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High xylosyltransferase activity in children and during mineralization of osteoblast-like SAOS-2 cells

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Abstract Skeletal growth and tissue remodelling processes are characterized by an elevated collagen and proteoglycan biosynthesis. The xylosyltransferases I and II are the rate-limiting step enzymes in proteoglycan biosynthesis and serum xylosyltransferase (XT) activity has been shown to be a biomarker for the actual proteoglycan biosynthesis rate. Here, XT, alkaline phosphatase (ALP), bone ALP (BALP) activities were measured in 133 juvenile Caucasians. Serum XT activities in juveniles were elevated and significantly correlated with ALP and BALP. In an osteoblast-like cell model using SAOS-2 cells mineralization and bone nodule formation were induced and XT-I, XT-II and ALP were monitored. Induction of mineralization in SAOS-2 cells resulted in a long-term increase of XT-I mRNA and enzyme activity, which could be paralleled with elevated ALP activity. In addition, HGH and IGF-I treatment of SAOS-2 cells led to an increased expression of XT-I and ALP. These results point to skeletal growth and tissue remodeling as a cause of the high XT activity in children.

Keywords Xylosyltransferase · Alkaline phosphatase · Osteogenesis · Proteoglycan · Glycosaminoglycans

Abbreviations

ALP	alkaline phosphatase
BALP	bone alkaline phosphatase
IGF-I	insulin-like growth factor I
HGH	human growth hormone
XT-I	xylosyltransferase I
XT-II	xylosyltransferase II
XT	xylosyltransferase

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Introduction

Growth-related processes essentially include the formation and remodeling of extracellular matrix in bones and other organ systems. Bone metabolism includes the coordinated action of osteoblast and osteoclast activity, which is regulated by hormones as well as by locally acting factors, which control cell proliferation and differentiation of progenitor cells. Many of the regulatory cytokines involved in these processes, like the fibroblast growth factors, TGF-β_1 or members of the bone morphogenic protein family, are heparin-binding growth factors, which interact with heparin and cell surface heparan sulfate chains [1]. Heparan sulfate proteoglycans are synthesized by virtually every animal cell and are crucially involved in many biological processes. They mediate diverse cellular functions through interaction with a variety of protein ligands. In most of these bindings electrostatic interactions with the glycosaminoglycan chains attached to the core protein are involved [2]. Thus, the biological activity of proteoglycans is intimately related to glycosaminoglycan biosynthesis.

In bone metabolism proteoglycans contribute to the compressive strength of skeletal bones and play a role as early placeholders for bone development [3]. Besides their ability to bind cytokines and growth factors and to regulate the local availability of these effector molecules, proteoglycans have been shown to stabilize collagen-fibrils in the extracellular matrix and to orientate fibrillogenesis [4, 5]. The importance of proteoglycans for bone metabolism is supported by observations that genetic defects in proteoglycan core proteins or in other enzymes involved in the biosynthesis

of lateral glycosaminoglycan chains result in pronounced phenotypes with severe skeletal impairment [6, 7].

Proteoglycans consist of a core protein which is posttranslationally modified by the addition of glycosaminoglycan chains. The polyanionic glycosaminoglycans chondroitin sulfate, heparan sulfate, heparin and dermatan sulfate are bound to the proteoglycan core protein by a xylose–galactose–galactose binding region. The xylosyltransferases I and II (XT-I, XT-II, EC 2.4.2.26) are the chain-initiating enzymes involved in the biosynthesis of glycosaminoglycan-containing proteoglycans [8]. The enzymes catalyze the transfer of D-xylose from UDP-D-xylose to specific serine residues of the core protein and are regulatory factors in glycosaminoglycan biosynthesis [9]. XT activity was found to be present in the early Golgi compartments [10], and we have shown that the enzyme is secreted from the Golgi apparatus into the extracellular space attached to large proteoglycans [11]. Consequently, XT activity present in the peripheral blood was proposed to be a marker for the determination of an enhanced proteoglycan biosynthesis rate [11, 12].

Osteoblast cell models are useful for the analysis of cytokine effects on bone mineralization and formation. The SAOS-2 osteosarcoma cell line is a widely-spread model of osteoblastic function [13]. These cells have multiple osteoblastic features as they express ALP, parathyroid hormone-linked adenylate cyclase, osteonectin, 1,25-dihydroxyvitamin D_3 and exhibit osteogenic properties [14, 15]. SAOS-2 cells have been shown to synthesize a prominent extracellular matrix and to be capable of mineralization in the presence of exogenous phosphate donors [13]. This process is regulated by a variety of growth factors and cytokines including insulin-like growth factor I (IGF-I) and human growth hormone (HGH).

