Different Doses and Routes of Administration of Methimazole Affect Thyroid Status in Methimazole-induced Hypothyroidism in Rats

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ABSTRACT

Objective: *It is agreed that methimazole (MMI) can be administered to induce hypothyroidism. However, there are conflicting data about its effect on thyroid function and development in rats through different administrations. In the present study, we established and compared differences of the rat hypothyroid model induced by MMI added to drinking water or given through an intragastric tube.*

Methods: *Sixty-four male Wistar rats were randomly divided into seven groups. Methimazole was added to the drinking water (0.025%, 0.04% or 0.1% wt/vol), or through intragastric gavage (5 mg/100 g body weight (bw) or 8 mg/100 g bw) one time each day for 21 days. The rats were weighed every seven days. Blood samples were taken in order to detect the concentrations of serum triiodothyronine (T3), thyroxine (T4) and thyrotropin (TSH) at the end of the experiments.*

Results: *Our results indicate that the effect of methimazole on a rat's thyroid function and body weight is similar in both the group given 0.1% concentration in drinking water and the group which received 8 mg/100 g bw once daily through the intragastric tube. Also, a similar effect was observed in the 0.025%, 0.04% and 5 mg/100 g bw groups.*

Conclusion: *These findings suggest that a relationship between the concentration of MMI by oral administration and the dose of it through intragastric administration could exist, and may contribute to inducing hypothyroidism in rats.*

Keywords: Administration, hypothyroidism, methimazole, rat model, thyroid

Las Diferentes Dosis y Vías de Administración de Metimazol Afectan el Estado de la Tiroides en el Hipotiroidismo Inducido por Metimazol en Ratas

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RESUMEN

Objetivo: *Existe acuerdo en cuando a que el metimazol (MMI) puede ser administrado para inducir hipotiroidismo. Sin embargo, hay datos contradictorios en relación con su efecto sobre la función tiroidea y el desarrollo en ratas a través de diferentes administraciones. En el estudio actual, establecimos y comparamos las diferencias del modelo de hipotiroidismo en ratas. El hipotiroidismo es inducido añadiendo MMI al agua de beber, o suministrándolo a través de un tubo intragástrico.*

Métodos: *Sesenta y cuatro ratas Wistar machos fueron divididas aleatoriamente en siete grupos. El metimazol fue añadido al agua de beber (0.025%, 0,04% ó 0.1% p/v), o suministrado mediante sonda intragástrica (5 mg/100 g de peso corporal (pc) ó 8 mg/100 g pc) una vez cada día por 21 días. Las ratas se pesaron cada 7 días. Se tomaron muestras de sangre para detectar las concentraciones de triyodotironina (T3), tiroxina (T4) y tirotropina (TSH) en suero al final de los experimentos.*

Resultados: *Nuestros resultados indican que el efecto del metimazol sobre la función de la tiroides y el peso corporal de una rata es similar tanto en el grupo que recibió una concentración de 0.1% en el agua de beber como en el grupo que recibió 8 mg/100 g pc una vez al día a través de un tubo intragástrico. También se observó un efecto similar en los grupos de 0.025%, 0.04% y 5 mg/100 g pc.*

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Conclusión: *Estos resultados sugieren que podría existir una relación entre la concentración de MMI mediante administración oral y la dosis de la misma a través de administración intragástrica, lo cual puede contribuir a inducir hipotiroidismo en ratas.*

Palabras claves: Administración, hipotiroidismo, metimazol, modelo de rata, tiroides

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INTRODUCTION

Hypothyroidism is a condition in which the thyroid gland fails to produce sufficient thyroid hormone, indispensable (1). They can affect all main pathways of metabolism. In particular, their actions on carbohydrate, lipid and protein metabolism can cause an increase of basic energy expenditure (2).

In order to evaluate the influence of thyroid hormone on developmental changes in rats, hypothyroidism was induced by pharmacological means or surgery in many studies (1, 3) Methimazole (MMI), which is used to treat hyperthyroidism, can also be administered to induce. Hypothyroidism has been induced by MMI administration in drinking water in many studies. This treatment, while resulting in a hypothyroid state, also caused a significant reduction of body weight in animal models. On the other hand, it was also reported that the period of time of the induced model was different, depending on if rats were given different MMI doses in their drinking water. Furthermore, according to previous studies, hypothyroidism could also be induced by MMI administration through an intragastric tube. However, there are conflicting data about the effect of different thyroid statuses on the body weight. It was found in a study that the body weight was shown to increase in hypothyroidism induced by intragastric MMI administration in rats (9).

