immediately prior to harvest on the indicated day (4, 6, 10, 14, or 16) after confluence. Relative mRNA levels for $\alpha 1(\text{I})$ procollagen (A), fibronectin (B), and actins (C) were quantified from Northern blots using densitometric analysis as described in Materials and Methods. Sizes of RNA bands analyzed were given in the legend to Figure 1. Multiple RNA samples were isolated and analyzed for each experimental condition and the data from 3 independent cell preparations were compiled. Error bars indicate standard deviations, ($n = 3$). The difference between each gallium-treated group and the untreated control at each culture stage was analyzed using a two-tailed Student's t -test (* $P < 0.05$; ** $P < 0.002$).

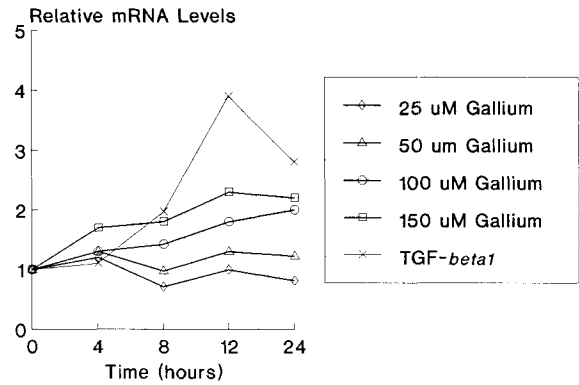


Fig. 3. Time and dose dependence of collagen mRNA levels in ROS 17/2.8 cells treated with gallium nitrate or TGF $\beta 1$. Confluent cultures were treated with 25–150 μM gallium nitrate or 200 pM TGF $\beta 1$ for 0, 4, 8, 12, or 24 h. Northern blots were run using 3–5 μg total RNA per lane. Densitometric analysis of $\alpha 1(\text{I})$ procollagen mRNA levels were normalized to the 28S ribosomal RNA bands as described in Materials and Methods.

classes of $\alpha 1(\text{I})$ procollagen transcripts. By this stage, a large proportion of cells had coalesced into dense clusters that were actively synthesizing collagenous matrix based on toluidine-blue staining (not shown). A small dose-dependent elevation in 6.5-kb fibronectin transcripts on day 10 was observed in all three experiments, although the differences were not significant due to high inter-experiment variability. No increase in procollagen or fibronectin mRNA was induced by gallium at day 14 or 16, although the steady-state levels of these mRNA species remained high. This stimulatory effect of gallium was not a generalized phenomenon since the yields of total RNA from treated and untreated cultures was comparable and neither size class of actin transcripts expressed by fetal calvarial cells responded to gallium at any stage tested. The effect of gallium on the mRNA profiles of calvarial cells was quantitative rather than qualitative, as we did not detect any difference in the sizes of specific transcripts between gallium-treated and control cultures.

Time Course and Dose-Dependence of the Gallium Response

The time course and dose dependence of procollagen mRNA accumulation was examined in greater detail using the osteoblastic osteosarcoma cell line ROS 17/2.8 (Fig. 3). At all effective concentrations of gallium (50–150 μM), procollagen mRNA levels were detectably elevated after as little as 4 h and peaked by 12–24 h. The magnitude and dose-dependence of the response

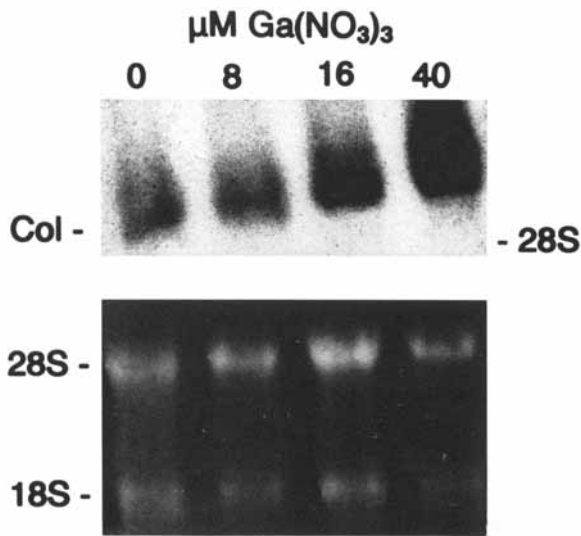


Fig. 4. The effect of gallium nitrate on $\alpha 1(I)$ procollagen mRNA levels in human dermal fibroblasts. Confluent cultures of nontransformed human dermal fibroblasts were treated with 0–40 μM gallium nitrate for 24 h. Total RNA was subjected to Northern blot analysis as described above. The ethidium bromide-stained ribosomal RNA bands demonstrate that approximately equal amounts of RNA were loaded in each lane.

in the ROS 17/2.8 cell line was comparable to the 48-h response of primary calvarial osteoblasts. In contrast, treatment with 200 pM TGF β 1, a physiologic mediator which is known to stimulate collagen synthesis [Centrella et al., 1987], exhibited a distinct kinetic profile: stimulation was first detected at 8 h, reached a transient peak at 12 h, and declined somewhat by 24 h.

