Anti-thyroid methimazole in an acidosis-induced retinopathy rat model of retinopathy of prematurity

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Purpose: Methimazole (MMI), an anti-thyroid drug known to reduce serum levels of L-thyroxine (T4) and insulin-like growth factor-1 (IGF-1), has been previously reported to increase the incidence of neovascularization (NV) in an oxygen-induced retinopathy (OIR) model of retinopathy of prematurity (ROP) in rats. We investigated the effect of MMI on the incidence and severity of NV in a non-oxygen-induced model of ROP, acidosis-induced retinopathy (AIR).

Methods: Newborn Sprague Dawley rats were raised in expanded litters of 25 in room air for four or ten days under one of the two following conditions: (1) Our established model of AIR (acidosis via NH4Cl gavage (10 mmol/kg) twice daily from days 2 to 7, followed by two days of recovery) or (2) MMI (given as a 0.1% solution to nursing mothers) in the above AIR model. Left eyes were fixed, and retinas were dissected and ADPase-stained. Flat mounted retinas were graded in a masked manner for presence and severity of NV, and retinal vascular areas were quantified. Serum IGF-1 and T4 concentrations were measured by radioimmunoassay on days 4 and 10. Arterial blood pH measurements were performed on day 4.

Results: The incidence and severity of NV were similar between AIR and MMI-AIR rats (incidence: 24% and 33%). Serum IGF-1 concentrations in 10 day MMI-AIR rats were significantly lower than untreated non-acidotic controls (medians: 158 ng/ml and 207 ng/ml; p=0.03). Serum IGF-1 concentrations were similar between 10 day AIR rats and untreated non-acidotic controls (medians: 189 ng/ml and 207 ng/ml; p>0.9).

Conclusions: MMI does not increase the incidence or severity of NV in an AIR neonatal rat model of ROP. Although serum IGF-1 has been considered permissive for NV in immature retinas, supranormal concentrations of serum IGF-1 may not be necessary for abnormal retinal angiogenesis. Further studies are warranted on the roles of serum IGF-1 and L-thyroxine in the pathogenesis of ROP.

Retinopathy of prematurity (ROP) is a blinding disease of premature infants characterized by abnormal retinal angiogenesis. Recently, decreased serum insulin-like growth factor-1 (IGF-1) [1] and L-thyroxine (T4) [2] concentrations have been implicated in the induction of abnormal angiogenesis in immature retinas and ROP. Hellstrom [1] reported lower initial concentrations of serum IGF-1 in premature infants who subsequently developed the most severe ROP. Berkowitz [2] reported an increased incidence of NV in an oxygen-induced retinopathy (OIR) model of ROP when hypothyroidism was induced in neonatal rats using methimazole (MMI) given to nursing mothers.

Although OIR is commonly used as a model for ROP, we have previously described an alternative model of ROP: Acidosis-induced retinopathy (AIR) in the neonatal rat [3-5]. We have also reported that MMI alone induces preretinal neovascularization (NV) in neonatal rats, associated with an early reduction of serum IGF-1 and T4 [6]. We hypothesized that exposing neonatal rats to MMI would increase the incidence and severity of acidosis-induced retinopathy. No previous study has addressed the possible effect of MMI on acidosis-induced retinopathy.

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METHODS

All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Care and Use Committee at our institution.

Animals: Pregnant Sprague Dawley rats were obtained from Harlan (Indianapolis, IN). Dams received a standard laboratory diet and either water or a 0.1% solution of methimazole (MMI) ad libitum for the entire duration of the study. Light was cycled on a 12 h on/12 h off schedule and the room temperature was maintained at approximately 21 °C. Newborn pups from dams delivering on the same day were raised in expanded litters of 25. We have previously shown that standardizing litter size is important in studies using rat models of ROP [7] and that raising neonatal rats in expanded litters results in increased incidence and severity of NV in rat models of ROP [8,9]. All animals were raised in room air, and all neonates were weighed daily.