In the present study we investigated XT, total ALP and BALP activity in serum samples of children and found high XT, ALP and BALP levels. In order to give a first insight in the molecular mechanisms underlying this correlation of the proteoglycan biosynthesis marker XT and the bone marker ALP we analyzed β-glycerophosphate and sodium ascorbate-induced mineralisation in the osteoblast-like SAOS-2 cells. A long-term induction of XT-I expression could be paralleled with ALP activity and bone nodule formation.

Material and methods

Patient characteristics

The study cohort comprised 133 Caucasian patients of German origin (75 males; age, 9.2±6.3) who attended the outpatient clinic. 24 of these young patients suffered from diabetes mellitus type 1 (15 males; age 14.6±5.3) and 109 were cardiac patients (60 males; age 7.9±5.9) with congenital heart defects (tetralogy of Fallot, ventricular septal defect, atrial septal defect; open ductus arteriosus Botalli, heart dysplasia; hypoplastic left heart syndrome; pulmonary atresia; n=67), heart transplantation (n=18), congenital valve defects (n=14), arrhythmia (n=6) and dilative cardiomyopathy (n=4). As these diseases are known to be not primarily associated with a highly altered proteoglycan metabolism or prominent bone remodeling these patients were both analysed in subgroups and a combined study cohort. Furthermore, all patients were without any apparent signs of fibrosis or sclerosis. Furthermore, all tested negative for antibodies against hepatitis C virus, thereby excluding an increased serum XT activity caused by hepatitis C virus-induced liver fibrosis. In order to exclude elevated serum ALP levels due to excessive liver damage, only samples with normal or moderately elevated levels of the liver enzymes alanine aminotransferase, aspartate aminotransferase or gamma-glutamyl transferase were included in our study. Serum samples from 363 blood donors (203 males, aged 18–60, 160 females, aged 18–60) were used as normal controls. All specimens used in our study were reserve materials, which were not needed for any other diagnostic procedure. No extra material or elevated sample volume was obtained from the patients. All patient materials were completely anonymized before being entered in the study procedures. The study was approved by the institutional review board.

Collection of serum samples

Venous blood samples were collected in serum monovettes (Sarstedt, Nümbrecht, Germany). After clotting and centrifugation at 4,000 g for 15 min the serum was stored at −70°C until assayed as described below.

Determination of serum parameters

Alkaline phosphatase (ALP) was determined using the Architect ci8200 analyzer (Abbott, Wiesbaden, Germany). Bone alkaline phosphatase (BALP) was quantified using the METRA BAP kit from Quidel (Heidelberg, Germany) as recommended by the manufacturer. Alkaline phosphatase isoenzyme electrophoresis was performed using the ISOPAL AP isoenzyme electrophoresis kit for the Paragon electrophoresis system (Beckman Coulter, Krefeld, Germany) according to the manufacturer's instructions.

XT activity assay

The method for determination of XT activity is based on the incorporation of [^{14}C]-D-xylose with recombinant bikunin as the acceptor. The reaction mixture for the assay contained a total volume of 100 µl: 50 µl of serum or cell

culture supernatant, 25 mM 4-morpholineethanesulfonic acid (pH 6.5), 25 mM KCl, 5 mM KF, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 1.0 μM UDP-[^{14}C]-D-xylose (Du Pont, Homburg, Germany) and 1.5 μM recombinant bikunin. After incubation for 1.25 h at 37°C, the reaction mixtures were placed on nitrocellulose discs. After drying, the discs were washed for 10 min with 10% trichloroacetic acid and three times with 5% trichloroacetic acid solution. Incorporated radioactivity was quantified after the addition of 5 ml of scintillation mixture (Beckman Coulter, Fullerton, CA) using a LS500TD liquid scintillation counter (Beckman Coulter). The enzyme activity was expressed in units (1 unit=1 μmol of incorporated xylose min^{-1}).

In vitro calcification of SAOS-2 cells

SAOS-2 cells were cultured in DMEM (Cambrex, St. Katharinen, Germany) containing 1 mmol/l sodium pyruvate supplemented with 10% FCS (Biowest, Nuaillé, France). After confluence, the medium was changed and replaced with DMEM containing 1 mmol/l sodium pyruvate supplemented with 10% FCS in the presence of 10 mmol/l β-glycerophosphate (Sigma-Aldrich, St. Louis, USA) and 50 μg/ml of ascorbic acid (Sigma) for 14 days. Thereby, the medium was replaced with fresh medium every 2 days.

Histological staining

Mineralization was confirmed directly by von Kossa staining using 5% silver nitrate solution (Sigma).

