Age Dependent Effects of Oxytetracycline in ob/ob Mice

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Summary. The effects of daily oxytetracycline injections on the metabolism and growth of prepubertal, 10 and 24 week old ob/ob and lean mice were examined. Drug treatment (100 mg/kg) between 17 and 28 days of age similarly reduced body weight gain and skeletal growth of both ob/ob and lean mice, but had little effect on obesity, serum glucose, or IRI levels. In older obese mice, 6 days of oxytetracycline led to substantial reductions of serum glucose, serum IRI, and obesity when compared with either ad libitum fed obese mice or food-restricted ob/ob mice serving as weight loss controls. In lean mice oxytetracycline also significantly reduced body weight and serum glucose, while no effects on obesity or IRI levels were noted. The present data show that oxytetracycline reduces the severity of many of the diabetic symptoms of ob/ob mice, but that only the ameliorative effects on obesity and hyperinsulinaemia appear to be specific to the ob/ob genotype.

Key words: Diabetes, obese-hyperglycaemic mice, insulin, hyperglycaemia, Terramycin, obesity, oxytetracycline.

Mature C57BL/6J ob/ob mice display many metabolic and behavioural disturbances including obesity, hyperinsulinaemia, hyperglycaemia, hyperphagia, insulin resistance, and reduced activity [1]. As yet, it is not clear which of these abnormalities are direct expressions of the mutation, but examination of the early development of the obese-hyperglycaemic syndrome indicates that obesity, hyperinsulinaemia, hyperphagia, and reduced activity precede the occurrence of hyperglycaemia and insulin

resistance [2–4]. Recently, the antibiotic oxytetracycline (Terramycin) has been shown to normalize the hyperglycaemia, hyperinsulinaemia, and insulin resistance of mature ob/ob mice [5, 6] and to potentiate the effects of exogenous insulin in the treatment of experimental diabetes mellitus in dogs [7, 8]. Since, in these studies, the drug's effectiveness appeared to stem from a modification of tissue sensitivity to insulin, we examined the effects of oxytetracycline on the growth and in vivo metabolism of ob/ob mice: 1) during the early development of the syndrome when insulin resistance and hyperglycaemia have not yet appeared (17–28 days of age), 2) during the 'dynamic' phase of the syndrome when basal glucose levels reach peak values (10 weeks of age), and 3) during the late phase of the syndrome when glucose levels return toward normal but hyperinsulinaemia and insulin resistance persist (24 weeks of age).

Methods

Male and female mice (C57BL/6J) were bred from heterozygous parents (+/ob) obtained from Jackson Laboratories (Bar Harbor, Me.). The offspring were weaned at 21 days of age and housed individually in a colony room that was maintained at $23 \pm 2^\circ \text{C}$ and had a 12 h light cycle.

In experiment 1, ob/ob mice and their lean littermates received either daily IP injections of oxytetracycline (100 mg/kg, Terramycin, Pfizer Laboratories) or drug diluent (2% w/v Lidocaine – propylene glycol, 1:3 by volume) over an 11 day period between the ages of 17 and 28 days. Twenty hours after the final injection of the series the animals were deprived of food (1.5–2 h) and blood samples were taken from the retro-orbital plexus under light ether anaesthesia.

In experiment 2, 10 week old, and in experiment 3, 24 week old ob/ob and lean mice received daily IP oxytetracycline injections (50 or 100 mg/kg) during a 6-day observation period. Obese and lean control animals received diluent injections only. These groups had free access to Purina Mouse Chow and tap water throughout the observation period. A second obese control group in each experiment was food restricted to 2–3 g chow/day. Blood samples were taken as described earlier.

Serum glucose levels were determined by a glucose oxidase method (Beckman glucose analyzer). Serum IRI was measured in individual 100 µl serum samples by solid phase radioimmunoassay (Pharmacia Diagnostics, Piscataway, New Jersey). The within and between coefficients of variation of this assay using normal rat serum were 4.0% and 15.6% respectively. IRI values are reported in terms of a mouse insulin standard (Novo, Copenhagen).

Statistical tests, as noted in Results, include two-way analysis of variance (2-way ANOVA), and one-way analysis of variance within phenotypes (1-way ANOVA) in conjunction with Duncan's New Multiple Range Test [9]. Values of $p < 0.05$ were considered significant.