Unfortunately so far, there has not been a study to demonstrate a comparison between the two different routes of administration and the effects of MMI on thyroid function and growth in rats. Thus, the purpose of this study was to compare the effects of MMI given by either adding it to the drinking water or *via* intragastric tube, while assessing thyroid function and body weight, in the process of creating a model of hypothyroidism in rats. This study may be beneficial to the establishment of a hypothyroidism model with MMI in rats.

SUBJECTS AND METHODS

Reagents

Methimazole was purchased from Beijing XJFT Biotech Development Co, Ltd (Beijing, China). Methimazole of EP quality standard was adopted in this study.

Animals

Male Wistar rats (six weeks old, initially weighing 175–200 g) were purchased from Shandong University School of Medicine. All the rats were kept in a room at a constant temperature of 22 ± 1 ˚C with a 12-hour light/12-hour darkness cycle (light on from 8 am to 8 pm daily). The animals were given free access to rodent laboratory chow and water. The use and treatment of animals followed the guidelines of the International Animal Care and Use Committee of Shandong University. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Shandong University.

Induction of hypothyroidism

The rats were randomly divided into seven groups after seven days of acclimatization (day 0). Control A rats (n = 10) drank regular water. Thirty animals received MMI (0.025%, 0.04% or 0.1% wt/vol) added to their drinking water, and 16 rats were treated with MMI (5 mg/100 g body weight [bw] or 8 mg/100 g bw) by intragastric tube once daily for 21 days, respectively. The control animals (control B; n = 8) received untreated water by intragastric tube administration once daily.

Blood samples and serum levels of thyroid hormones

At the end of the experiments (day 21), and after 12 hours overnight fasting at 8:00 hours, venous blood from the rats was obtained *via* jugular sinus puncture and was collected in test tubes with coagulant. Serum fractions were separated by centrifugation. The serum obtained was used for an assay of triiodothyronine (T3), thyroxine (T4) and thyroid-stimulating hormone (TSH). Serum concentrations of T_3, T_4 and TSH were measured using the automated chemiluminescent immunoassay (Bayer Corp. Diagnostics).

All rats were weighed once weekly from day 0 to the end of the experiments (day 21).

Statistical analysis

The data analysis was performed with SPSS 11.5 software package (SPSS 11.5, Chicago, IL, USA). Data were statistically analysed using a one-way analysis of variance followed by a Student-Newman-Keuls multiple test to compare the groups. A value of $p < 0.05$ was accepted as significant.

RESULTS

Serum levels of T_3, T_4 and TSH

Serum concentrations of T_3, T_4 and TSH in the control A, control B and experiment groups are shown in the Table.

The T_3 and T_4 concentrations were significantly lower and the TSH concentration was significant higher in rats that received MMI *via* drinking water or *via* intragastric gavage administration compared to control A or control B, respectively

Table: Serum T_3, T_4 and TSH in control and hypothyroid rats

Group	T_3 (nmol/l)	T_4 (nmol/l)	TSH (mIU/L)
Control A (n = 10)	0.9 ± 0.14	59.92 ± 6.23	0.78 ± 0.45
0.025% MMI (n = 10)	0.52 ± 0.12[ab]	13.46 ± 2.32 [ab]	43.05 ± 9.71 [ab]
0.04% MMI (n = 10)	0.54 ± 0.11[ab]	11.26 ± 1.56 [ab]	45.79 ± 3.52 [ab]
0.1% MMI (n = 10)	0.3 ± 0 [abcd]	7.63 ± 0.97 [abcd]	45.2 ± 6.6 [ab]
Control B (n = 8)	0.95 ± 0.14	58.93 ± 10.21	1.06 ± 0.54
5 mg/100 g MMI (n = 8)	0.39 ± 0.84 [ab]	13.64 ± 6.33 [ab]	41.45 ± 7.43 [ab]
8 mg/100 g MMI (n = 8)	0.33 ± 0.05 [abcd]	9.98 ± 2.57 [ab]	39.56 ± 6.4 [ab]

T_3: triiodothyronine; T_4: thyroxine; TSH: thyroid-stimulating hormone
Numbers in parentheses are sample sizes. Data represent mean ± SD.
[a]$p < 0.01$ *vs* control A; [b]$p < 0.01$ *vs* control B; [c]$p < 0.05$ *vs* 0.025% MMI; [d]$p < 0.01$ *vs* 0.04% MMI

(T_3 $p < 0.01$; T_4 $p < 0.01$; TSH $p < 0.01$). The lowest concentrations of T_3 and T_4 were obtained in rats given 0.1% MMI added to the drinking water, which showed about a 67% decrease of T_3 and about an 86% decrease of T_4 compared with control A.