Effect of cytokines on the XT-I and XT-II expression in SAOS-2 cells

SAOS-2 cells were cultured in six-well plates in 2 ml DMEM with 10% FCS. When cells reached subconfluence, the medium was changed to FCS-free DMEM and preincubated for 12 h. HGH and IGF-I (Strathmann Biotec, Hamburg, Germany) were than added and incubated for 48 h. SAOS-2 cells were supplemented with HGH at a dosage of 25 to 50 ng/ml. IGF-I was used in a final concentration of 10 to 25 ng/ml.

RNA extraction

The total RNA was isolated using a commercial kit with additional on-column DNase I treatment for removing contaminating genomic DNA according to the manufacturer's recommendations (Qiagen, Hilden, Germany) and stored at −80°C.

LightCycler real-time quantitative RT-PCR analysis

The mRNA expression of XT-I and XT-II was analyzed by a fluorogenic RT-PCR assay using the LightCycler System (Roche, Mannheim, Germany). The PCR reaction for the mRNA quantification was performed using a SYBR green Taq-DNA polymerase mixture (Platinum SYBR Green qPCR SuperMix-UDG, Invitrogen, Karlsruhe, Germany). Thermal cycling conditions included enzymatic degradation of uracil-containing DNA at 50°C for 2 min, activation of the DNA polymerase at 95°C for 2 min, followed by 45 cycles at 94°C for 5 s, at 58°C for 15 s and at 72°C for 15 s. The transcriptional levels of all target genes were normalized to constant mRNA levels of ubiquitin. Primers used for the specific amplification were described previously [16].

Statistical analysis

Statistical analysis was performed using the *t*-test and the Kolmogoroff–Smirnoff test where appropriate. Normality testing for Gaussian distribution of values was performed using the *F*-test. ANOVA was used to compare multiple

Table 1 Serum levels of XT, alkaline phosphatase and bone alkaline phosphatase

Age group	*n*	XT activity (mU/l)	ALP activity (U/l)	BALP activity (U/l)	BALP/ALP ratio
Male					
0–5 years	26	1.34±0.22	172.2±76.7	99.6±53.4	0.56±0.17
6–10 years	15	1.51±0.21	170.5±56.1	104.4±51.2	0.59±0.11
11–15 years	17	1.37±0.39	230.9±96.9	131.0±48.6	0.58±0.10
16–20 years	17	1.32±0.27	90.7±45.0	55.4±32.3	0.62±0.21
Adult controls	203	1.11±0.25	78.4±48.2	28.2±17.9	0.37±0.14
Female					
0–5 years	19	1.38±0.22	170.7±74.3	98.0±52.0	0.54±0.08
6–10 years	12	1.45±0.17	163.3±42.6	94.2±40.0	0.56±0.14
11–15 years	11	1.57±0.29	177.8±72.5	123.8±44.3	0.75±0.42
16–20 years	16	1.26±0.29	86.3±42.3	40.6±27.3	0.46±0.13
Adult controls	160	0.84±0.17	76.9±51.1	27.6±18.5	0.38±0.21

