Differential regulation of MMPs and matrix assembly in chicken and turkey growth-plate chondrocytes

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The growth plate is a highly organized cartilaginous structure located between the epiphyseal and metaphyseal bone at the distal ends of the long bones. This is the place where longitudinal growth takes place by the endochondral ossification process, replacing cartilaginous scaffold with bone in a coordinated fashion (23). The growth-plate chondrocytes are organized in several horizontal zones: the reserve zone, the proliferative zone, with flattened cells; the prehypertrophic or transition zone; and the hypertrophic zone, with a partially calcified matrix and invading capillaries (17). The avian growth plate contains longer columns of chondrocytes than the mammalian growth plate; more cells are found in each zone, and it is highly vascularized (40, 43). The proliferative and prehypertrophic zones are vascularized from the proximal side by penetrating epiphyseal vessels and from the distal side by the metaphyseal blood vessels. Vascularization of the hypertrophic region involves matrix degradation by enzymes secreted by chondrocytes, chondroclasts, osteoclasts, and endothelial cells (5).

The matrix metalloproteinases (MMPs) are a large family of zinc-dependent proteases whose principal substrates are basement membrane proteins and the extracellular matrix (ECM) (32, 35). Members of the MMP family have been implicated in physiological and pathological processes that involve proteolytic cleavage or remodeling of the ECM (54). They regulate angiogenesis by several mechanisms: they break down ECM components thus allowing endothelial cell invasion and vascular growth, release ECM-bound angiogenic growth factors, generate promigratory ECM-component fragments, and cleave endothelial cell-cell adhesions (48). At least 24 distinct MMPs have been cloned in humans, and they can be grouped into four subclasses based on substrate specificity and domain structure: collagenases, which cleave fibrillar collagens; stromelysins, which digest laminin, fibronectin, and proteoglycans; membrane-type MMPs, which have a carboxy-terminal transmembrane domain and are anchored to the extracellular surface of the plasma membrane; and gelatinases, which can hydrolyze denatured collagens (gelatins) with high efficiency (6).

The MMPs involved in endochondral ossification are the collagenases, MMP-1 and MMP-13; the gelatinases, MMP-2 and MMP-9 (gelatinase A and B, respectively); the stromelysins, MMP-3 and MMP-10; and the membrane-type MMP-14 (30, 62). Gelatinase B/MMP-9 is a key regulator of growth-plate angiogenesis and apoptosis of hypertrophic chondrocytes (55). Its molecular mode of action includes releasing vascular endothelial growth factor or other growth factors from the matrix (13, 49) and degrading type II collagen. The avian 75-kDa-like gelatinase B, which shows low sequence similarity to the mammalian enzyme (59% at the protein level) (18) seems to function similarly in the context of endochondral bone formation in the avian growth plate (52). Gelatinase A/MMP-2, a crucial enzyme for angiogenesis (25), has been shown to degrade native collagen types I, IV, and V (1). Mutation in this gene causes multicentric osteolysis and arthritis syndrome in humans (31). In the chicken’s growth plate, gelatinase A is expressed in the proliferative zone and in areas surrounding the blood vessels (39). MMP-13 (collagenase 3) plays a crucial role in bone formation and remodeling. Its substrates include collagen types I and II, as well as agranec,
and it can synergize with gelatinase B in their degradation (13, 50). Mutation in human MMP-13 causes the Missouri variant of spondyloepimetaphyseal dysplasia with abnormalities in the development and growth of endochondral skeletal elements (26). MMP-13-deficient mice show altered endochondral bone development and profound defects in growth-plate cartilage with markedly increased hypertrophic domains (24, 50). In chicken, MMP-13 gene expression has been found to be induced during chondrocyte hypertrophy, and its processing to the active 55-kDa form is dependent on gelatinase A activity (11). MMPs 1, 10, and 14 have still not been identified in avian species, and MMP-3 was recently predicted using cDNA libraries (XM_417175).

