ROLE OF PENTOXIFYLLINE IN PREVENTING RADIATION DAMAGE TO EPIPHYSEAL GROWTH PLATE CHONDROCYTES

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INTRODUCTION

Multimodality therapy including surgery, chemotherapy and radiation, has become the standard of care for a number of life-threatening childhood malignancies. A severe, dose-limiting complication of radiation therapy to the adolescent skeleton is premature closure of the active growth plate and subsequent defects in bone formation and growth. The radiosensitivity of proliferating growth plate chondrocytes has been well documented in both animal studies and in pediatric patients with abnormal endochondral ossification and skeletal development and limb shortening following radiotherapy.

At the ORS meeting in 2001 (and recently published) we used a well established in vitro adolescent chick growth plate chondrocyte (GPC) model to demonstrate that radiation selectively inhibits mitogenic factors without affecting protein or proteoglycan synthesis. In particular, irradiation of GPC led to a decrease in PTHrP and PTHrP receptor mRNA levels without affecting other mitogenic growth factors (FGF-2 and TGF-β isoforms). We also demonstrated a decrease in bcl-2 mRNA and a significant increase in caspase 3 activity, thus suggesting increased apoptosis in irradiated GPC. Additionally, our study demonstrated that the rise in cytosolic calcium levels following irradiation was probably responsible for the decrease in PTHrP and increase in apoptosis. Treating GPC with EGTA (a calcium chelator) before irradiation prevented the post-irradiation decrease in cytosolic calcium and the subsequent decrease in PTHrP mRNA and apoptosis, thus suggesting that preventing the post-irradiation increase in cytosolic calcium levels may curtail the deleterious effects of radiation on GPC.

The aim of the current study was to find a non-toxic pharmacologic agent that could lower cytosolic calcium levels and prevent the radiation associated decrease in PTHrP mRNA synthesis. We investigate the cytosolic calcium lowering effect of pentoxifylline on irradiated chick GPC and its effectiveness in preventing the radiation-induced decrease in proliferation and increase in apoptosis.

METHODS

GPC were isolated from 4-6 week old chicks by sequential enzymatic digestion, irradiated 2-3 hours after monolayer plating, and maintained in short term monolayer cultures. Proliferative activity was examined by thymidine assays. RNA was isolated using TRIzol reagent (Gibco BRL) and used for northern analysis or reverse transcribed for competitive PCR for determination of PTHrP levels. Northern blots were performed for bcl-2, PTHrP receptor, and TGF-β. Cytosolic calcium levels were measured using fura-2 and digital fluorescence microscopy.

RESULTS

Proliferation of chondrocytes, as measured by 3H-thymidine, was inhibited by seventy percent 24-h after irradiation. Given the known mitogenicity of PTHrP, treating chondrocytes with it caused a 5-fold increase in proliferation compared to untreated controls. Addition of exogenous PTHrP prior to irradiation diminished the inhibitory effect of irradiation and caused a 3-fold increase when compared to untreated controls. Treatment of chondrocytes with pentoxifylline caused a 2-fold increase in proliferation. Addition of exogenous pentoxifylline prior to irradiation did not prevent the radiation associated decrease in proliferation, but it did maintain proliferation at the same rate as untreated controls.

Consistent with previous results, an approximate 25-fold decrease in PTHrP mRNA levels was seen in chondrocytes 24-h after irradiation (Fig. 1). Because previous work has shown the importance of cytosolic calcium in regulating PTHrP mRNA levels, the cytosolic calcium lowering agent pentoxifylline was used to treat chondrocytes before irradiation. Treating chondrocytes with exogenous pentoxifylline increased the basal expression of PTHrP mRNA levels by approximately 7-fold. Irradiating the pentoxifylline treated chondrocytes caused an approximate 50% decrease in PTHrP mRNA levels compared to unirradiated pentoxifylline treated chondrocytes (Fig. 1). Interestingly, PTHrP mRNA levels in pentoxifylline treated irradiated chondrocytes were 3-fold higher than untreated controls and approximately 35-fold higher than irradiated chondrocytes that were not treated with pentoxifylline.

Cytosolic calcium levels were measured in chondrocytes 24-h after irradiation (Fig. 2). Irradiated chondrocytes demonstrated an approximate 20 nM increase in cytosolic calcium levels. In contrast, chondrocytes treated with pentoxifylline had an approximate 25 nM decrease in cytosolic calcium levels compared to unirradiated controls. Treating chondrocytes with exogenous pentoxifylline before irradiation did not prevent the radiation-induced increase in cytosolic calcium and resulted in an approximate 25 nM increase when compared to pentoxifylline treated chondrocytes. However, the intracellular calcium concentration in pentoxifylline treated irradiated chondrocytes (117 nM) was similar to untreated chondrocytes (120 nM). These data suggest that pentoxifylline’s effects on PTHrP mRNA expression might be mediated through its effects on cytosolic calcium levels.

bcl-2 mRNA levels were analyzed to examine apoptotic activity in irradiated chondrocytes. Treating chondrocytes with pentoxifylline caused an increase in bcl-2 mRNA levels when compared to untreated controls. Consistent with previous results, irradiation caused a dose-dependent decrease in bcl-2 mRNA levels. However, treating chondrocytes with exogenous pentoxifylline prior to irradiation prevented the decrease in bcl-2. These results suggest that pentoxifylline not only lowers the basal apoptotic activity in GPC, but also lowers the radiation associated apoptosis in GPC.

Pentoxifylline also lowered the basal expression of PTHrP receptor mRNA levels compared to untreated controls. Consistent with previous results, there was a dose-dependent decrease in PTHrP receptor in irradiated chondrocytes. However, chondrocytes treated with exogenous pentoxifylline before irradiation did not demonstrate a decrease in PTHrP receptor mRNA levels. To determine whether the effects of pentoxifylline receptor mRNA levels were specific for the PTHrP pathway, we also examined TGF-β1 mRNA levels in irradiated chondrocytes and those treated with exogenous pentoxifylline prior to irradiation. Pentoxifylline treatment did not alter basal TGF-β1 mRNA levels or in irradiated GPC when compared untreated chondrocytes.

DISCUSSION

Pentoxifylline prevents the radiation induced increase in cytosolic calcium, and the subsequent decrease in PTHrP mRNA and GPC apoptosis. Given the importance of PTHrP in endochondral ossification, pentoxifylline may prove to be an effective pharmacologic agent to help minimize and prevent the deleterious effect of irradiation on the adolescent growth plate.

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