The values shown are mean values±standard deviation or numbers

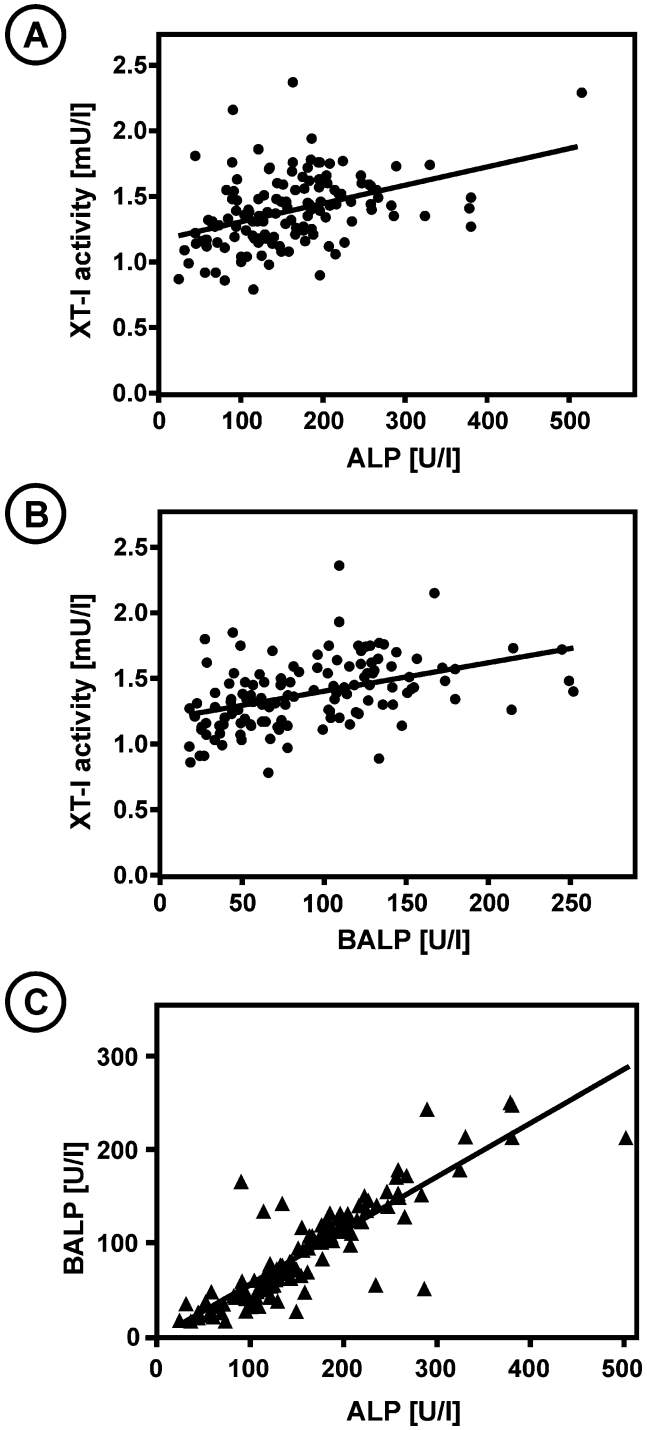

Fig. 1 Correlation of XT activity, ALP and BALP in the serum of juvenile patients. **a** Serum levels of XT activity and alkaline phosphatase in 133 juvenile patients, aged 0 to 20 years, were correlated. A moderate but highly significant correlation (correlation coefficient r=0.405, p<0.0001) between these two parameters was observed. **b** The correlation of serum XT activity and bone-specific alkaline phosphatase levels was similar to that of XT and ALP (correlation coefficient r=0.428, p<0.0001). **c** A good correlation between BALP and total ALP was observed in the serum specimens of our cohort (correlation coefficient r=0.872, p<0.0001). The average ratio of BALP and total ALP was determined as 0.58

data sets and multiple linear regression analyses was used to assess the independent role of the serum XT activity and patient characteristics. p values of 0.05 or less were considered significant. Values are given as mean±standard deviation.

Results

Determination of serum xylosyltransferase activity

We determined XT activities in serum samples obtained from 133 juvenile patients (Table 1). XT activities in the juvenile males (n=75) were significantly elevated in comparison to adults (p<0.0001). The mean value and 90% range were 1.38 mU/l (SD 0.28) and 0.98–1.78 mU/l in juvenile males and 1.11 mU/l (SD 0.25) and 0.70–1.55 mU/l in adult males (n=203), respectively. XT activities in serum specimens from juvenile females were also significantly increased compared to adult women (p<0.0001). In juvenile females (n=58) the mean value and 90% range were 1.40 mU/l (SD 0.27) and 0.99–1.76 mU/l, respectively. In the corresponding group of adult women (n=160) the mean XT activities were calculated as 0.84 mU/l (SD 0.17) and the 90% range as 0.61–1.15 mU/l. The observed differences of serum XT activities remained significant after adjustment for the different diseases in the juvenile patients. No significantly different serum XT levels were observed in the different age groups in juvenile patients. However, we observed a tendency towards higher serum XT activities in boys and girls aged 6–15 years compared to the other groups. No sex-related differences in serum XT activities were observed in the juvenile patient group. The serum XT activities in young patients with diabetes mellitus type 1 (1.38±0.34 mU/l, mean±SD) and in juvenile cardiac patients (1.39±0.26 mU/l) were not significantly different. Within the latter patient group the XT activities did not differ between the subgroups: congential heart failure, 1.40 mU/l (SD 0.24); heart transplantation, 1.42 mU/l (SD 0.35); congential valve defects, 1.35 mU/l (SD 0.25); arrhythmia, 1.33 mU/l (SD 0.19); dilative cardiomyopathy, 1.16 mU/l (SD 0.09) (p>0.05 for all). Furthermore, adult patients with diabetes mellitus type 1, coronary heart disease, dilative cardiomyopathy, heart transplantation (N=50) had normal serum XT activities comparable to the adult blood donors.