Results

Weanling Mice

Table 1 summarizes the effects of 100 mg/kg oxytetracycline administered daily between the ages of 17 and 28 days, on the glucose, IRI, and growth of ob/ob and lean mice. Two-way analysis of variance of the data demonstrated that obese mice maintained significantly higher rates of body weight gain than did the lean mice during the experiment ($p < 0.001$), but that oxytetracycline treatment significantly reduced weight gain during this period ($p < 0.025$). The absence of a significant treatment by phenotype interaction on body weight gain indicated that the depressions of body weight which resulted from oxytetracycline treatment were virtually identical in obese and lean mice. Skeletal growth was also significantly reduced by oxytetracycline ($p < 0.001$), and ob/ob mice were smaller than their controls ($p < 0.001$). Again, no significant treatment by phenotype interaction was noted. The reductions of skeletal growth noted during drug treatment were proportional to the changes of body weight, since no differences in the Lee Index¹ [10] of obesity were

observed between drug-treated and sham-treated mice. However, the elevated Lee Index values of ob/ob mice were maintained during the drug treatment period ($p < 0.001$).

Analysis of the glucose and IRI effects of oxytetracycline showed that the drug had only marginal effects on serum glucose ($0.05 < p < 0.1$), while no effects were noted on the IRI concentrations of either phenotype. Ob/ob mice however, did maintain higher levels of circulating IRI than their appropriate lean controls during the experiment ($p < 0.001$).

10 Week Old Mice

The effects of food restriction or of daily injections of oxytetracycline (50 or 100 mg/kg) on the growth, and glucose and IRI levels of post pubertal mice were examined during a six-day period (Table 2). Oxytetracycline had significant and apparently dose-dependent effects on the rates of body weight gain in both phenotypes but the drug did not substantially alter the skeletal growth of the groups of obese mice. In contrast food restriction of ob/ob mice caused similar reductions of body weight gain, but also depressed the rate of skeletal growth significantly ($p < 0.05$, 1-way ANOVA).

A significant effect of oxytetracycline was noted on both the serum glucose and IRI levels of ob/ob mice (all $p < 0.001$), while only the glucose concentrations of lean mice were affected by the drug. Application of Duncan's test to the ob/ob glucose and IRI values revealed that there were no reliable effects of drug dose on the systemic concentrations; nor were differences noted in the glucose levels or IRI of lean mice receiving 50 or 100 mg/kg/day.

Oxytetracycline effects were most pronounced in reducing the serum glucose of ad libitum fed ob/ob mice, as the glucose concentrations of these groups were virtually identical with those of drug-treated lean mice and were lower than those of either the untreated lean mice or of the food-restricted obese mice. The IRI levels of oxytetracycline-treated ob/ob mice were substantially lower than those of either ad libitum fed or restricted-fed ob/ob mice, but the values remained significantly higher than those of both the treated and untreated lean littermates.

24 Week Old Mice

The effects of daily 50 or 100 mg/kg oxytetracycline injections (6 days) or of food restriction on fully mature obese and lean mice are presented in Table 3.

¹ Lee Index = $\text{Body weight (g)}^{1/3} \times \text{Nasoanus length (mm)}^{-1} \times 10^3$

Table 1. Effects of daily oxytetracycline administered between 17–28 days of age on the glucose, IRI, and growth of ob/ob and lean mice

Group	N	Treatment	Δ Body wt (g) ^{b,c} (per 11 days)	Δ Nasoanus ^{b,c} Length (mm) (per 11 days)	Final ^c Lee Index	Final Serum glucose (mg/dl)	Final Serum IRI ^b (μ U/ml)
Lean	6	100 mg/kg	4.2 $\pm$ 1.0 ^a	10.3 $\pm$ 0.8	293 $\pm$ 2	198 $\pm$ 9	42 $\pm$ 11
Lean	7	sham	7.9 $\pm$ 0.7	17.1 $\pm$ 1.0	290 $\pm$ 2	208 $\pm$ 7	32 $\pm$ 10
Obese	9	100 mg/kg	6.2 $\pm$ 0.7	7.7 $\pm$ 0.6	325 $\pm$ 2	166 $\pm$ 14	181 $\pm$ 26
Obese	6	sham	10.1 $\pm$ 0.9	13.3 $\pm$ 0.5	323 $\pm$ 4	226 $\pm$ 12	251 $\pm$ 29