Tibial dyschondroplasia (TD) is the most prevalent skeletal abnormality associated with rapid growth rate in many avian species, such as chickens and turkeys. TD is characterized by the presence of a nonvascularized, nonmineralized lesion that extends from the epiphyseal growth plate into the metaphysis (27, 28). The mechanisms underlying TD development are not known; however, the lesion is initiated when the chondrocytes’ transition from prehypertrophy to hypertrophy is disrupted. This inhibition produces a layer of cartilage tissue that is resistant to vascularization (9, 41, 60). Rath et al. (44) measured low collagenolytic and gelatinolytic activities in conditioned media derived from cartilage-explant cultures of TD growth plates. In-situ hybridization analysis showed reduction in MMP-9 and MMP-13 expression in thiram-induced TD lesions (39).

There are several nutritional factors that influence TD incidence (2, 29, 47). Furthermore, skeletal tissue appears to be extremely sensitive to dithiocarbamates such as thiram (45), which serve as a research model in chicken.

In this paper, we induced TD in chickens and turkeys using thiram and found differences in the concentration and duration required for TD induction. We found downregulation of MMP activity in TD lesions from chickens, as well as in thiram treated primary cultured growth plate chondrocytes from chickens and turkeys, but again, the later required higher concentrations of thiram. Furthermore, we found differences in MMP expression and activity in those primary cultures under different conditions. These findings suggest that MMP down-regulation is involved in TD development and that mechanisms of MMP regulation differ in the growth plates of chickens and turkeys, resulting in altered matrix assembly.

MATERIALS AND METHODS

Materials. ATP (32P, 6,000 Ci/mmol) was purchased from the Radio-Chemical Center (Amersham, UK). DMEM, trypsin-EDTA solution (0.25, 0.02%), and FBS were purchased from Biological Industries (Beth-Haemek, Israel). Thiram, phorbol 12-myristate 13-acetate (PMA) and retinoic acid were purchased from Sigma Chemical (St. Louis, MO).

Animals and diets. One-day-old broiler chicks (Cobb strain) were obtained from a commercial hatchery (Ramat, Hadera, Israel) and raised for 10 days under the recommended temperature regime and fed according to National Research Council (NRC) recommendations, ad libitum. Birds were fed either 1) a regular diet (control group) or 2) the same diet containing 50 ppm tetramethylthiuram disulfide (thiram) to induce TD. One-day-old turkey chicks (B.U.T. strain) were obtained from a commercial hatchery (Ramtim, Ramit, and B.U.T. strain) were obtained from a commercial hatchery (Ramit, Ramit, (control group) or

Thiram differentially induces TD in chicken and turkey growth plates and downregulates MMPs activity in vivo. Genetic selection for rapid growth and heavy body weight have
increased the incidence of spontaneous TD in chickens and turkeys over the past decade. TD lesion can serve as a model for studying matrix assembly in the growth plate. Nontoxic amounts of thiram have been shown to induce high incidence of severe TD lesions in chickens (45); however, in turkeys, no such model has been described for TD induction, so far (19, 20).

We used thiram to induce the disorder in chickens and turkeys. In the former, after 10 days of thiram administration, 56% of the tibias showed severe TD lesions (scores 3 and 4), 34% of the tibias showed light TD lesions (scores 1 and 2), and the rest of the group had no TD lesions. In the control group, all the tibias had a normal phenotype (Fig. 1A). This treatment had no effect in turkeys (data not shown); therefore, we...
increased the amount of thiram to 100 ppm and 400 ppm and the time period to 11 wk. Until week 7, no TD was observed. At the age of 7 wk, 70% of the tibias in the 400 ppm group showed light TD lesions (score 1), and both of the other groups (control and 100 ppm thiram) had a normal phenotype (Fig. 1E). At the age of 11 wk, 40% of the tibias in the control group showed spontaneous TD of light severity (scores 1 and 2), and in the 100 ppm group, 30% of the tibias had light-severity TD lesions (score 1). These two groups were not statistically different. In the 400 ppm group, 54% of the tibias had severe TD lesions (scores 3 and 4), 30% of the tibias had light TD lesions (scores 1 and 2), and the rest had normal growth-plate phenotypes. This group was statistically different from the others (Fig. 1E).