Correlation of XT activity and ALP levels

Total ALP activity and bone-specific ALP activity were determined in the serum samples of juvenile men and women (Table 1). As expected significantly higher ALP levels were observed in young patients, aged 15 or under, compared to patients aged 16 to 20. The bone-specific

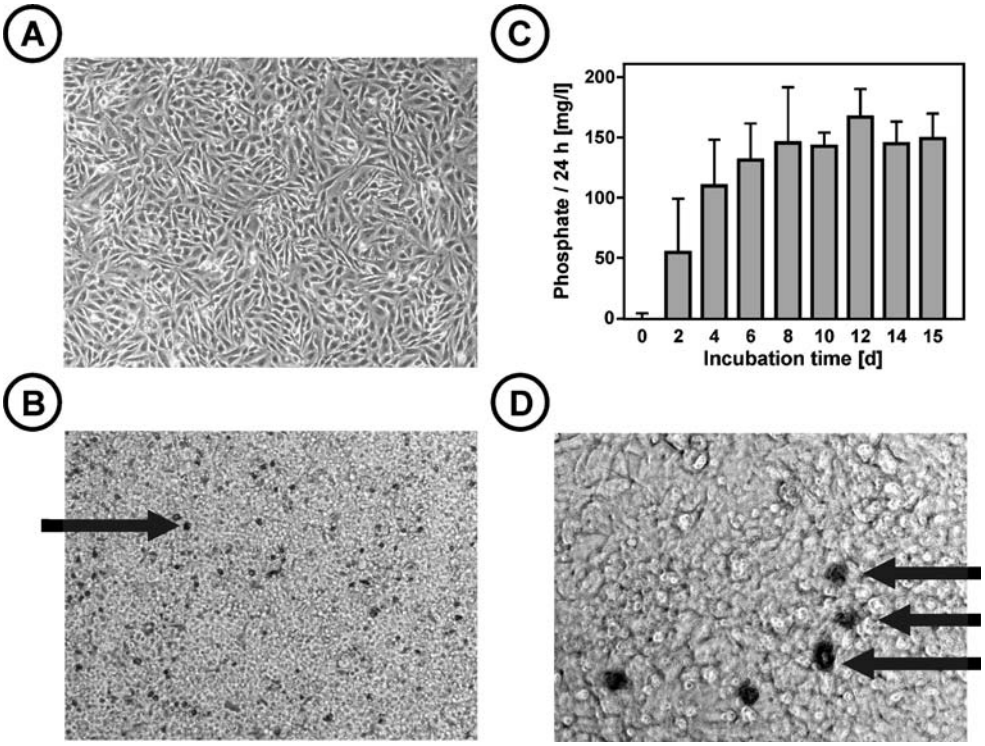

Fig. 2 Calcium-phosphate deposition in SAOS-2 cells. The presence of calcium-phosphate containing bone nodules (*arrows*) was analyzed in von Kossa stained cells 0 and 15 days after the induction of mineralization by phase-contrast microscopy. **a** Day 0, magnification 100×; **b** day 15, magnification 100×; **d** day 15, magnification 400×. In panel **c** the phosphate levels in the cell culture supernatant of SAOS-2 cells after induction of mineralization are shown. Cell culture medium was changed every 48 h

isoform represented approximately 50–60% of the total ALP activity in the investigated samples. A very good correlation between serum ALP and BALP was noted (correlation coefficient 0.872, $p<0.0001$; Fig. 1). Furthermore, a significant positive correlation of serum XT activity and ALP levels (correlation coefficient 0.405, $p<0.0001$) and BALP levels (correlation coefficient 0.428, $p<0.0001$) was detected (Fig. 1). ALP and BALP levels in young patients with diabetes mellitus type 1 (ALP, 141±79 U/l; BALP, 86±50 U/l, mean±SD) and in juvenile cardiac patients (ALP, 162±79 U/l; BALP, 95±56 U/l) were not significantly different. Neither ALP nor BALP levels differed in the cardiac subgroups. A significant correlation of serum XT activity with ALP (correlation coefficient 0.436, $p<0.001$) and BALP levels (correlation coefficient 0.529, $p<0.001$) was detected in the cardiac patients. A similar correlation was observed in the juvenile diabetic patients, which just failed to show significance, due to the small cohort size (correlation coefficients 0.315 and 0.349, respectively). These observed correlations remained significant after adjustment for the different diseases in the juvenile patients. No significant correlation between XT and other serum parameters was observed.

Increased XT-I and ALP expression in mineralizing osteoblast-like SAOS-2 cells

In order to validate the observed correlation of serum XT and ALP levels to be a result of growth processes we analysed the expression of XT-I and XT-II in the osteoblast-like SAOS-2 cell line under conditions that permit mineralization. Diffuse calcification was induced by the addition of β-glycerophosphate and sodium ascorbate, and the formation of bone nodules was visualized by von Kossa stain for a period of 15 days. The nodules were evenly distributed throughout the culture dish and could be visualized as discrete black nodules. Phosphate levels increased throughout the incubation period (Fig. 2c) and were in parallel with the ALP activity in the culture supernatant (Fig. 3a). A long-term increase in the ALP secretion was observed in the mineralizing SAOS-2 cells. The maximum was reached after 15 days where the ALP formation per day was more than 25-fold higher than at the beginning of the bone nodule induction. The XT activity secreted by the mineralizing SAOS-2 cells increased in parallel with the ALP activity and reached its maximum after 10 days (Fig. 3b). From day 10 to 15 the XT formation rate remained at a constant level, which was fourfold higher than in the controls. In order to elucidate whether the increased XT secretion rate is also accompanied by an elevated XT-I and XT-II biosynthesis, we analyzed the relative mRNA abundance of XT-I and XT-II after different time points (Fig. 4). β-glycerophosphate- and sodium ascorbate-induced bone nodule formation resulted in a long-term increase in XT-I expression. XT-I expression was more than 1.5-fold increased after 4 days and more than 2-fold elevated after 10 days ($p<0.001$) compared to the constantly expressed housekeeping gene ubiquitine. On