Retinopathy study animals: Rats were exposed to one of two conditions and sacrificed on either day 4 or day 10 of life. In the first condition, our standard model of AIR [3], NH4Cl gavage was given (10 mmol/kg) twice daily to each newborn rat (n=125), from either days 2 to 4 (4 day AIR rats; n=25) or days 2 to 7, followed by two days of recovery (10 day AIR rats, n=100). In the second condition, rats were exposed to MMI (Sigma-Aldrich, St. Louis, MO) given as a 0.1% solu-
tion in the drinking water of nursing dams for the duration of the study, in addition to the acidosis regime induced by gavage to the neonatal rats described above (n=50 for 4 day MMI-AIR rats and n=100 for 10 day MMI-AIR rats). The AIR rats thereby served as controls for the MMI-AIR rats, specifically to investigate whether MMI had an additive effect on acidosis. MMI solution was changed every other day and opaque bottles were used to prevent photo-deterioration. To compare these new AIR data and new MMI-AIR data to untreated controls, we chose to use previous untreated control data from animals studied in a concurrent parallel experiment [6]. These secondary untreated non-acidotic controls have been previously published [6] and served only as concurrent retinal grading controls, and controls for T4 and IGF-1 levels, in the present study.

Rats were sacrificed on day 10 because day 10 was found to be the time of maximum neovascularization in previous studies of AIR [5]. The intermediate time point, day 4, was chosen to represent the condition during drug exposure.  

**Serum IGF-1, serum T4, and blood gas animals:** Additional parallel litters were studied to determine serum IGF-1, serum T4, and arterial blood gas levels. It was not possible to collect serial blood samples across days on any single animal since obtaining sufficient blood from these neonatal rats is a terminal event and all animals were sacrificed immediately after blood collection.

275 rats were raised in expanded litters of 25 and exposed to identical treatments as described above (4 day AIR, n=50; 10 day AIR, n=25; 4 day MMI-AIR, n=75; 10 day MMI-AIR, n=25). Additional control data from untreated non-acidotic rats were taken from another study [6] which ran concurrently with the current study. These new AIR data and new MMI-AIR data to untreated controls (n=25). Additional control data from untreated non-acidotic rats have been previously published [6] and served only as concurrent retinal grading controls, and controls for T4 and IGF-1 levels, in the present study.

The incidence of neovascularization was similar between 10 day AIR rats and 10 day MMI-AIR rats, despite the MMI-rats having a smaller ratio of vascularized to total retinal area. Any small discrepancies, between number of rats surviving and the denominator for incidence, were due to 3 retinas being ungradable. AIR represents acidosis-induced retinopathy, MMI represents Methimazole. The asterisks denote non-acidotic control values taken from a previously published study [6] that ran simultaneously with the current study.

### Table 1. Incidence and Severity of Neovascularization

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Survival</th>
<th>Incidence of NV</th>
<th>Severity of NV in affected rats in clock hours</th>
<th>Ratio of vascularized to total area (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 day AIR</td>
<td>19 of 25 (76%)</td>
<td>1 of 19 (5%)</td>
<td>1</td>
<td>41 ± 6%</td>
</tr>
<tr>
<td>10 day AIR</td>
<td>69 of 100 (69%)</td>
<td>16 of 67 (24%)</td>
<td>1 to 6 (median 1.5)</td>
<td>91 ± 7%</td>
</tr>
<tr>
<td>4 day MMI-AIR</td>
<td>43 of 50 (86%)</td>
<td>0 of 43 (0%)</td>
<td>N/A</td>
<td>36 ± 7%</td>
</tr>
<tr>
<td>10 day MMI-AIR</td>
<td>52 of 100 (52%)</td>
<td>17 of 52 (33%)</td>
<td>1 to 2 (median 1)</td>
<td>79 ± 8%</td>
</tr>
<tr>
<td>4 day Control</td>
<td>24 of 25 (96%)*</td>
<td>0 of 23 (0%)*</td>
<td>N/A</td>
<td>50 ± 6%*</td>
</tr>
<tr>
<td>10 day Control</td>
<td>23 of 25 (92%)*</td>
<td>0 of 23 (0%)*</td>
<td>N/A</td>
<td>91 ± 4%*</td>
</tr>
</tbody>
</table>

The incidence of neovascularization was similar between 10 day AIR rats and 10 day MMI-AIR rats, despite the MMI-rats having a smaller ratio of vascularized to total retinal area. Any small discrepancies, between number of rats surviving and the denominator for incidence, were due to 3 retinas being ungradable. AIR represents acidosis-induced retinopathy, MMI represents Methimazole. The asterisks denote non-acidotic control values taken from a previously published study [6] that ran simultaneously with the current study.
nal areas were traced in a masked manner using Analyze image analysis software (version 6.0.3b; AnalyzeDirect, Inc., Lenexa, KS) [13] and the ratio of vascular to total retinal area was calculated [6].