Serum T_3 and T_4 concentrations in rats given 0.025% MMI and 0.04% MMI in drinking water did not show a significant difference in comparison to that of rats given 5 mg/100 g bw MMI by intragastric tube administration.

It was also shown that serum T_3 and T_4 concentrations in rats given 8 mg/100 g bw MMI were lower than those of rats receiving 5 mg/100 g bw MMI through intragastric tube administration, respectively, but there were no significant differences. There was also no significant difference in the levels of T_3, T_4 and TSH concentrations between 0.1% MMI and 8 mg/100 g bw MMI groups.

Both the control A and control B groups had similar levels of T_3, T_4 and TSH concentrations. Serum TSH concentrations in animals in each hypothyroid group did not show a significant difference.

Body weight

A summary of the body weight in all groups in this study is presented in the Figure.

Hypothyroid rats progressively lost weight, developed dry fur, and were observed to be less active than euthyroid rats (control A or control B). At the beginning of this study, there was not a significant difference in the body weight of animals in each group.

The body weight of all animals in the study began increasing. The fastest increase in body weight was obtained in rats of control A and control B groups, which showed about a 20% increase compared with their respective baseline body weight, and the slowest rate of increase was evident in 0.1% MMI group rats, which showed about an 11% increase compared with their baseline body weight during the first week. There were significant differences between the body weights of rats in the 0.1% MMI group compared to those of the control A group on the 7th day (259.20 ± 16.59 *versus* 293.80 ± 21.22 g; $p < 0.05$).

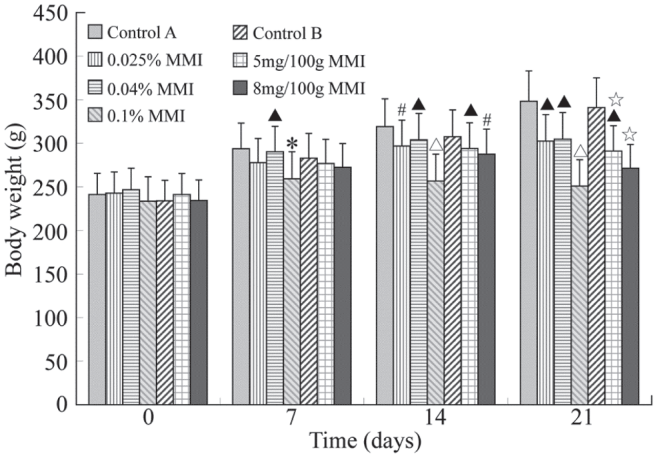

Figure: The body weight profiles in control and hypothyroid rats during the experiment. Rats in each group were weighed every seven days. Data are expressed as mean ± SD.
[*]$p < 0.05$ *vs* control A; [Δ]$p < 0.01$ *vs* control A; $p < 0.05$ *vs* control B; [#]$p < 0.05$ *vs* 0.1% MMI; [▲]$p < 0.01$ *vs* 0.1% MMI

The body weight of rats in the 0.1% MMI group began to decrease after one week. We also observed that rats in both the 0.025% MMI group and in the 0.04% MMI group virtually stopped growing after two weeks. However, during this same time period, the body weight of rats in the 5 mg/100 g bw group and in the 8 mg/100 g bw group began to decrease.

On the 21st day, compared with control B, those receiving either 5 mg/100 g bw MMI or 8 mg/100 g bw MMI exhibited a significant decrease in body weight ($p < 0.01$). The mean body weight in rats of the 8 mg/100 g bw group was lower than that of the rats in the 5 mg/100 g bw group, but this was not a significant difference (271.50 ± 33.24 *versus* 291.13 ± 14.68 g; $p > 0.05$). Compared with rats in 0.025% MMI, 0.04% MMI and 5 mg/100 g bw MMI groups, body weight was significantly lower in the 0.1% MMI group ($p < 0.05$). No significant difference in body weight was found between the 0.1% MMI and 8 mg/100 g bw MMI groups (251.00 ± 16.53 *versus* 271.50 ± 33.24 g; $p > 0.05$).

