Evaluation of the efficacy of vitamin D3 or its metabolites on thiram-induced tibial dyschondroplasia in chickens

N.C. Rath a,*, L. Kannan b, P.B. Pillai b, W.E. Huff a, G.R. Huff a, R.L. Horst c, J.L. Emmert b

a Agricultural Research Service, USDA, Poultry Science Center, University of Arkansas, Fayetteville, AR 72701, United States
b Department of Poultry Science, Poultry Science Center, University of Arkansas, Fayetteville, AR 72701, United States
c National Animal Disease Center, USDA, Ames, IA 50010, United States

Accepted 12 December 2006

Abstract

Two trials were conducted to determine if thiram-induced tibial dyschondroplasia (TD) in chickens was linked to a vitamin D deficiency and calcium homeostasis dysregulation, and whether feeding vitamin D fortified diets may prevent it. Day-old chickens were given grower diets containing different vitamin D products throughout the experiment until necropsy on day 16. Half of the birds in each feed group received thiram at levels of 100 ppm (trial 1) or 50 ppm (trial 2) between days 7–9 to induce TD. The birds were weighed, bled, and euthanized to determine TD incidences and severity by examining the growth plates. Tibial bones were used to measure biomechanical strength and ash content. Blood concentrations of 25-hydroxyvitamin D, Ca, P, alkaline phosphatase, and creatine kinase were measured in serum that showed no differences between different groups. Thiram reduced body weight and induced TD regardless of any vitamin D treatment to the same extent as untreated birds.

Published by Elsevier Ltd.

Keywords: Chicken; Tibial dyschondroplasia; Vitamin D; Thiram

1. Introduction

Tibial dyschondroplasia (TD) is a metabolic cartilage disease in which parts of the growth plate cartilage in the proximal tibia and tarsometatarsus fail to form bone resulting in the retention of a plug of avascular cartilage (Leach and Nesheim, 1965). Tibial dyschondroplasia is a major leg problem in poultry that causes lameness and deformity (Thorp, 1994; Crespo and Shivaprasad, 2003). The natural etiology of TD is not known; however, a range of hypotheses which include genetic susceptibility to nutritional imbalance, and environmental exposure to mycotoxin and pesticide intoxications, have been suggested as possible causes of this disease (Leach and Lilburn, 1992; Orth and Cook, 1994; Edwards, 2000; Pines et al., 2005). Because of inherent difficulties in determining the origin of the naturally occurring disease, experimental models have been used to understand the possible mechanisms of TD. Using broilers fed diets containing low Ca to P ratios or vitamin D deficient diets, impaired Ca homeostasis has been hypothesized as a cause of TD since broilers under these conditions show an increased incidence of the disease that can also be partially remedied by feeding these birds vitamin D3 or
some of its metabolites (Edwards, 1990, 2000; Whitehead, 1995; Rennie and Whitehead, 1996; Mitchell et al., 1997; Ledwaba and Roberson, 2003; Whitehead et al., 2004). However, there is no compelling evidence to show that deficiency of vitamin D metabolism is the primary reason of TD. Moreover, in experimental models that uses manipulated dietary levels of Ca: P ratio or vitamin D deficiency to induce TD, it is hard to discriminate whether TD is secondary to rickets since it can be reversed by correcting the problems of Ca homeostasis. On the other hand, there are other experimental models such as thiram or disulfiram-induced TD which show all the pathological characteristics of a naturally occurring disease (Vargas et al., 1983; Edwards, 1990; Orth and Cook, 1994; Rath et al., 2004, 2005; Pines et al., 2005) that have not been shown to be linked to calcium homeostasis problems. We have shown that a brief exposure of young broiler chickens to thiram or similar such dithiocarbamates for only 24–48 h is enough to increase the incidence and severity of TD (Rath et al., 2004, 2005). Therefore, this model appears not only suitable to study the mechanisms of TD but also to assay for factors that may control or prevent the disease. Thus, the objectives of the current study were to determine whether thiram-induced TD was the result of any disruption in overall Ca homeostasis and whether the disease can be controlled by diets fortified with vitamin D3 or its metabolites.