Analysis of serum IGF-1 and T4: Carotid blood samples were obtained from randomly selected pups (8 to 27 samples per condition at day 4 and day 10). As described previously [6], for the blood collection, pups were anesthetized with inhaled CO₂. Under dissecting microscopy, the left carotid artery was exposed through a skin incision. The artery was transected and 100 to 400 µl of arterial blood was collected with a 21-gauge Vacutainer blood collection set (Beckton Dickinson and Co., Franklin Lakes, NJ). Blood samples were allowed to clot on ice prior to centrifugation at 3000x g for 5 min. Serum was removed and stored at -80 °C until analyzed.

Acid-ethanol extraction was performed to remove IGF-1 binding proteins [14] as described previously [6]. Briefly, 200 µl of acid-ethanol mixture (87.5% ethanol: 12.5% (v/v) 2 M hydrochloric acid) was added to 50 µl of serum in 1.5 ml polypropylene tubes. The mixture was vortexed and incubated at 4 °C for 30 min and centrifuged at 13000x g for 15 min. 200 µl of supernatant was neutralized with 80 µl of 0.86 M Tris, vortexed, and incubated at -20 °C for 1 h, followed by centrifugation at 13000x g for 10 min. Supernatant was removed, aliquoted, and sent to the National Hormone and Peptide Program in Torrance, CA, where IGF-1 radioimmunoassays were performed.

Frozen, whole serum was shipped to the Yerkes Core Endocrine Laboratory (Atlanta, GA) where serum T4 radioimmunoassays were performed (6 to 18 samples per condition). The sensitivity of the T4 radioimmunoassay was 1.0 µg/dl.

Arterial blood gas study: Pups were randomly selected from studies ending on day 4 (5 to 8 samples per condition). Arterial blood samples were collected as described previously [4]. All pups underwent urethane anesthesia via an intraperitoneal injection (1.5 g/kg) in the flank, near the lumbar vertebrae and hind limb, while breathing room air. Body temperature was preserved using 39 °C warming pads (Deltaphase isothermal pad, Braintree Scientific, Braintree, MA). The left carotid artery was exposed through a skin incision. The artery was transected and 50 µl of arterial blood was collected in a heparinized microhematocrit capillary tube (Fischer Scientific, Pittsburg, PA) and analyzed immediately using a blood gas analyzer (Synthesis 10, Instrumentation Laboratory, Lexington, MA).

Statistical analysis: Incidence of NV was compared between groups using Fisher’s exact tests. Severity of NV was compared using Wilcoxon tests. Rat weights were compared at each day using ANOVA and post hoc Student’s t-tests with Bonferroni corrections. Bonferroni-corrected p values of less than 0.05 were considered statistically significant. The ratios of vascularized to total retina area, and blood pH values, were compared between groups of interest using ANOVA and post hoc Student’s t-tests with Bonferroni corrections. Serum IGF-1 and T4 concentrations were compared between groups with Kruskal-Wallis tests and post hoc Wilcoxon tests with Bonferroni corrections. All statistical analysis was performed using SAS (release 6.12 for Windows, SAS Institute, Cary, NC).

RESULTS

Animal survival and retinas analyzed: As in our previous studies using expanded litters, not all rats survived (Table 1). All gradable retinas from rats surviving to study completion were included in the analyses. Only 1.3% (3 of 230) of ADPase-stained retinas were ungradable and were therefore excluded.
from analysis: Two from the 10 day AIR rats and one from a 4
day control rat. One rat from the 10 day MMI-AIR group was
excluded from analysis because it failed to develop a left eye.

**Incidence of neovascularization:** The incidence of retin-
opathy at day 10 was similar between AIR and MMI-AIR rats (24% and 33%, respectively; p=0.3; Table 1; Figure 1). Only
one retina (1 of 19) from the 4 day AIR rats was graded as
positive for NV, while none of the 4 day MMI-AIR and none
of the untreated non-acidotic control retinas [6] (4 day or 10
day) had NV.

**Severity of neovascularization:** Severity of NV in the 10
day AIR retinas ranged from 0 to 6 clock hours, with the ma-
jority of retinas (14 of 16) having 1 or 2 clock hours of NV
when present. In the 10 day MMI-AIR rats, severity ranged
from 0 to 2 clock hours, with the majority (13 of 17 retinas)
having 1 clock hour of NV when present (Table 1).

