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Biophysical parameters of rats' skin after the administration of methimazole

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Abstract

The paper describes the influence of oral administration of methimazole on biophysical skin parameters. Wistar rats of different sex (220–260 g) were used in the experiment. Biophysical skin parameters, such as transepidermal water loss (TEWL), corneometry, and pH were examined at seven-day intervals. Significant changes in the parameters were observed on the 7th d of methimazole administration. The changes were observed in both sex but males appeared to be less sensitive in that respect. Changes in the parameters in the females showed rapid mechanisms, which normalised transepidermal water loss and skin hydration, as well as restored skin barrier functions. TEWL, skin hydration, and skin pH measurements allow an early assessment of skin barrier dysfunction after administration of this drug.

Key words: skin, methimazole, transepidermal water loss, corneometry, pH.

Introduction

Methimazole (1-methyl-2-mercapto imidazole) is an antithyroid drug used worldwide as the first choice in chronic treatment of hyperthyroidism in humans (31), dogs (39), and cats (15, 16, 21, 28, 29). Its mechanism of action is similar to other thioamides that block the activity of thyroperoxidase, leading to reduced biosynthesis of thyroid hormones; however, uncontrolled methimazole treatment (overdosing) results in hypothyreosis (5). This medicine has been known for more than 60 years and used in humans despite numerous side effects. The most often observed dermatological complications are aplasia cutis congenita and dystrophic fingernails in children born by mothers treated with methimazole during pregnancy (1, 24, 32), as well as drug-induced vasculitis (42). No side effects were observed in patients with local treatment of *i.e.* post-inflammatory hyperpigmentation (17) and psoriasis (10). In cats subcutaneous treatment of hyperthyreosis with methimazole also did not cause any local side effects (15), but auricular erythema was reported. This type of treatment was not accompanied by facial pruritus, which could be noted in the case of oral administration (15).

Assessment of skin condition can be conducted using non-invasive measurements of biophysical skin parameters. It allows to obtain crucial information on the condition of epidermis and dermis. The most common methods include transepidermal water loss (TEWL), skin hydration (corneometry), and skin pH. Correct skin hydration values are known in human medicine (10, 22), and they are examined thoroughly in the course of atopic dermatitis (7, 12) and contact dermatitis (20). In veterinary medicine, the mentioned parameters have been examined in dogs (14, 27, 30, 36) and cats (4, 37). TEWL, also known as insensible perspiration, defines the permeability of the epidermal barrier and consists of the diffusion of water into the atmosphere of relatively low humidity (10, 26, 35).

It describes the ability of the skin to retain water and indicates the integrity of the dermal and epidermal barrier (10, 12, 14, 27, 34, 36). An increase in TEWL was observed in humans (6, 7, 12) and dogs with atopic dermatitis (14, 23). An open chamber and a close chamber methods can be used. Drafts and air currents have no impact on the results obtained, when the close chamber method is applied. However, in practice, the open chamber method has been used more frequently in the early diagnosis of atopic dermatitis in dogs (23). Corneometry, in turn, assesses the electric potential of the stratum corneum, which depends on its hydration at the depth of 10 to 100 μm (10, 33). A decrease in its value has been observed in humans (33) and dogs (36) with atopic dermatitis in areas with skin changes. An increase of skin pH has been noted in humans with atopic dermatitis, seborrheic dermatitis, acne, ichthyosis, contact dermatitis, and *Candida albicans* infections (7, 25, 34), and in dogs with pyoderma (30).

The aim of the study was to assess TEWL, skin hydration, and skin pH potential changes in rats during the course of experimental administration of methimazole resulting in hypothyroidism (experimental model of overdosing methimazole).

Material and Methods

Animals. Wistar rats of both genders (220-260 g) were divided into four groups: C1 (control group) – healthy males (n = 6); C2 (control group) – healthy females (n = 6); E1 (experimental group) – males receiving methimazole (n = 6), and E2 (experimental group) – females receiving methimazole (n = 6). The rats were kept in an air-conditioned room with average humidity of 45%-47%, temperature of 22-23°C and 12/12 light cycle. The experiment was approved by the Local Ethics Committee at the University of Life Sciences in Lublin. The rats underwent 14 d adaptation prior to the experiment. During this period, they were fed a commercial diet for laboratory animals (Agropol, Poland), and had an access to tap water *ad libitum*. Then, the rats from groups E1 and E2 were given 0.05% methimazole (Sigma-Aldrich) solution, administered *ad libitum* instead of water. Fresh solution was prepared daily.

Measurements of biophysical skin parameters. The measurements of biophysical skin parameters were performed on the left side of the chest, five times at 7-d intervals. TEWL (expressed in g/h/m^2), corneometry (expressed in corneometer units – CU), and pH were measured in this region. Hair was clipped with Metzenbaum scissors to 1 mm in length 48 h prior to the measurements. Each time, the mean value from six measurements was calculated. A Courage Khazaka Multi Probe Adapter 5 equipped with the following heads: Tewameter TM 300 (to measure TEWL - the open chamber measurement method), Corneometer CM 825 (to measure skin hydration), and Skin-pH-Meter PH 905 (to measure skin pH) was used. The same device was previously used in dogs (36) and cats (37). Each time, the measurements were taken in the room where the animals were kept, between 8 and 10 a.m.