In view of the fact that TD is associated with impaired blood penetration into the growth plate (28) and with impaired matrix turnover, two processes mediated by MMP activity (50, 55, 62), we checked the gelatinolytic and caseinolytic activities of cartilage taken from control or TD-affected growth plates (scores 3 and 4) induced by thiram in 10-day-old chickens (pooled from ten different birds). Using gelatin or casein impregnated zymography analysis gels, we detected reductions in the activities of the 64-kDa gelatinase A and the 60-kDa gelatinase A (an active degradation product), as well as the activities of the 75-kDa gelatinase B and the 55-kDa MMP-13 (Fig. 1, C and D), suggesting a role for MMP downregulation in TD development. MMP activities of turkey TD lesions were technically unfeasible due to the late age and immense size of the developed lesion.

Thiram reduces gelatinase A expression and activity, and MMP-13 activity in cultured chondrocytes from chicken and turkey growth plates. As shown by us and by others, the copper chelator thiram induces TD in chickens, via an unknown mechanism (45). To examine our hypothesis that thiram inhibits growth-plate vascularization by downregulating MMPs and the possible involvement of MMPs in TD etiology, we examined the effect of thiram on gelatinases gene expression in primary cultured chondrocytes from chicken and turkey tibial growth plates by Northern blot analysis with probes for gelatinase A and B mRNA. At a concentration of 10 μM, thiram downregulated gelatinase A expression in chickens, whereas 100 μM was required in turkeys to achieve the same effect (Fig. 2, A and B). Gelatinase B gene expression was not induced by thiram in either species.

We then wanted to verify the effect of thiram on the gelatinases activity. Chicken and turkey chondrocytes were cultured with a range of thiram concentrations in serum-free DMEM for 24 h. The gelatinolytic activity of conditioned media was analyzed in gelatin-impregnated gels by zymography analysis. Corresponding with the gene expression pattern and the in vivo effect, thiram reduced gelatinase A activity in both chicken and turkey chondrocytes, with maximal effect at 10 and 100 μM, respectively. Gelatinase B activity was not induced in either chicken or turkey chondrocytes (Fig. 2C).

The activity of other members of the MMP family in the conditioned media was analyzed in casein-impregnated gels by zymography analysis. In chicken and turkey chondrocytes, thiram reduced the activity of the 55-kDa MMP-13 with a maximal effect at 10 μM in chicken chondrocytes and 100 μM in turkeys (Fig. 2D).

Retinoic acid and PMA differentially regulate MMPs in chondrocytes derived from chicken and turkey growth plates. To examine the regulatory pathways involved in MMP expression and activity in chicken and turkey growth plate, we examined two pathways that are known to induce MMPs; retinoic acid (RA), which is a differentiation factor in chon-

![Fig. 2. Down-regulation of gelatinase A expression and activity and MMP-13 activity by thiram in cultured chondrocytes. Primary cultured chicken or turkey chondrocytes were treated with the indicated concentrations of thiram for 24 h. (A) RNA was subjected to Northern blot analysis with 32P-labeled probe of avian gelatinase A (GEL A). The amounts of RNA on the membranes were visualized by methylene blue staining of the 28 S ribosomal RNA. (B) Gene expression was analyzed by scanning densitometry, relative to the expression of 28 S ribosomal RNA. C,D: Conditioned media were separated under nonreducing conditions on zymography gels. (C) Band corresponding to the 64-kDa gelatinase A (64 kDa GEL A) was detected by gelatin-impregnated gel. (D) Band corresponding to the 55-kDa MMP-13 was detected by casein-impregnated gel.](http://ajpregu.physiology.org/doi/abs/10.1152/ajpregu.00745.2006)
drocytes (3, 61) and an inducer of vascular invasion and premature fusion of the epiphyseal plate (12), and PMA, which is a protein kinase C activator that has been shown to induce transcription of most MMP genes (4).

The primary growth plate chondrocytes culture that we used is a well-defined system, known to display characteristics that are similar in both morphological and developmental terms to that of chick chondrocyte in vivo and therefore offers an excellent in vitro model for endochondral ossification (14). These cells when cultured under different conditions change their differentiation stage, which can be monitored by the expression of collagen types II and X. As a monolayer, the cells sustain their proliferative properties (57).