2. Materials and methods

2.1. Diets and chickens

A corn soybean-based broiler starter diet was prepared according to NRC specification (National Research Council, 1994) and used throughout the duration of the experiments. Except for 1,25-dihydroxyvitamin D3 (Hoffmann La Roche Company, Nutley, NJ) which was stored prepared, as a stock solution of 100 µg/ml in corn oil, other vitamin D preparations were obtained as premixes and added to diets at specified concentrations over and above the levels of vitamin D already present in the feed. Vitamin D3 (cholecalciferol, Alpharma, Fort Lee, NJ), Hy-D (25-hydroxycholecalciferol, DSM Nutritional Products, Parsippany, NJ), and Solbone A (SA) and PAM (Herbonis AG, Switzerland), two herbal vitamin D products prepared from the dried leaves of Solanum glaucophyllum that contain >90% glycosidic forms of 1,25-dihydroxyvitamin D3 (Cheng et al., 2004), were added to the feed according to manufacturer recommended concentrations. All premixes were added in a graded sequence to the basal diet for uniform mixing. HyD concentration was based on studies by Yarger et al. (1995) whereas the concentrations for both SA and PAM were based on biological equivalency studies of these products against synthetic 1,25-dihydroxyvitamin D3 (H. Bachmann, Personal communication). Based on a preliminary trial which showed synthetic 1,25-dihydroxyvitamin D3 to reduce body weight at concentrations of 10 µg/kg feed we reduced its concentration to 5 µg for subsequent tests. The experiments were approved through the institutional animal care and use guidelines.

Two trials were conducted several months apart using day-old male broiler chicks (Cobb-Vantress, Sialom Springs, AR) that were raised in Petersime batteries at a density of 12 birds/cage under a constant daily light period of 23 h and given diet and water ad libitum unless and otherwise specified. All dietary treatments were initiated at day of hatch. For each diet treatment, two experimental groups were formed one with and one without thiram added to the feed to induce TD. All dietary treatments were done as replicates with n = 24 chicks/replicate. In the first trial thiram was given at a concentration of 100 ppm whereas it was reduced to 50 ppm in the second trial.

2.2. Induction of TD

TD was induced according to an earlier protocol (Rath et al., 2004) by including thiram (tetrathiomethylthiuram disulfide, Sigma, St. Louis, MO) in the respective diets. On day 7 feed was withdrawn from all birds over night for a period of 14–16 h after which one group of birds in a feed replicate received thiram in the feed and the other control feed only. Forty eight hours after refeeding all the chicks in a feed replicate received the same diet until the end of the experiment on day 16.

2.3. Blood chemistry

Prior to euthanasia, blood from 12 birds in each group (6 from each replicate cage) was collected by heart puncture using Vacutainer tubes (BD Bioscience, Franklin Lakes, NJ) for serum clinical chemistry. Blood chemistry was determined using a clinical chemistry analyzer (Chiron Corporation, San Jose, CA). The serum concentrations of Ca, P, and alkaline phosphatase (ALP) were used as indicators of calcium homeostasis and bone problems. Serum iron and magnesium concentrations were determined as indicators of the divalent ion sequestering properties of thiram (Rath et al., 2004); alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), creatine kinase, and ALP were assayed as the markers of muscle, bone or liver damage.

2.4. Determination of serum 25-hydroxyvitamin D

25-Hydroxyvitamin D level in the blood is considered to reflect overall vitamin D status (Zittermann, 2003). Hence, we measured the serum concentration of 25-hydroxyvitamin D from 8 birds in a group which received a basal control diet with or without 100 ppm thiram, using an enzyme linked protein binding assay kit (ALPCO Diagnostics, NH, USA) according to the manufacturer suggested protocol.
Serum from thiram fed birds in trial #1 which had severe TD with a severity score of 2 was selected along with control fed birds which had normal growth plates (Fig. 1). Standards, calibrator controls, and samples were identically treated and assayed according to the manufacturer suggested protocol.