^a Mean $\pm$ SEM^b = Significant ($p < 0.05$) treatment effects (2-way Analysis of Variance)^c = Significant ($p < 0.05$) phenotype effects (2-way Analysis of Variance)**Table 2.** Effects of 6 days of daily oxytetracycline on the glucose, IRI, and growth of 10 week old ob/ob and lean mice

Group	N	Treatment	Δ Body wt. (g) (per 6 days) ^{a,b}	Δ Nasoanus Length (mm) (per 6 days) ^{a,b}	Final Lee Index ^{a,b}	Final Serum glucose (mg/dl) ^{a,b}	Final Serum IRI (μ U/ml) ^{a,b}
Lean	7	sham	− 0.6 $\pm$ 0.2 ^c	0.9 $\pm$ 0.1 ^c	299 $\pm$ 1 ^c	191 $\pm$ 10 ^c	55 $\pm$ 10 ^c
Lean	6	50 mg/kg	− 3.4 $\pm$ 0.4 ^d	0.7 $\pm$ 0.1 ^{cd}	294 $\pm$ 1 ^c	140 $\pm$ 11 ^d	55 $\pm$ 16 ^c
Lean	7	100 mg/kg	− 4.8 $\pm$ 0.7 ^d	0.4 $\pm$ 0.1 ^d	290 $\pm$ 1 ^c	129 $\pm$ 7 ^d	36 $\pm$ 5 ^c
Obese	12	sham	1.5 $\pm$ 0.2 ^c	1.0 $\pm$ 0.2 ^c	368 $\pm$ 4 ^c	371 $\pm$ 50 ^c	384 $\pm$ 70 ^c
Obese	13	50 mg/kg	− 3.3 $\pm$ 0.5 ^d	0.9 $\pm$ 0.1 ^c	358 $\pm$ 2 ^{cd}	151 $\pm$ 16 ^{de}	167 $\pm$ 29 ^d
Obese	13	100 mg/kg	− 6.3 $\pm$ 0.4 ^e	0.9 $\pm$ 0.2 ^c	353 $\pm$ 3 ^d	112 $\pm$ 21 ^d	93 $\pm$ 17 ^d
Obese	4	Diet restriction	− 3.2 $\pm$ 0.4 ^d	0.3 $\pm$ 0.1 ^d	364 $\pm$ 4 ^{cd}	217 $\pm$ 29 ^c	335 $\pm$ 33 ^c

^a All values presented as Mean $\pm$ SEM^b Values in columns having different superscripts are statistically different (One-way Analysis of Variance within phenotypes – Duncan's New Multiple Range Test)**Table 3.** Serum glucose, IRI, and growth of mature (24 week) ob/ob and lean mice following daily oxytetracycline for 6 days

Group	N	Treatment	Δ Body wt. (g) (per 6 days) ^{a,b}	Δ Nasoanus Length (mm) (per 6 days) ^{a,b}	Final Lee Index ^{a,b}	Final Serum Glucose (mg/dl) ^{a,b}	Final Serum IRI (μ U/ml) ^{a,b}
Lean	3	sham	− 3.0 $\pm$ 1.6 ^c	0.5 $\pm$ 0.2 ^c	298 $\pm$ 4 ^c	206 $\pm$ 19 ^c	42 $\pm$ 6 ^c
Lean	4	50 mg/kg	− 7.6 $\pm$ 1.4 ^d	0.0 $\pm$ 0.2 ^c	305 $\pm$ 5 ^c	135 $\pm$ 5 ^d	31 $\pm$ 8 ^c
Lean	3	100 mg/kg	− 9.3 $\pm$ 1.8 ^d	0.3 $\pm$ 0.1 ^c	293 $\pm$ 9 ^c	153 $\pm$ 4 ^d	32 $\pm$ 9 ^c
Obese	11	sham	− 1.2 $\pm$ 0.3 ^c	1.1 $\pm$ 0.2 ^c	372 $\pm$ 3 ^c	160 $\pm$ 9 ^c	655 $\pm$ 73 ^c
Obese	10	50 mg/kg	− 5.6 $\pm$ 0.6 ^d	1.3 $\pm$ 0.3 ^c	358 $\pm$ 4 ^d	127 $\pm$ 8 ^d	172 $\pm$ 18 ^d
Obese	9	100 mg/kg	− 8.5 $\pm$ 0.7 ^e	1.2 $\pm$ 0.3 ^c	349 $\pm$ 5 ^d	117 $\pm$ 11 ^d	183 $\pm$ 20 ^d
Obese	7	Diet restriction	− 4.6 $\pm$ 0.3 ^d	0.4 $\pm$ 0.1 ^d	367 $\pm$ 4 ^c	152 $\pm$ 8 ^c	596 $\pm$ 79 ^c