Effect of Gallium on Human, Nontransformed Fibroblasts

To determine whether the stimulatory effect of gallium nitrate on type $\alpha 1(I)$ collagen and fibronectin mRNA levels was limited to osteoblast cells or was a more general property of mesenchymally derived cells, confluent monolayers of nontransformed human dermal fibroblasts were treated with various doses of gallium for 24 h. Messenger RNA levels for type $\alpha 1(I)$ collagen were increased by 16–40 μM gallium (Fig. 4). The observed increase in fibroblast procollagen mRNA levels was similar in magnitude to that seen with 25–150 μM doses of gallium in the osteoblast cultures. A similar increase in fibronectin mRNA levels was also seen in response to treatment with gallium nitrate (data not shown).

DISCUSSION

The data presented here demonstrate that treatment of mesenchymally derived cells with gallium nitrate can increase the rate of collagen synthesis and steady-state matrix protein mRNA levels in these cells. Specifically, ROS 17/2.8 osteosarcoma cells, primary rat osteoblasts, and nontransformed human fibroblasts responded to exposure of gallium nitrate with increased levels of $\alpha 1(I)$ collagen and fibronectin mRNAs. In addition, explanted newborn rat calvaria exposed to similar, noncytotoxic doses of gallium nitrate demonstrated an increase in ^3H -proline incorporation into collagenase digestible protein and hydroxyproline, signifying an increase in collagen synthesis.

The reported effects of gallium on bone formation are varied. Explanted mouse calvaria treated with 12–20 μM gallium for 96 h showed a 50% decrease in the rate of collagen synthesis when pulse-labeled during the last 2 h of culture [Lakatos et al., 1992]. The difference between these results and ours may reflect species differences in responsiveness, duration of treatment, or other experimental parameters. An *in vivo* study of 8-week-old rats treated with 0.9 mg/kg elemental gallium showed decreased histomorphometric parameters of bone formation [Wakley et al., 1992]; however, these authors have subsequently questioned the suitability of their own model for histomorphometric analysis after finding a similar decline in bone formation indices following treatment with estrogen [Turner et al., 1992]. Jenis and co-workers recently reported a small stimulatory effect of 50 μM gallium on ROS 17/2.8 that concurs with our present findings, but failed to detect changes in Type I procollagen mRNA levels in the femurs of 10-week-old rats treated with 0.5 mg/kg gallium nitrate for 3 weeks [Jenis et al., 1992]. It should be noted that the dose of gallium used by Jenis and co-workers was only one-tenth of the dosage used in earlier studies which showed an increase in bone mineral, hydroxyapatite crystal size, and perfection after 2 weeks in rats of a comparable age [Bockman et al., 1986; Repo et al., 1988]. Direct measurement of collagen protein, synthetic rates, or mRNA levels have not been performed after *in vivo* therapy with the higher dosages of gallium that have been shown to augment bone mineral levels.

The critical cellular target for gallium in the osteogenic lineage may reflect a particular stage

of differentiation. With respect to fibronectin and Type I collagen mRNA levels, only calvarial cell cultures that were actively laying down collagenous matrix (circa day 10 post-confluence) showed a significant net response to gallium. The failure of cells to respond before day 6 may be interpreted as the nonresponsiveness of immature pre-osteoblasts which are morphologically and metabolically distinct from more differentiated osteoblasts. Up to day 6 post-confluence, cultures were composed predominantly of flattened cells with little accumulated matrix, and mRNA for osteocalcin and osteopontin was barely detectable by Northern blotting. Older cultures (beyond day 12) appeared more heterogeneous with respect to cell type or differentiated state, containing mature osteoblasts in nodules and immature cells in the internodular regions. Cells at both stages as well as intermediate phenotypes probably contain fibronectin and procollagen mRNA, but may be differentially responsive to gallium so that mass biochemical measurements are difficult to interpret. Since the clonal osteoblast-like cell line ROS 17/2.8 responds to gallium with an increase in $\alpha 1(1)$ procollagen transcripts [Jenis et al., 1992; and the present study] and a concomitant decrease in osteocalcin synthesis, it may provide a particularly useful model for exploring the molecular basis for of gallium action on bone [Guidon et al., 1993]. The difference between the kinetic profile for gallium and TGF- $\beta 1$ elevation of $\alpha 1(1)$ procollagen mRNA suggests that these two effectors (one pharmacologic and one physiologic) utilize distinct intracellular signalling pathways, an area we are currently exploring.

To date, many of the studies concerning the outcome of different effector treatments on collagen synthesis have utilized tissues and cells derived from fetal, newborn, or juvenile animals. In these model systems, collagen synthesis would be expected to be at maximal or near maximal rates, since these animals are actively growing. In diseases such as osteoporosis, however, osteoblast synthesis of new bone matrix components such as collagen is markedly reduced in both older animals and humans. Therefore, we are currently investigating whether gallium nitrate can effect collagen synthesis in trabecular cells from 2-year-old rats, which would appear to be a more appropriate model system in which to examine the potential therapeutic benefits of this drug. Finally, the effect of gallium on $\alpha 1(1)$ procollagen mRNA levels in

dermal fibroblasts suggests gallium may affect Type I collagen synthesis by connective tissues other than bone and suggests that gallium therapy may have additional clinical applications.

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