The effect of RA on gelatinase gene expression was studied in cultured chondrocytes from turkey growth plate. RA induced the expression of gelatinase A from undetectable levels to a maximal effect at 0.1 μM. In contrast, gelatinase A gene expression was detectable in the untreated chondrocytes and was elevated by the treatment with a peak at 0.1 μM RA (Fig. 3, A and B). A similar effect of RA on chicken chondrocytes has been previously demonstrated (52).

To verify the effect of RA on gelatinases activity, chicken and turkey chondrocytes were cultured with a range of RA concentrations in serum-free DMEM for 24 h. The gelatino-lytic activity of the conditioned media was analyzed in gelatin-impregnated gels by zymography analysis. In chicken and turkey chondrocytes, the 64-kDa gelatinase A activity was slightly increased by RA treatment. The 75-kDa gelatinase B was induced by RA in the chicken chondrocytes, as shown previously (52), but not in the turkey chondrocytes (Fig. 3C), in contrast to the gene expression studies (Fig. 3, A and B).

The activity of other members of the MMP family in those conditioned media was analyzed in casein-impregnated gels by zymography analysis. RA treatment slightly increased the 55-kDa MMP-13 activity in a dose-dependent manner in both chicken and turkey chondrocytes (Fig. 3D).

The effect of PMA on gelatinases gene expression was studied in cultured chondrocytes from chicken and turkey growth plates. RNA was extracted and probed for gelatinase A and B mRNA. PMA dramatically and dose dependently induced the expression of gelatinase B, in both chicken and turkey, from undetectable levels. Gelatinase A gene expression was detected in the untreated chondrocytes and was slightly increased, in a dose-dependent manner, by PMA treatment in chicken and to a lesser extent in turkey chondrocytes (Fig. 4, A and B).

To verify the effect of PMA on gelatinases activity, chicken and turkey chondrocytes were cultured with a range of PMA concentrations in serum-free DMEM for 24 h. The gelatino-lytic activity of the conditioned media was analyzed in gelatin-impregnated gels by zymography analysis. PMA did not affect the 64-kDa gelatinase A activity in chickens or turkeys (Fig. 4C), although the gene transcription was slightly increased (Fig. 4, A and B). In chicken chondrocytes, PMA slightly induced gelatinase B activity, but its levels were hardly detectable (Fig. 4C), again in spite of the strong induction of gene transcription (Fig. 4, A and B). In contrast, in turkey chondrocytes, PMA induced the activities of the 75-kDa gelatinase B (Fig. 4C).

The activity of other members of the MMP family in the conditioned media was analyzed in casein-impregnated gels by zymography analysis. PMA treatment increased the 50- and 55-kDa MMP-13 activities, in a dose-dependent manner, in both chicken and turkey chondrocytes, but in the latter, the 50-kDa MMP-13 activity was much stronger (Fig. 4D).

Differential regulation of MMPs in chicken and turkey chondrocytes by combined treatments of RA, PMA, and thiram. The expression of gelatinase B is inducible, and thus a possible inhibitory effect of thiram on this gene cannot be studied unless it is induced by other factors. For this study, chicken and turkey chondrocytes were cultured with a range of thiram and RA concentrations or thiram and PMA concentrations in serum-free DMEM for 24 h. MMP activity of conditioned media was analyzed in gelatin or casein-impregnated gels by zymography analysis. Band densities were analyzed by scanning densitometry and are given as arbitrary values in Table 1. In chicken chondrocytes, the combined effect of 10 μM thiram and RA abolished the upregulating effect of RA on gelatinase A activity. Thiram had the same effect on the activity of MMP-13, which was up-regulated by RA. The combined treatment did not affect gelatinase B activity in chicken chondrocytes (Fig. 5A, Table 1). In contrast, in turkey chondrocytes, the combined effect of 10 μM thiram and RA also abolished the upregulating effect of RA on gelatinase A activity but induced the activity of gelatinase B and reinforced the upregulating effect of RA on the activity MMP-13 (Fig. 5B, Table 1).