DISCUSSION

Due to the fact that thyroid hormone has been recognized as an important factor in the development of rodents (1), the establishment of a robust hypothyroid animal model has become increasingly important in order to study the effects of thyroid hormone on developmental changes in animals.

There are many animal models in the literature through which thyroid hormone could be studied. In recent years, some gene knockout hypothyroidism models have been established successfully in mice. Thyroid hormone receptors (TRs), located in cell nuclei, mediate the action of thyroid hormone by positive and negative regulation of thyroid hormone responses genes (10). The TRβ knockout mouse with deletion of both $TR\beta_1$ and $TR\beta_2$ gene has been used to research the role of thyroid hormone receptors, which mediate the action of thyroid

hormone (11). In addition, the hypothyroid (hyt/hyt) mouse is also a model system of evaluating the actions of thyroid hormone on the development of gene expression in mice (1). A TSHR knockout (TSHR-KO) mouse as a model of studying TSH receptor function was generated by Marians *et al* through the methods of homologous recombination. In this study, it was found that the development and growth of TSHR-KO mice was delayed, they were profoundly hypothyroid, with no detectable thyroid hormone, TSH increased and they had an abnormal thyroid histopathology (12). On the other hand, central hypothyroidism, defined as low plasma total and free T4 concentrations in the presence of normal TSH values, is a rare disorder. A mouse which is mutant deficient in the TRH-receptor 1 (TRH-R1) gene was generated by Rabeler *et al* through an approach of homologous recombination, which presents a model for studying central hypothyroidism in animals (13).

However, in rats, the hypothyroidism model is usually induced by chemicals (propylthiouracil or MMI), by surgical thyroidectomy or by ^{131}I (14–18). Methimazole administration in drinking water is a common method of inducing hypothyroidism in rats: Inuwa and Williams induced hypothyroidism in rats by replacing their drinking water with a solution of 0.02% wt/vol MMI for six weeks (4); Bruno *et al* carried out a study that examined how hypothyroidism affects ATP, ADP and AMP hydrolysis in rat hippocampal and cortical slices with hypothyroid rats treated by having MMI (0.05% wt/vol) added to their drinking water for 14 days (19); and Leal *et al* induced hypothyroidism by MMI (0.03% wt/vol) treatment in the drinking water for 21 days (8). Moreover, it was also reported that hypothyroidism was able to be induced if the rats received a daily administration of MMI through their intragastric tube for 28 days (20).

The results reported here demonstrate that hypothyroidism is able to be induced not only by MMI (0.025%, 0.04% or 0.1% wt/vol) in drinking water, but also by MMI administration (5 mg/100 g bw or 8 mg/100 g bw) through an intragastric tube once daily for 21 days. The hypothyroid groups showed significantly different decreases of serum T_3 and T_4 concentration and a significant increase of TSH concentration. In addition, these treatments led to an obvious reduction of body weight during the process of inducing the hypothyroid state. This suggests that other factors, such as dietary factors, might be included in these treatments (16). Based on these results, it was concluded that similar effects on thyroid function and body weight result from MMI administration *via* 0.1% wt/vol in drinking water and *via* 8 mg/100 g bw daily intragastric tube administration. Also, the effects of MMI on thyroid function and body weight in rats were similar in the groups that were given 0.025% or 0.04% wt/vol in drinking water, or given 5 mg/100 g bw once daily through the intragastric tube. Interestingly, in this study, it was found that the amount of water consumed by MMI-water drinking rats was dependent on the concentration of MMI in the drinking water itself; the higher the concentration of MMI in the drinking water, the less amount of water the rats drank. This may be an important observation to support the idea that the hypothyroid rat's response was not dependent on the MMI dose completely.

In conclusion, hypothyroidism in rats was successfully induced by MMI both by administration in drinking water and by administration through the intragastric tube. Our findings suggest that a relationship between the concentration of MMI by oral administration and the dose of it through intragastric administration could exist, which may contribute to the induction of a hypothyroid model in rats.