**Retinal vascular areas:** Quantitative analysis of ADPase-
stained retinas revealed significantly reduced vascular areas
in the 4 day AIR and 4 day MMI-AIR retinas compared to 4
day untreated non-acidotic controls (41±6% and 36±7% com-
pared to 50±6%; p<0.001 for each comparison; Table 1). At
day 10, the retinal vascular areas were similar in AIR and un-
treated non-acidotic controls [6] (91±7% and 91±5%, respec-
tively [6]), but significantly less in MMI-AIR rats (79±8%)
compared to both AIR and untreated non-acidotic controls
(p=0.0003).

**Serum IGF-1:** At day 10, MMI-AIR rats had significantly
lower serum IGF-1 concentrations compared to untreated non-
acidotic controls [6] (medians: 158 ng/ml and 207 ng/ml [6];
p=0.03; Figure 2). However, serum IGF-1 concentrations were
not significantly different between 10 day AIR rats and un-
treated non-acidotic controls (medians: 189 ng/ml and 207 ng/
ml [6]; p>0.9). In contrast to differences seen at day 10, serum
IGF-1 concentrations were similar among groups at day 4
(medians: 161 ng/ml for AIR, 196 ng/ml for MMI-AIR, and
133 ng/ml for untreated non-acidotic controls [6], p=0.07).
These IGF-1 concentrations from the non-acidotic animals
treated with MMI had been previously published [6].

**Serum T4:** At day 10, T4 concentrations in MMI-AIR rats
were significantly lower than AIR rats (medians: <1 µg/
dl compared to 2.08 µg/dl; p=0.01, Figure 3). These values
were also lower than previous published non-acidotic controls
[6] (medians: <1 µg/dl compared to 1.89 µg/dl; p=0.02, Fig-
ure 3). In contrast, serum T4 concentrations measured at day
4 in AIR, MMI-AIR, and untreated non-acidotic controls were
not significantly different from each other (Figure 3).

**Arterial blood pH:** MMI-AIR rats were no more or no
less acidic than AIR rats with mean arterial pH values of
7.11±0.11 and 7.18±0.12, respectively (p=0.9; Table 2). Age-
matched controls had normal mean arterial blood pH values
of 7.45±0.04. In previous studies, we have found that rats
treated with MMI alone had normal arterial blood pH mea-
surements (7.42±0.04 mm Hg) [15].

**Animal growth:** Growth retardation was significant in
MMI-AIR rats compared to both AIR and untreated, non-aci-
dotic controls [6] beginning on day 3 of life (p=0.005 for each
day). AIR rat weights were similar to untreated non-acidotic
controls [6] on all but day 4 (Figure 4).

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**Figure 2.** Serum insulin-like growth factor-1 (IGF-1) levels. Serum insulin-like growth factor-1 (IGF-1) concentrations from individual neonatal rat blood samples are represented by box-and-whiskers plots (19, 27, and 19 samples for AIR, MMI-AIR, and untreated non-acidotic controls, respectively, at day 4; 8 samples for each condition at day 10). Serum IGF-1 was lower in MMI-AIR rats compared to both AIR and untreated non-acidotic controls at day 10. No difference in serum IGF-1 is seen between AIR and control rats. The untreated non-acidotic control values were taken from a previously-published study [6], which ran simultaneously with the current study.

**Figure 3.** Serum L-thyroxine (T4) levels. Serum L-thyroxine (T4) concentrations from individual neonatal rat blood samples are represented by box-and-whiskers plots (11, 12, and 18 samples for control, AIR, and MMI-AIR, respectively, at day 4; 6 samples for each condition at day 10). Serum T4 concentrations were below detectable levels in MMI-AIR rats by day 10. No difference in T4 concentrations was seen between AIR and control rats. The untreated non-acidotic control values were taken from a previously-published study [6], which ran simultaneously with the current study.
**DISCUSSION**

In the present study, we found that treatment with the anti-thyroid drug, MMI, which decreases serum concentrations of T4 and IGF-1, did not increase the incidence or severity of NV in our neonatal rat AIR model of ROP. Neovascularization was seen in the presence of reduced serum concentrations of IGF-1 in the 10 day MMI-AIR rats and normal concentrations of IGF-1 in AIR rats. We conclude from these data that AIR may not be mediated by serum IGF-1. Given the similar incidence and severity of NV in AIR and MMI-AIR rats, it is possible that acidosis and MMI may be working through a common pathway in the pathogenesis of preretinal NV in immature rats.