2.5. Evaluation of TD and other bone parameters

At necropsy the proximal tibia and tarsometatarsus from each bird were shaved and the incidence and severity of TD was assessed. The severity of TD was scored as 0 (growth plate normal), 1 (growth plate broadened to twice the normal width), or 2 (growth plate broadened beyond twice the normal size). The tarsometatarsal joints however, were not scored. TD index was defined as incidence times the severity as described earlier (Rath et al., 2004). After scoring for TD both left and right tibia from 12 birds in each group were harvested free of connective tissue to determine biomechanical strength and bone ash content as described earlier (Rath et al., 1999, 2000). The biomechanical strength (the load and stress at failure, the strain, and Young’s modulus) of right tibia from individual birds was determined by a three point flexural bending method using an Instron 4502 material testing machine (Instron Corp., Canton, MA). The mid diaphyseal diameter of the bones at the site of impact was measured using a dial caliper. The ash percentage was calculated using an approximately 1.5–2 cm section of mid diaphyseal bone from left tibia. The marrow was removed by flushing with water, washing thoroughly with successive changes of water and 95% ethanol, and dried for 10 h at 110 °C to record dry weight. Individual pieces of bones were then subjected to a temperature of 750 °C for 18 h in a furnace after which the ash percentage was calculated with respect to their dry weights (Rath et al., 1999, 2000).

Representative growth plates from 4 to 5 birds in each group were fixed in neutral buffered formalin for histology. Sections (5 μm) of growth plates were stained with haematoxylin and eosin for light microscopy.

2.6. Statistics

Quantitative results were expressed as mean ± SEM, analyzed by GLM procedure and separated using Duncan’s multiple range tests with SAS statistical software (SAS Institute, 1994). Differences were considered significant at p < 0.05.

3. Results

3.1. Body weight and TD index

The results from both trials 1 and 2 using 100 and 50 ppm thiram are shown in Table 1. In trial 1 when the birds were given 100 ppm thiram, their body weight gain was significantly affected regardless of dietary supplements whereas in trial 2 when thiram was reduced to 50 ppm level the BW differences were not significantly different from the control. However, in both trials, all those birds receiving thiram showed a significantly higher TD index most of which were severe. All birds that were affected with TD invariably showed the presence of the lesion in the proximal growth plates of both tibiotarsal and tarsometatarsal bones. The TD index of birds in the second trial was lower. In none of the trials was there any difference in the TD index of birds receiving any of the vitamin D supplements from control birds, and did not prevent the incidence of TD induced by thiram. Histological examination revealed large numbers of dying chondrocytes in the post-proliferative region exhibiting reduced nuclear volume, empty cytoplasm, and atrophying capillary vessels (data not shown). It was noteworthy that even when birds showed severe forms of TD in the proximal growth plate, the distal growth plate invariably appeared normal (Fig. 1).

3.2. Vitamin D levels

Measurement of blood concentrations of 25-hydroxyvitamin D in birds that received basal control diet with or without thiram showed no differences. The control birds with normal growth plate had a vitamin D concentration of 4.37 ± 0.67 nmol/L whereas those that received thiram and had severe TD had a vitamin D concentration of 4.55 ± 0.98 nmol/L (n = 8 per group).

Fig. 1. Tibial bones of chickens given control (left) or a thiram containing diet (right). Proximal growth plate (top) of the thiram-fed bird shows tibial dyschondroplasia where as the distal growth plate remains free of disease.
3.3. Serum chemistry

The results of the second trial are shown in Table 2. Except in SA group in the trial #2 where thiram significantly decreased both Ca and P levels, there were no substantial differences in serum concentrations of Ca, P, alkaline phosphatase (ALP) between other treatment groups that would indicate any disorders in mineral metab-

Table 2
Blood chemistry of birds fed different vitamin D metabolites with or without 50 ppm thiram (trial #2) (n = 8)