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REFERENCES

1. Green RP, Birkenmeier EH, Beamer WG, Maltais LJ, Gordon JI. The hypothyroid (hyt/hyt) mouse: a model system for studying the effects of thyroid hormone on developmental changes in gene expression. Proc Natl Acad Sci USA 1988; **85:** 5592–6.
2. Hapon MB, Varas SM, Jahn GA, Giménez MS. Effects of hypothyroidism on mammary and liver lipid metabolism in virgin and late-pregnant rats. J Lipid Res 2005; **46:** 1320–30.
3. Wu S, Tan G, Dong X, Zhu Z, Li W, Lou Z et al. Metabolic profiling provides a system understanding of hypothyroidism in rats and its application. PLoS One 2013; **8:** e55599.
4. Inuwa I, Williams MA. Morphometric study on the uterine horn and thyroid gland in hypothyroid, and thyroxine treated hypothyroid rats. J Anat 1996; **188:** 383–93.
5. Işman CA, Yeğen BC, Alican I. Methimazole-induced hypothyroidism in rats ameliorates oxidative injury in experimental colitis. J Endocrinol 2003; **177:** 471–6.
6. Siqueira CC, Rossoni RR, Tiengo AN, Tufik S, Schenberg LC. Methimazole-induced hypothyroidism inhibits the panic-like behaviors produced by electrical stimulation of dorsal periaqueductal gray matter of rats. Psychoneuroendocrinology 2010; **35:** 706–16.
7. Blennemann B, Leahy P, Kim TS, Freake HC. Tissue-specific regulation of lipogenic mRNAs by thyroid hormone. Mol Cell Endocrinol 1995; **110:** 1–8.
8. Leal AL, Pantaleão TU, Moreira DG, Marassi MP, Pereira VS, Rosenthal D et al. Hypothyroidism and hyperthyroidism modulates Ras-MAPK intracellular pathway in rat thyroids. Endocrine 2007; **31:** 174–8.
9. Xu J, Lin HH, Sun XF. Changes of glucose transporter 2 in rat livers with different thyroid state. Chin J Public Health 2005; **21:** 442–3.
10. Lazar MA. Thyroid hormone receptors: multiple forms, multiple possibilities. Endocr Rev 1993; **14:** 184–93.
11. Weiss RE, Murata Y, Cua K, Hayashi Y, Seo H, Refetoff S. Thyroid hormone action on liver, heart, and energy expenditure in thyroid hormone receptor beta-deficient mice. Endocrinology 1998; **139:** 4945–52.
12. Marians RC, Ng L, Blair HC, Unger P, Graves PN, Davies TF. Defining thyrotropin-dependent and -independent steps of thyroid hormone synthesis by using thyrotropin receptor-null mice. Proc Natl Acad Sci USA 2002; **99:** 15776–81.
13. Rabeler R, Mittag J, Geffers L, Rüther U, Leitges M, Parlow AF et al. Generation of thyrotropin-releasing hormone receptor 1-deficient mice as an animal model of central hypothyroidism. Mol Endocrinol 2004; **18:** 1450–60.

14. Kumar R, Hegde KS. Influence of thyroid hormone on the phospholipid composition of lung tissue and surfactant of rats. Indian J Pharmacol 1983; **27:** 203–8.
15. Mishkin S, Morris HP, Yalovsky MA, Murthy PV. Inhibition of the growth of Morris hepatoma N0.44 in rats after induction of hypothyroidism: evidence that Morris hepatomas are thyroid dependent. Gastroenterology 1979; **77:** 547–55.
16. Giudetti AM, Leo M, Siculella L, Gnoni GV. Hypothyroidism down-regulates mitochondrial citrate carrier activity and expression in rat liver. Biochim Biophys Acta 2006; **1761:** 484–91.
17. Gamboa R, Regalado JC, Huesca-Gómez C, Posadas-Romero C, Verdejo Paris J, Vargas-Alarcón G et al. Decreased activity of lecithin: cholesterol acyltransferase and hepatic lipase in chronic hypothyroid rats: implications for reverse cholesterol transport. Mol Cell Biochem 2003; **246:** 51–6.
18. Guajardo-Salinas GE, Carvajal JA, Gaytan-Ramos AA, Arroyo L, López-Reyes AG, Islas JF et al. Effects of bone marrow cell transplant on thyroid function in an I131-induced low T4 and elevated TSH rat model. J Negat Results Biomed 2007; **6:** 1.
19. Bruno AN, Diniz GP, Ricachenevsky FK, Pochmann D, Bonan CD, Barreto-Chaves ML et al. Hypo-and hyperthyroidism affect the ATP, ADP and AMP hydrolysis in rat hippocampal and cortical slices. Neurosci Res 2005; **52:** 61–8.
20. Xu J, Lin HH. Changes of glucose transporter 2 in rat livers under different status of thyroid function. Chin J Endocrinol Metab 2004; **20:** 172–3.