Previous studies have suggested that IGF-1 has an important role in the development of normal retinal vasculature [16] and in the pathogenesis of ROP [1]. Early sustained low concentrations of serum IGF-1 in human premature infants, followed by a subsequent increase, is strongly associated with development of severe ROP characterized by preretinal NV [1]. We found that in AIR rats, serum IGF-1 concentrations do not differ significantly from untreated non-acidotic controls at days 4 or 10, despite a 24% incidence of NV in AIR rats at day 10. These data suggest that AIR is not mediated by changes in serum IGF-1, in contrast to the association reported in clinical ROP [1]. In addition, MMI-AIR rats, with a 33% incidence of NV, had significantly lower serum IGF-1 concentrations compared to controls [6] at day 10. In fact, serum IGF-1 concentrations at day 10 were no higher than concentrations measured at day 4, again suggesting that NV in these rats may not be mediated solely by increasing serum IGF-1. Nevertheless, we have not addressed changes in local retinal IGF-1. It is possible that either or both MMI-induced retinopathy or acidosis-induced retinopathy might be mediated by local increases in IGF-1, but this is beyond the scope of the present study.

In the present study, we found no difference in the incidence or severity of NV with the addition of MMI to the AIR model. This result is surprising in light of our recent report that MMI alone induces NV [6,15] and that of Berkowitz et al. [2] who reported that MMI exposure in a neonatal OIR model of ROP increases the incidence of NV. It is possible that these disparate results may have been influenced by the source of research animals. Specifically, the source of our Sprague Dawley rats used in MMI studies (Harlan, Indianapolis, IN) was different from that of Berkowitz (Hilltop, Scottsdale, PA). We have previously reported differences in NV response between rats from different vendors [17]. The difference between OIR and AIR in response to MMI might also be explained by the AIR model reaching some threshold of maximum NV. Nevertheless, we feel this is unlikely since we have previously reported an incidence of NV up to 59% in a similar model [4], illustrating both biologic variability of the model and potential for more severe NV. In addition, in Berkowitz’s study of “OIR of low retinal neovascular incidence” [2] the litter sizes were smaller than the standardized expanded litters of our study. Nevertheless, we had not reached a maximum insult based on body weight, mortality rate or incidence of neovascularization. Another possible explanation for the lack of an additive effect of MMI on AIR is that both hypothyroidism [18] and acidosis [19] similarly increase retinal vascular permeability and break down the blood-retina barrier. Such an increase in permeability may be a threshold effect resulting in leakage of growth factors across the blood-retina barrier, as observed in other proliferative vascular diseases, such as diabetic retinopathy [20]. However, increased vascular permeability as the cause of NV in these models is speculative, and potentially supportive studies [18] are based on models of different duration from ours.

Another possible explanation is that MMI may be playing dual and opposing roles by (1) independently inducing NV [6,15] and (2) ameliorating the effects of acidosis by acting as an anti-inflammatory [21-24] and an anti-oxidant [25,26]. It is also possible that MMI and acidosis are acting via a common molecular pathway and so their effects are not additive. OIR, on the other hand, may be acting through a different pathway, and thus, the addition of MMI in OIR causes an increase in preretinal NV, as reported by Berkowitz [2].

It is noteworthy that MMI-AIR rats had similar levels of acidosis compared to AIR alone rats. In previous studies [15] we found that MMI alone does not induce acidosis. Since MMI does not appear to induce NV via acidosis, it is intriguing that MMI and acidosis are not additive. It is also noteworthy that MMI-AIR rats had similar bicarbonate ion concentrations compared to AIR alone rats (Table 2, p=0.18), suggesting that the bicarbonate ion concentration alone is not predictive of NV. Further speculation on the molecular pathways of MMI-induced NV and acidosis-induced NV is premature and awaits further studies.