Surprisingly, the combined treatment of thiram and PMA had similar effects; in chicken chondrocytes, 10 μM thiram and
PMA decreased gelatinase A activity and slightly decreased MMP-13 activity (Fig. 5C, Table 1). There was no effect on gelatinase B activity. In contrast, in turkey chondrocytes, the combined effect of 10 μM thiram and PMA also decreased gelatinase A activity but induced the activity of gelatinase B and slightly increased the activity of MMP-13 compared with the control and PMA treatments (Fig. 5D, Table 1).

**DISCUSSION**

The proper regulation of MMPs is highly important because of their involvement in a variety of processes, such as angiogenesis, morphogenesis, and wound healing. This family of proteases is finely tuned by integration of various levels of regulation, which include gene expression, enzyme activation, inhibition, and degradation. The expression of most MMPs is normally low in tissues and is induced when remodeling of the ECM is required; this is primarily regulated at the transcriptional level (58), permitting both coordinated and cell-type-specific expression of MMPs (4).

In this study, we demonstrate the differential regulation of MMP gene expression in a specific cell type, the chondrocyte, from two related species, chicken and turkey. We found that in chicken and less in turkey chondrocytes, PMA treatment upregulates the transcription of gelatinase A despite the fact that this gelatinase does not contain any known PMA response element within its promoter region (21). Gelatinase B gene expression was also induced by PMA treatment in chondrocytes of both species. These findings are consistent with the observation that inducible MMPs (MMP-1, 3, 7, 9, 10, 12, and 13) contain one or more activator protein-1 (AP-1) binding sites which are known to respond to PKC signaling (4) in their promoter, whereas the promoter region of the constitutive

**Table 1. Combined effect of thiram and PMA or thiram and RA on MMP activity**

<table>
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<tr>
<th>RA, μM</th>
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<th>Gelatinase B</th>
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Primary cultured chicken or turkey chondrocytes were treated with the indicated concentrations of thiram and retinoic acid (RA) or phorbol 12-myristate 13-acetate (PMA) for 24 h. Conditioned media were separated under nonreducing conditions on gelatin/casein-impregnated zymography gels. Band densities were analyzed by scanning densitometry and given as arbitrary values. MMP, metalloproteinase.
In contrast, PMA also induces MMP activity in chickens. As well as in turkey chondrocytes, MMP activity is inhibited by tissue inhibitors of metalloproteinases (TIMPs), which bind to the MMP activity in the extracellular space is inhibited by tissue inhibitors of metalloproteinases (TIMPs). Among various activators such as plasmin (33) and activated MMPs, which can participate in processing other pro-MMPs (53). Most MMPs are secreted as latent precursors (zymogens) that are proteolytically activated in the extracellular space by various activators such as plasmin (33) and activated MMPs, which can participate in processing other pro-MMPs (53). MMP activity in the extracellular space is inhibited by tissue inhibitors of metalloproteinases (TIMPs), which bind to the highly conserved zinc-binding site of active MMPs (53).

Differential regulation between chickens and turkeys was found in MMP activity level. Although RA induces gelatinase B expression in chicken (52), it only induces the corresponding enzyme activity in chickens. In contrast, PMA also induces gelatinase B expression in both species, but it only induces its activity in turkey. These differences in activity could be attributed to differential activation or inhibition. The primary activator of pro-MMP-9 is MMP-3 (37), although it can be activated by MMP-2, 7, and 13 as well (16). Both RA and PMA have been shown to upregulate MMP-3 transcription (15, 59). Could it be that in one species, but not in the other, RA or PMA treatment up-regulates an activator MMP?