<table>
<thead>
<tr>
<th>Additives (per kg basal diet)</th>
<th>Ca (mg/dL)</th>
<th>P (mg/dL)</th>
<th>ALP (×10^3 U/L)</th>
<th>CK (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10.6 ± 0.2&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>5.73 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.5 ± 4.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>151.1 ± 24.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>+Thiram</td>
<td>11.2 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.2 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.2 ± 4.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>157.9 ± 11.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin D3, 4000 IU</td>
<td>10.4 ± 0.3&lt;sup&gt;c,e&lt;/sup&gt;</td>
<td>6.3 ± 0.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>36.6 ± 6.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>188.1 ± 28.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin D3, 4000 IU + thiram</td>
<td>11.3 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.4 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.3 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>127.4 ± 11.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hy-D, 63 μg</td>
<td>10.1 ± 0.2&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>6.1 ± 0.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>29.9 ± 3.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>126.2 ± 12.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hy-D, 63 μg + thiram</td>
<td>10.8 ± 0.4&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>6.2 ± 0.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>46.6 ± 5.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>111.8 ± 11.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1,25 (OH)&lt;sub&gt;2&lt;/sub&gt;D&lt;sub&gt;3&lt;/sub&gt;, 5 μg</td>
<td>10.6 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.3 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.5 ± 4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>171.2 ± 12.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1,25 (OH)&lt;sub&gt;2&lt;/sub&gt;D&lt;sub&gt;3&lt;/sub&gt;, 5 μg + thiram</td>
<td>11.2 ± 0.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>6.0 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.5 ± 4.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>151.0 ± 21.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PAM, 10 μg</td>
<td>10.6 ± 0.2&lt;sup&gt;c,e&lt;/sup&gt;</td>
<td>6.3 ± 0.3&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>42.9 ± 6.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>157.7 ± 25.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PAM, 10 μg + thiram</td>
<td>10.0 ± 0.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.7 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.6 ± 3.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>125.4 ± 23.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Solbone A, 100 μg</td>
<td>10.5 ± 0.3&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>6.7 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.9 ± 4.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>123.5 ± 7.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Solbone A, 100 μg + thiram</td>
<td>9.3 ± 0.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.7 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.1 ± 4.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>140.1 ± 9.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values in a column with no common superscripts denote significant differences (P ≤ 0.05).
olism or bone problems. Similarly, there were no dramatic differences in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), creatine kinase, ALP concentrations to suggest muscle, liver, or bone damaging effects of thiram. Serum magnesium and iron concentrations between different groups were not different except that PAM and SA treated birds showed lower Fe values (data not shown).

3.4. Bone parameters

The effects of different feed treatments on bone diameter, mechanical strength and ash percentage in trial #2 are shown in Table 3. In trial 1, with 100 ppm thiram, the bone diameters were significantly lower than their control fed cohorts reflecting the trends of body weight differences and a decrease in bone strengths by thiram as indicated by lower yield stress values (data not shown). However, in the second trial with a moderate level of thiram the differences in the bone diameters were less pronounced although there existed some sporadic differences in various parameters for example the bone strength of SA treated control birds were highest and Young’s modulus values in PAM treated birds were significantly different between control and thiram treated birds. Nonetheless, the trends did not reflect clear cut changes (Table 3). Thiram feeding also decreased the bone ash content of birds that were treated with either vitamin D3, or Hy-D, or 1,25-dihydroxyvitamin D3.

4. Discussion

The results of these studies show that a brief exposure to thiram induces significantly high levels of TD and decreases the body weights at higher concentrations but it does not affect blood 25-hydroxyvitamin D concentration nor Ca, P, or alkaline phosphatase levels. Also, none of the vitamin D supplements were able to provide protection against the TD-inducing effects of thiram. The possibility of involvement of Ca homeostatic mechanisms in the pathogenesis of TD has been suggested to include vitamin D deficiency due to either a systemic or a local dysregulation of vitamin D metabolism (Farquharson, 2002). Inadequate dietary vitamin D3 or its metabolism in organs such as liver and kidney because of xenobiotic interactions or other adverse environmental constraints can lead to insufficient hydroxylation and a disruption of local metabolism in the growth plate due to vitamin D receptor dysfunction leading to the pathogenesis of TD (Edwards, 1990, 2000; Berry et al., 1996; Xu and Soares, 1997; Webster et al., 2003). In light of the fact that vitamin D regulates many aspects of growth plate development including calcification (Brinthurst et al., 2003; Nilsson et al., 2005) and that the chondrocytes actively metabolize vitamin D (Boyan et al., 2002) such propositions are logical. However, a majority of the experimental studies reported on the pathogenesis of TD were conducted using chicks that received either a Ca or vitamin D compromised diet or used tissues from affected growth plates (Edwards, 1990, 2000; Mitchell et al., 1997; Rennie and Whitehead, 1996; Farquharson, 2002; Xu and Soares, 1997; Webster et al., 2003; Webster et al., 2004). Studies in birds with naturally occurring TD or in experimental models such as used in the current experiment, there is no evidence of dysregulations of Ca homeostasis (Leach and Lilburn, 1992; Rath et al., 2004). The apparent down regulation in the levels of vitamin D receptors or other growth factors, and enzymes relating to growth plate development in the dyschondroplastic tissues or its resistance to the actions of vitamin D (Thorp, 1994; Xu and Soares, 1997; Farquharson, 2002; Webster et al., 2003) can be attributed to nonviable chondrocytes in the lesion (Rath et al., 1994). Because atypical cell death in the TD-affected growth plate being a major feature of both naturally occurring and experimentally-induced TD (Praul et al., 1997, 2000; Rath et al., 1998, 2005), a down regulation of vitamin D or other cellular metabolism observed in the TD-affected tissues are likely to be secondary to the disease than a primary cause.