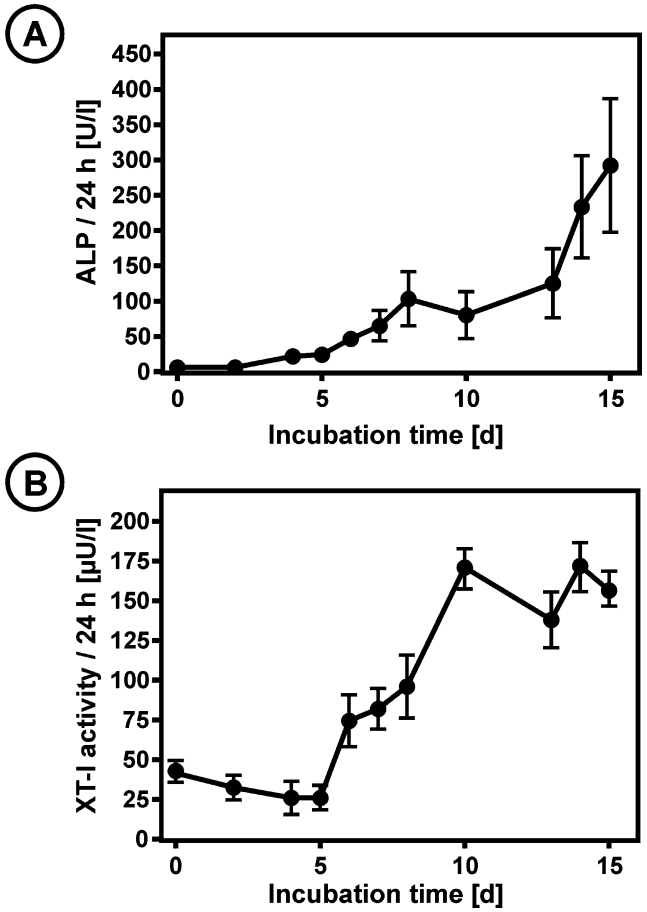

Fig. 3 ALP and XT activity after induction of mineralization in the osteoblast-like cell line SAOS-2. Calcification of the osteosarcoma cell line SAOS-2 was induced by addition of β-glycerophosphate and sodium ascorbate and ALP (**a**) and XT activity (**b**) were determined in the cell culture supernatant. Both enzymes show a similar long-term increase after induction of mineralization. Values are shown as enzyme activity synthesized per 24 h

the other hand XT-II mRNA remained at a constant level during the whole period (Fig. 4).

Induction of XT-I expression in SAOS-2 cells after HGH and IGF-I treatment

HGH is a major determinant of bone growth and acts either through IGF-I, whose expression is controlled by HGH, or directly by affecting osteoblast activity. SAOS-2 cells have been shown to be responsive to HGH and IGF-I [17, 18]. Therefore, we investigated whether addition of HGH or IGF-I results in an upregulation of ALP, XT-I and XT-II. Both ALP and XT activities were upregulated by HGH and IGF-I treatment and the increased XT-I mRNA expression could be paralleled with an elevated XT enzyme activity (Fig. 5). IGF-I supplementation always resulted in a less prominent induction of the bone metabolism markers compared to the HGH-induced elevation. The XT-II mRNA expression was not significantly altered by HGH or IGF-I.

Discussion

Bone formation and growth are complex biological processes, which are characterized by an increased production of collagen and proteoglycans. It has been clearly shown that disturbance of the proteoglycan biosynthesis leads to severe impairment of bone formation and skeletal development [6, 7]. The xylosyltransferases XT-I and XT-II are the initial enzymes in the posttranslational biosynthesis of the glycosaminoglycan chains and were shown to be the rate-limiting step enzymes in proteoglycan biosynthesis [9, 19]. As the chemical and biophysical properties of proteoglycans are mainly determined by the lateral glycosaminoglycan chains, their biological function is closely related to XT activity.