In both species, PMA upregulates the activity of a 55-kDa MMP, which, based on its molecular weight, has been suggested to be MMP-13 (11). Mostly in turkey, PMA induces the activity of a 50-kDa MMP, another active form of MMP-13 (63). We suggest that in turkey, MMP-13 plays an important role in pro-MMP-9 activation. This would explain the duality between induction of gene expression and enzyme activity between these two species. Moreover, because gelatinase B is known to be inactivated by members of the TIMP family (53), an alternative possibility is that rather than induction, the differential regulation seen between these two species is due to differential inhibition by TIMPs or other gelatinase B inhibitors, such as RECK (51). Finally, we cannot exclude the possibility that the enzyme activity was induced at lower levels, which are not detected by the method; this suggestion is also in line with our hypothesis regarding the differential regulation between the two species.

Differences in sensitivity to thiram were found between chicken and turkey in vivo, as well as in vitro. Whereas chicken developed severe TD within 10 days of 50 ppm thiram administration, turkeys did not respond to this dosage, and 11 wk of 400 ppm thiram administration were needed to induce severe TD in this species. On the basis of morphological characteristics, TD is addressed as the same disorder in chicken and turkeys (42). Here, for the first time, we show differences in thiram sensitivity and in TD development between these two related species. Reduction in gelatinase A, gelatinase B, and MMP-13 activities in TD lesions taken from 10-day-old chickens fed with thiram suggests a possible role for MMPs in TD etiology. Furthermore, in primary cultured chondrocytes, from both chicken and turkey, thiram downregulated the expression and activity of gelatinase A and the activity of MMP-13. However, a 10-fold higher concentration of thiram was needed to achieve these effects in turkey cells, compatible with our in vivo findings. It has been previously shown that thiram directly inactivates PKC isozymes, thereby inhibiting their signaling pathway (10). PKC acts as an important messenger for the transcriptional regulation of MMP genes (8). Consequently, we assume that thiram has a direct effect on MMP transcription in those chondrocytes.

The mechanism for TD development is still unclear; Rath et al. (46) suggests that a metabolic dysfunction leads to destruction of blood capillaries in the transition zone. Leach suggests that the lesion occurs when the transition of chondrocytes from prehypertrophy to hypertrophy is inhibited (43). Farquharson also suggests that the lesion is filled with transitional chondrocytes that are unable to differentiate to hypertrophic chondrocytes (56); Orth suggests that the chondrocytes secrete an immature cartilage that becomes highly cross-linked and is resistant to resorption and vascularization by the metaphyseal vessels (38). On the basis of our results, we suggest a complementary hypothesis in which MMPs are involved in TD etiology. We speculate that, in vivo, the defective chondrocytes fail to synthesize and secrete MMPs, and thus the matrix is not properly degraded, less blood vessels penetrate into the lesions.
into the growth plate, calcification is inhibited and the nonvascularized, nonmineralized TD lesion is formed.

Further support for our hypothesis of the role of MMP deficiency in TD etiology is contributed by the MMP-9 knockout mice that exhibit an abnormal pattern of growth-plate vascularization and ossification resulting in progressive lengthening of the growth plate (55); the MMP-13 knockout mice in which chondrocytes differentiate normally but their exit from the growth plate is delayed (24, 50); and mostly, by the extreme phenotype in mice lacking both MMP-9 and MMP-13 that exhibit severely impaired endochondral bone, characterized by diminished ECM remodeling and delayed vascular recruitment (50). The growth-plate phenotypes in these mice, which resemble TD lesions, together with our results, suggest that MMP-9 and -13 play a crucial role in the development of thiram-induced TD.

The combined treatment of RA or PMA with thiram induces gelatinase B activity in turkeys but not in chickens; moreover, the combined treatments have a synergistic effect on the upregulation of MMP-13 in turkey, while downregulating this enzyme activity in chicken. This may suggest that in the turkey growth plate, thiram, combined with local factors, elevates gelatinase B and MMP-13 activities and possibly other MMP activities that serve to protect from the inhibitory effect of thiram, leading to an in vivo phenotype, whereby turkeys are less susceptible to thiram-induced TD than chickens.

GRANTS

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REFERENCES


58. White La Maute C, Brinkerhoff CE. ETS sites in the promoters of matrix metalloproteinases collagenase (MMP-1) and stromelysin (MMP-3) are auxiliary elements that regulate basal and phorbol-induced transcription. Connect Tissue Res 36: 321–335, 1997.