Hormone analysis. Blood samples (0.5 mL) for hormone tests were collected from day 0 five times at 7-d intervals between 8 and 10 a.m. from the lateral tail vein, and allowed to clot. Next, the samples were centrifuged for 10 min to obtain serum. In each sample total thyroxine (T4) concentration was measured with ELISA (GenWay Biotech Inc., San Diego), to assess methimazole-induced hypothyreosis. Due to a limited amount of serum samples, only one parameter describing the functional state of the thyroid gland was selected for examination. The sera were kept at -70°C for further analysis.

Statistical analysis. For all parameters, the mean and standard deviation (SD) were calculated. Statistical analysis was conducted by the Mann-Whitney U test at $P \leq 0.05$ (Statistica 6.0 software). For each parameter, statistically significant differences were calculated between control and experimental groups on days: 0, 7, 14, 21, 28, and each day was compared to day 0.

Results

Hormone tests results confirmed hypothyreosis in the examined animals. Total thyroxine (T4) levels in rats treated with methimazole (E1 and E2) were statistically lower compared to the control groups (C1 and C2) beginning from day 7 of observation. On days 0, 7, 14, 21, and 28 the mean values amounted, respectively, to 78.2, 74.8, 76.6, 75.0, 77.6 nmol/L in control animals, and 77.5, 58.1, 41.7, 13.4, and 4.6 nmol/L in experimental animals - without statistically significant differences between groups before the experiment.

TEWL results are presented in Table 1. In the case of the control C1 group, the TEWL values remained at a stable level throughout the entire experiment. In the experimental group of males (E1), statistically significant increase in the parameter, comparing to day 0 values, was observed on the 28th d of the experiment (18.5 g/h/m^2). In control (C2) and experimental (E2) females, TEWL values remained on a stable level throughout the entire experiment. However, on the 7th d of the experiment a statistically significant increase in the parameter (14.28 g/h/m^2) was observed in experimental females (E2), when compared to the control group (C2).

Corneometry measurement results are presented in Table 2. There were no statistically significant differences in corneometry results in both males and females, except females of the experimental group on the 7th d, in which a decrease in skin hydration

(10.38 CU) was observed when compared to control females group (C2) (12.58 CU).

Skin pH results are presented in Table 3. A gradual statistically significant increase in skin pH was observed in control males (C1), starting from day 7 of the experiment compared to day 0 values. In the experimental group of males (E1), there was a statistically significant growth in the parameter, compared to day 0, and its slight fluctuation was observed from day 7. Compared to the control group (C1), the values were statistically significantly higher on the 21th d of the experiment.

In control females (C2) and experimental females (E2), skin pH remained on a stable level throughout the entire experiment. However, in the experimental group of females (E2), the value of the parameter was statistically significantly higher than in control females (C2) on the 7th and 28th d of the experiment.

Discussion

The diffusion of water across the skin notably increased in males on the 28th d of administering methimazole. As in the case of males, transepidermal water loss in females was observed already on 7th d, but such alterations had no permanent effect. The observed changes in TEWL result from damaging the integrity of epidermis/dermis barrier. Hence, males appear to be less sensitive in that respect. Similarly, corneometry assessment indicated a positive effect of methimazole on skin hydration in females on 7th d of the experiment (only compared to control group).

Table 1. TEWL on the left side of the chest (g/h/m^2)

	Day of study									
	0		7		14		21		28	
	x	SD	x	SD	x	SD	x	SD	x	SD
C1	13.83	1.48	14.53	3.88	14.19	2.98	13.25	3.83	14.90	3.11
E1	11.97	1.76	13.62	4.95	12.02	5.59	9.90	1.1.75	18.50*	5.90
C2	11.57	0.92	10.13	2.56	11.13	5.64	11.37	5.06	13.83	3.98
E2	11.73	2.12	14.28^	2.17	10.90	3.68	7.18	5.35	11.40	2.74

x - mean, SD - standard deviation, * - statistically significant compared to day 0, ^ - statistically significant compared to control group (E1:C1 and E2:C2), C1 – control group of male rats, E1 – experimental group of male rats, C2 – control group of female rats, E2 – experimental group of female rats

Table 2. Corneometry/skin hydration on the left side of the chest (CU)

	Day of study									
	0		7		14		21		28	
	x	SD	x	SD	x	SD	x	SD	x	SD
C1	10.50	3.25	12.10	2.38	11.13	0.70	8.67	2.52	11.96	6.17
E1	10.29	3.88	8.27	5.58	7.58	1.81	9.15	1.35	10.10	1.88
C2	10.30	0.76	12.58	3.20	11.04	3.64	9.05	3.14	8.97	3.46
E2	10.41	3.93	10.38^	4.54	6.20	3.17	6.75	0.69	4.42	0.66

x - mean, SD - standard deviation, ^ - statistically significant compared to control group (E1:C1 and E2:C2), C1 – control group of male rats, E1 – experimental group of male rats, C2 – control group of female rats, E2 – experimental group of female rats