In the present study, feeding birds with extra levels of different vitamin D3 products failed to prevent birds from getting TD due to thiram. These results are in contrast with the conclusions obtained by other investigators who, using diets with compromised levels of Ca: P to induce TD, have obtained a certain degree of reprieve from the disease particularly using the active metabolite 1,25-dihydroxyvitamin D3 (Edwards, 1990, 2000; Rennie and Whitehead, 1996; Xu and Soares, 1997). It is likely that under those conditions where TD is induced using hypocalcemic or low vitamin D diets the TD may be secondary to rickets which could be mitigated by correcting calcium homeostasis.

A remarkable aspect of TD-induced by thiram is its overall presentation both in terms of morphological and histological features including its lack of effect in the distal end of both tibial or tarsometatarsal joints which remain unaffected as in the naturally occurring disease. This could mean that if there were systemic errors of vitamin D metabolism associated with TD, it should cause pathological changes in other growth plates of skeletal system. It appears to affect exclusively the weight bearing joints. However, no such effects have been reported either in naturally occurring or in experimentally-induced disease. By moderating the dose of thiram, there was no large effect on bone biomechanical strength although some sporadic changes in bone ash contents or other parameters were noted without any clear cut trend. Besides, the birds did not cease to grow albeit they failed to gain full body weight on treatment with higher concentrations of thiram.

TD is a disease of pluricausal origin. A wide variety of factors, including genetic predisposition, nutritional deficiencies, and environmental constraints like over crowding, poor litter conditions, mycotoxins and pesticide contaminations, have been linked to the etiology of TD (Huff, 1980; Leach and Lilburn, 1992; Orth and Cook, 1994; Sanotra et al., 2001; Pines et al., 2005). Regardless of the
origin of the disease at the downstream, they all lead to the same stereotypic lesions in the proximal growth plates of leg bones suggesting that some uniform developmental disturbances may underlie the malformation. Ischemia of the growth plate due to chronic immobility may cause localized vascular atrophy leading to a failure of osteogenesis and causation of chondrocyte death. Inadequate mobility of birds because of environmental or experimental conditions that cause leg weakness and reluctance to walk may lead to prolonged rest and angiogenesis dysfunction. Osteomalacia and rickets are some of the major causes of muscle weakness and pain (Skaria et al., 1975; Prabhalia et al., 2000; Bringhurst et al., 2003) as are several other nutritional deficiencies (Motlagh et al., 2005), and poor environmental conditions (Dawkins et al., 2004) which lead to leg problems. Sciatic neuropathy causes muscle weakness and pain that are induced on exposure to thiram or similar other dithiocarbamates (FIFRA report, 2001). Local ischemia of proximal growth plates during the period of intense growth leading to nutrient deprivation and localized cell death may result in the failure of osteogenesis and the retention of growth plate cartilage because the dead cells may not be easily accessible to phagocytes to be removed promptly. We have shown that there is a certain age dependency in the induction of TD by thiram (Rath et al., 2004) and that treatment with thiram leads to death in capillary vessels within 48 h which later lead to chondrocyte death (Rath et al., 2004, 2005).

While the thiram-induced TD appears to be similar to its naturally occurring counterpart, it is possible that there are other mechanisms that cause the failure of transitional chondrocytes to complete the differentiation process leading to TD-like lesion. In spontaneously occurring disease multiple factors may participate and deficiencies of certain nutritional factors such as vitamin D could precipitate the disease. In conclusion, these results show that vitamin D deficiency may not be a primary cause for the development of TD nor it may be a remedy for all forms of TD.

Acknowledgements

We thank Dr. K. Powell (DSM) for the supply of 25-hydroxyvitamin D (HyD premix), Dr. H. Bachmann (Harbonis AG, Switzerland) for SA and PAM. We acknowledge the technical assistance of David Horlick, Sonia Tsai, Scott Zornes, Dana Bassi, and Wally McDonner.

References