^a All values presented as Mean $\pm$ SEM^b Values in columns having different superscripts are statistically different (One-way Analysis of Variance within phenotypes – Duncan's New Multiple Range Test)

All animals lost weight during the injection period, but significantly greater weight losses were noted in the drug-treated obese and lean groups when compared with ad libitum fed control mice ($p < 0.05$, 1-way ANOVA). Obese mice food-restricted to less than 3 g/day had body weight losses similar to obese mice receiving the 50 mg/kg/day dose, but less than those receiving 100 mg/kg/day. No differences in weight loss were observed within phenotypes for the

50 and 100 mg/kg doses, nor were significant differences noted between phenotypes in their weight responses to treatment.

The rates of skeletal growth of oxytetracycline-treated obese mice were similar to sham-treated ob/ob mice, but were significantly greater than those of the drug-treated lean mice or of food restricted obese mice. Lee Index values were lower in drug-treated obese mice ($0.05 < p < 0.1$), while no con-

sistent differences were observed in the lean mice groups or between the ad libitum fed and food-restricted obese controls.

Oxytetracycline treatment reduced serum glucose levels in lean mice ($p < 0.025$) and also in obese mice when compared with either ad libitum fed or restricted-fed controls ($p < 0.05$). There were highly significant reductions of serum IRI in both the 50 and 100 mg/kg-treated ob/ob mice ($p < 0.05$), although dose had no consistent effect on the insulin concentrations. Again, as noted in 10 week old obese mice, the drug was most effective on the serum glucose levels, since the IRI levels remained significantly higher than those of the lean controls.

Discussion

Earlier reports have demonstrated that the long-term administration of oxytetracycline to animals having either experimental diabetes or genetic diabetes-obesity results in a reduced severity of the diabetic symptoms [5–8]. Since oxytetracycline has been shown to increase the *in vitro* insulin sensitivity of ob/ob mouse muscle, to “normalize” *in vivo* glucose and insulin tolerance in ob/ob mice [5, 6], and to potentiate the effects of exogenous insulin in depancreatized dogs [7, 8], the drug appears to act by increasing target cell sensitivity to the catalytic actions of insulin.

In a previous study we have described the early development of the obese-hyperglycaemic syndrome and shown that hyperinsulinaemia, obesity, and growth deficits are manifested by 17 days of age, while significant abnormalities of *in vivo* glucose metabolism, i.e. hyperglycaemia, glucose intolerance, and “insulin resistance”, do not appear until the ages of 24 to 28 days (2, unpublished observations). It is likely therefore, that the development of insulin resistance in the muscle, fat, and livers [1, 11] of these animals at approximately 4 weeks of age accounts for the *in vivo* expressions of hyperglycaemia and glucose intolerance at this time.

Considered jointly, our previous findings of a delayed development of insulin resistance in ob/ob mice and the proposed mechanisms of action of oxytetracycline suggest that the drug would be ineffective in significantly altering the metabolic disturbances of weanling ob/ob mice, but would progressively increase in effectiveness as the animal matured. Accordingly, we found here that the administration of oxytetracycline to ob/ob mice between 17 and 28 days of age had little effect on their