Table 3. Skin pH on the left side of the chest

	Day of study									
	0		7		14		21		28	
	x	SD	x	SD	x	SD	x	SD	x	SD
C1	5.48	0.28	6.06*	0.26	6.17*	0.49	6.50*	0.38	6.68*	0.29
E1	5.51	0.27	6.25*	0.32	6.16*	0.19	7.22*^	0.07	6.69*	0.18
C2	5.97	0.50	5.73	0.17	5.86	0.35	5.96	0.43	5.96	0.03
E2	5.83	0.35	6.18^	0.22	5.86	0.37	5.89	0.68	6.13^	0.08

x - mean, SD - standard deviation, * - statistically significant compared to baseline day 0, ^ - statistically significant compared to control group (E1:C1 and E2:C2), C1 – control group of male rats, E1 – experimental group of male rats, C2 – control group of female rats, E2 – experimental group of female rats

Changes in the parameters in female rats indicate the existence of rapid mechanisms that normalise TEWL and skin hydration, as well as restore skin barrier functions. A significant increase in skin pH on the 21st d of the experiment in male rats was observed. However, contrary to females, this parameter was not a constant value in healthy animals. In the case of the latter specimens, a significant increase in skin pH was observed on days 7 and 28 of administering of methimazole. Thus, skin pH changes, similarly to TEWL, skin and hydration changes, were different in males and females. It seems that although females are more sensitive to overdosing methimazole than males, they have mechanisms to normalise biophysical skin parameters rapidly.

Administration of methimazole results in hypothyroidism and its characteristic symptoms: lowering body weight gain, body temperature, systolic blood pressure, and heart rate (3). The most frequently occurring complications of methimazole treatment are agranulocytosis, granulocytopenia, aplastic anaemia, thrombocytopenia, systemic lupus erythematosus, drug-induced vasculitis, and toxic liver injury. The drug may cause biophysical skin disorders due to blocking the biosynthesis of thyroid hormones (5, 32). Methimazole also significantly modulates the production of reactive oxygen species such as a superoxide anion (O^{-2}), and stimulates the mechanisms of anti-free radical defence (11). Thyroid hormones have a direct or indirect influence on the epidermis and stimulate formation of epidermal barrier in foetal rats (13). In the present study a change in water balance occurred in rats' skin due to experimentally evoked hypothyroidism. Previous reports have demonstrated that it can lead to hyaluronic acid accumulation, resulting in disturbance of the transcapillary fluid balance, increased interstitial fluid pressure, and "wash-down" of interstitial proteins (40, 41). Changes similar to those in human and canine hypothyreosis, including skin drying, inhibiting cell proliferation in hair follicles, telogenisation and follicular atrophy, disabling hair regrowth, as well as epidermal thinning can also be observed in rats. However, histopathological changes can be noted only after 12 weeks from removing the thyroid gland, and, contrary to what is observed in humans and dogs, no alopecia or hair thinning occurs in rats (38). In our experiment, the changes in the tested parameters in females were noted as early as 7 d after hypothyroidism induction. This indicates that the assessment of biophysical skin parameters allows to detect disorders in the integumentary system sooner than by histopathological examination.

The changes in the tested parameters may also be caused by the beyond-thyroid mechanisms of action of methimazole. It induces skin changes in newborns of mothers treated with this drug during pregnancy (1, 24, 32). However, after local methimazole administration, a species-specific reaction to the is demonstrated. In humans, it induces skin discoloration (17) and inhibits keratinocyte proliferation (7), which was not observed in cats (15). A significant influence of methimazole on melanocyte functions appears (17, 19) despite the lack of cytotoxicity even in high concentrations (2). This phenomenon is explained by post-drug blocking of peroxidase, present in melanocytes, which takes part in various phases of melanogenesis (18). Methimazole, similarly to other thioamids, reduces the expression of proliferative cell nuclear antigen (PCNA) in the examined bioptates. This explains its antiproliferative effect on keratinocytes and changes in biophysical skin parameters (8, 9).

Overdosing of methimazole, without monitoring the level of thyroid hormones or compliance with medical recommendations, may change the integrity of the skin barrier through two pathological mechanisms: dermal action of the drug and hypothyroidism. The changes can be observed in both sex but males are more resistant. Therefore, in this situation it is necessary to apply a moisturising treatment, which enables repairing the skin barrier, retaining/increasing water content, reducing TEWL, restoring the lipid barriers' ability to absorb and redistribution of water. In the course of using thionamides, there is a potential risk that the described changes will appear, especially when applying an increased dosage that leads to hypothyroidism. However, the risk is decreased in the case of controlled hyperthyroidism treatment (maintaining euthyreosis). TEWL, skin hydration, and skin pH measurement allow the early assessment of skin barrier dysfunction after the treatment.

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