The role of the thyroid hormone axis in the development of the central nervous system [27,28], the eye [29], and the retina [29-32] has been the focus of several previous studies. Congenitally hypothyroid rats had significantly smaller and thinner retinas, with fewer dividing progenitor cells [31,32]. In addition, Berkowitz et al. [2] reported a decrease in peripheral vascular density in neonatal rats exposed to MMI. In our present study, both AIR and MMI-AIR rats had significantly reduced retinal vascular areas compared to untreated non-acidotic controls at day 4. By day 10, retinal vascular areas continued to be reduced in MMI-AIR when compared to non-acidotic controls, whereas AIR rats had similar retinal vascular areas compared to untreated non-acidotic controls. Since the incidence of NV in AIR and MMI-AIR was similar, we speculate that early retardation of retinal vasculature, rather than late or prolonged retardation, is important in the develop-

![Table 2. Arterial blood pH and blood gases](https://molvis.org/molvis/v11/a108/)

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>pH</th>
<th>PP oxygen (mm Hg)</th>
<th>PP carbon dioxide (mm Hg)</th>
<th>Bicarbonate (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 day AIR (n=8)</td>
<td>7.18 ± 0.12</td>
<td>100 ± 7</td>
<td>28.6 ± 6.6</td>
<td>11.1 ± 3.5</td>
</tr>
<tr>
<td>4 day MMI-AIR (n=5)</td>
<td>7.11 ± 0.11</td>
<td>104 ± 19</td>
<td>23.4 ± 2.1</td>
<td>7.7 ± 1.3</td>
</tr>
<tr>
<td>4 day Control (n=8)</td>
<td>7.45 ± 0.04</td>
<td>78 ± 6</td>
<td>42.8 ± 4.5</td>
<td>30.0 ± 1.4</td>
</tr>
</tbody>
</table>

The severity of induced acidosis was similar between AIR and MMI-AIR rats.

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opment of subsequent preretinal NV. This suggestion is also supported by our previous observations that in MMI-induced retinopathy [6] retinal vascular areas were retarded early (day 4), but were no different than untreated non-acidotic controls at day 10, when NV was observed. Taken together, these observations lead us to hypothesize that early retardation of retinal vascular development may be part of a common pathway between acidosis-induced and hypothyroidism-induced NV.

Regarding animal growth, early growth retardation was observed in both AIR and MMI-AIR rats, whereas severe growth retardation continued only in the MMI-AIR rats. Such profound growth retardation would be expected based on the systemic hypothyroid effect of MMI. Again, it is noteworthy that the incidence and severity of NV is similar in AIR and MMI-AIR. This finding suggests that continued growth retardation is not necessary for induction of NV and that growth retardation per se does not entirely account for differences in neovascularization. Nevertheless, the early growth retardation in both AIR and MMI-AIR rats is consistent with our hypothesis that early retardation of retinal vascular development is part of a common mechanism for acidosis and hypothyroidism-induced NV.

One weakness in this present study is the significant mortality rate in 10 day MMI-AIR and 10 day AIR rats. It is possible that NV was actually underestimated in these animals since the sickest rats may be more likely to develop NV. Unfortunately, it is not possible to control for survival rate in these studies, and autolysis of retinal tissues precludes analysis of NV in rats that die during the course of an experiment. Our study would also have been improved if IGF-1 and T4 could have been compared between rats with and without NV. It is also possible that different effects might be seen in alternative (i.e., NH4Cl-independent) AIR models and future studies might address these questions in such alternative models (e.g., acetazolamide-induced AIR [4]). It should also be noted that our model of using MMI postnatally to reduce serum IGF-1 in neonatal rats is not entirely similar to the human premature infant who is born with already subnormal serum IGF-1. Further studies might also investigate the interaction of VEGF and IGF-1 in these conditions.

In summary, we found that exposure of neonatal rats to MMI and acidosis does not have an additive effect in either incidence or severity of preretinal NV, in contrast to a previous report of an additive effect of oxygen and MMI [2]. Although we found a significant suppression of serum IGF-1 and T4 in MMI-AIR rats, this suppression was not seen in AIR, suggesting that NV in AIR may not be mediated by changes in serum IGF-1 that have been reported in human ROP [1]. Further studies are warranted to investigate the role of IGF-1 and T4 in the pathogenesis of ROP.

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Figure 4. Animal growth. Rat weights (mean±SD) for all neonatal animals in the 10 day experiments where measurements were made daily (5 AIR litters and 5 MMI-AIR litters). Previously published data [6] from 2 untreated non-acidotic control litters are also provided for comparison. Growth retardation was significant in all MMI-AIR rats beginning at day 3 (asterisk represents p<0.005). Number of surviving rats at each day is given in the table below the graph. The first row corresponds to the previously-published control animals [6], the second to the current AIR animals, and the third to the current MMI-AIR animals.
REFERENCES