hyperinsulinaemia, glucose levels, or on their obesity. In contrast, oxytetracycline treatment of the older ob/ob groups significantly reduced the hyperinsulinaemia, hyperglycaemia, body weights, and obesity. Thus, the relative ineffectiveness of oxytetracycline in young ob/ob mice and its therapeutic effects in older ob/ob mice appear to be fully consistent with an effect of the drug on abnormal insulin resistance. However, since we noted virtually identical drug-induced changes on the weight and glycaemia of the older lean mice during the treatment period, it appears that oxytetracycline also affects other aspects of metabolism. For example, if we consider that the body weight and the glycaemic effects of oxytetracycline on mature ob/ob mice are mediated via a reversal of the insulin insensitivity of specific tissues we would not expect similar consequences of the drug in lean mice. Since we consistently found in this study that only the reductions of circulating IRI and obesity were specific to the ob/ob genotype, it is apparent that oxytetracycline has at least two discrete effects on *in vivo* metabolism. One effect is likely to be on the abnormal insulin secretion, and consequently on the maintenance of obesity in ob/ob mice [11]. Another effect, which is common to both phenotypes, appears to specifically alter some aspect of glucose metabolism, and is possibly related to a diminution of hepatic glycogenolysis in response to certain endogenous agents [8]. Finally, previous reports indicate that oxytetracycline treatment causes transiently reduced food intake and body weight loss in ob/ob mice [5, 6]. Since hyperphagia has been implicated in the maintenance of the obese-hyperglycaemic syndrome [12], hypophagia could partially account for the drug effects reported here. However, the programme of food restriction of obese mice that we employed caused body weight changes comparable to those of oxytetracycline-treated obese mice, but led only to marginal differences in their obesity, hyperglycaemia, and hyperinsulinaemia. Thus, neither reduced food intake nor body weight loss appears to contribute significantly to the metabolic effects of oxytetracycline.

In summary, the present data corroborate previous studies by demonstrating that oxytetracycline is an effective agent in ameliorating many of the diabetic symptoms of an animal model of maturity-onset diabetes. Along with supporting previous findings, our data additionally show that oxytetracycline: 1) is relatively ineffective in altering the hyperinsulinaemia and obesity manifested by ob/ob mice in the periweaning period, and 2) appears to have more than one discrete effect on *in vivo*

metabolism, since it affects growth and glycaemia in mature animals of each phenotype, but affects the basal insulin concentrations and obesity of only ob/ob mice.

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References

1. Bray, G. A., York, D. A.: Genetically transmitted obesity in rodents. *Physiol. Rev.* **51**, 598–645 (1971)
2. Dubuc, P. U.: The development of obesity, hyperinsulinemia, and hyperglycemia in ob/ob mice. *Metabolism* **25**, 1567–1574 (1976)
3. Bergen, W. C., Kaplan, M. L., Merkel, R. A., Leveille, G. A.: Growth of adipose and lean tissue mass in hindlimbs of genetically obese mice during preobese and obese phases of development. *Am. J. Clin. Nutr.* **28**, 157–161 (1975)
4. Joosten, H. F. P., van der Kroon, P. H. W.: Growth pattern and behavioral traits associated with the development of the obese-hyperglycemic syndrome in mice (ob/ob). *Metabolism* **23**, 1141–1147 (1974)
5. Begin-Heick, N., Heick, H. M. C.: The effect of oxytetracycline on the response to insulin of diaphragm muscle and on lipid synthesis *in vivo* and *in vitro* in the ob/ob mouse. *Diabetologia* **12**, 35–42 (1976)
6. Begin-Heick, N., Bourassa, M., Heick, H. M. C.: The effects of oxytetracycline on insulin resistance in obese mice. *Biochem. J.* **142**, 465–475 (1974)
7. Hiatt, N., Bonorris, G.: Insulin response in pancreatectomized dogs treated with oxytetracycline. *Diabetes* **19**, 307–310 (1970)
8. Hiatt, N., Bonorris, G., Coverdale, M. G.: Oxytetracycline and hypoglycemia with convulsions in pancreatectomized dogs. *Proc. Soc. Exp. Biol. Med.* **122**, 489–493 (1966)
9. Duncan, D. B.: Multiple range and multiple F tests. *Biometrics* **11**, 1–42 (1955)
10. Bernardis, L. L.: Prediction of carcass fat, water, and lean body mass for Lee's nutritive ratio in rats with hypothalamic obesity. *Experientia* **26**, 789–790 (1970)
11. Assimacopoulos, F., Jeanrenaud, J. B.: The hormonal and metabolic basis of experimental obesity. *Clin. Endocrinol. Metab.* **5**, 337–365 (1976)
12. Genuth, S. M., Przybylski, R. J., Rosenberg, D. M.: Insulin resistance in genetically obese, hyperglycemic mice. *Endocrinology* **88**, 1230–1238 (1971)

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