Here, we could show that the XT activity in serum of juveniles is elevated compared to adults. The patients that were included in our study attended the outpatient clinic because of diabetes mellitus type I or congenital heart defects and a subgroup analysis of our data revealed that the elevated XT activity is not linked to any special disease or treatment but merely to young age. In addition, this is supported by our finding that adult patients with diabetes mellitus type I, dilated cardiomyopathy and other heart defects had normal serum XT activities. A highly significant correlation was observed between serum XT activity and ALP or BALP activity. ALP and BALP activity are well-known markers for osteogenic activity and bone formation in children and juvenile patients. This correlation pointed to juvenile bone formation and extracellular matrix synthesis as the cause for the elevated serum XT activities in our patient group. The correlation analysis of XT and ALP revealed only a moderate correlation with correlation coefficients of 0.4–0.5. This finding leads to the conclusion that the XT levels in the peripheral blood are derived

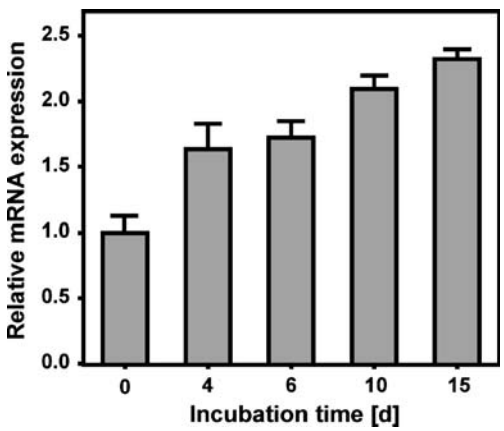

Fig. 4 mRNA expression of XT-I and XT-II in SAOS-2 cells after induction of mineralization. A long-term increase of the XT-I mRNA expression was observed in the cells, while XT-II mRNA remained at a constant level. The housekeeping gene ubiquitin was constantly expressed and the relative mRNA abundance of XT-I and XT-II was calculated. Data are shown as mean±SD

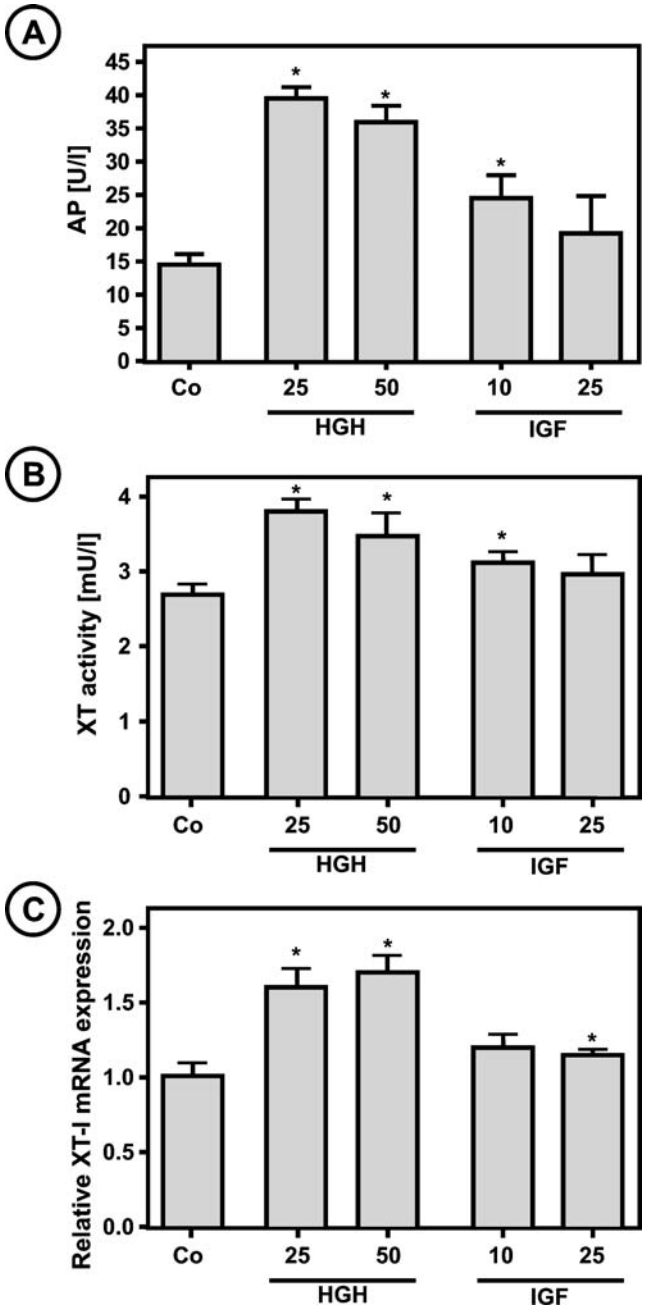

Fig. 5 Stimulation of alkaline phosphatase, XT-I mRNA and XT activity in SAOS-2 cells by HGH and IGF-I. SAOS-2 cells were supplemented with HGH (25 and 50 ng/ml) or IGF-I (10 and 25 ng/ml) and XT-I mRNA, XT and ALP activity were determined after 48 h. XT-I and ALP were induced by growth hormone and IGF-I. Addition of IGF-I always resulted in a weaker induction compared to growth hormone supplementation. Cells were counted prior to the analysis in order to exclude differences in cell numbers due to potential proliferative effects of the hormone treatment. No significant differences in cell numbers were observed. Data are shown as mean±SD and *asterisks* indicate a significant increase compared to the untreated control ($p<0.05$)

from multiple sources in the human body. Besides osteogenesis and bone growth, tissue remodeling and other proteoglycan-related processes contribute to the total serum XT activity.

Due to the observed correlation of serum XT and BALP we elucidated the role of XT-I and XT-II in bone formation in the osteoblast-like cell model SAOS-2. We could demonstrate that SAOS-2 cells mineralize under appropriate conditions and that this process increases the proteoglycan biosynthesis rate. XT activity and XT-I mRNA showed a long-term elevation after induction of bone-nodule formation, which was in parallel with the ALP activity. Interestingly, XT-II mRNA was not upregulated during this process. XT-I and XT-II catalyze an initial and rate-limiting step in the synthesis of glycosaminoglycan chains in proteoglycans and, therefore, represent key enzymes for extracellular matrix assembly and remodeling. Both enzymes are capable of initiating the biosynthesis of chondroitin sulfate-, dermatan sulfate- and heparan sulfate glycosaminoglycan chains [8, 20]. Until now we and others have not found any differences of both xylosyltransferases in regards of acceptor specificity or a preference for the biosynthesis of either chondroitin sulfate or heparan sulfte proteoglycans. XT-I was found to be the dominant xylosyltransferase in cardiac fibroblasts, to be regulated by profibrotic cytokines like TGF-β_1 and to play the major role in the elevated proteoglycan biosynthesis during cardiac fibrosis and heart tissue remodeling [19]. Here, we could show that XT-I is also the predominant xylosyltransferase in bone formation in the osteoblast-like cell model SAOS-2. The biosynthesis of proteoglycans and other extracellular matrix molecules is an important step during bone formation and mineralization. Proteoglycans contribute significantly to the strength of the long skeletal bones and play an important role during bone development [3]. Furthermore, they build up a scaffold during collagen fibrillogenesis and regulate the local availability of cytokines that are also involved in osteoblast stimulation. The biosynthesis of proteoglycans is closely related to xylosyltransferase activity, which has been shown to be a measure for the actual proteoglycan biosynthesis rate [8]. In conclusion, our results demonstrate that induction of bone nodule formation leads to an upregulation of proteoglycan biosynthesis and provide first evidence that the increased XT activities found in the serum of juveniles are suspected to be a result of a high proteoglycan biosynthesis rate during skeletal growth and extracellular matrix remodeling processes.

The control of osteoblast activity is a crucial element for bone homeostasis and development. HGH has been shown to be one of the major determinants in bone growth, both in juvenile skeletal bone growth and in the periodic bone remodeling in the adult skeleton [17]. HGH acts directly on the osteoblasts controlling their biological activity and in addition controls the expression of IGF-I. IGF-I is the predominant factor secreted by osteoblasts and stimulates bone formation by autocrine and paracrine mechanisms [21–23]. SAOS-2 cells have been shown to be capable of

cellular response to HGH [17] and IGF-I [18]. Therefore, we used SAOS-2 cells to study whether the XT expression in osteoblasts is also triggered by HGH and IGF-I. The IGF-I-mediated action of HGH on osteoblasts was proposed to be the critical factor for the anabolic effects of this cytokine in bone metabolism [17]. This is in concordance with our results, which showed a more prominent induction of XT-I mRNA, XT activity and ALP after HGH treatment compared to IGF-I treatment. This is probably due to a combined action of HGH on the SAOS-2 cells, which includes a paracrine induction of IGF-I synthesis and a direct activation of cellular bone formation processes. XT-II mRNA was not upregulated by HGH or IGF-I, which is in concordance with the results obtained after induction of mineralization in SAOS-2 cells. Our results demonstrate an induction of XT-I mRNA expression after supplementation with bone agonists, which was in parallel with the formation of XT enzyme activity and ALP. In general, the transcriptional level of mRNA and the corresponding total mRNA content, which is isolated from a cell, do not have to be identical. Consequently, our investigations are based on the cellular mRNA content and are not able to distinguish between an extended mRNA half-life and actually increased transcription rates. However, we do have first evidence for TGF-β_1-induced alterations of XT-expression levels that altered mRNA levels are rather a cause of different transcriptional activity than mRNA half-life alterations [19].

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