EFFECT OF ABOUTHIOULINE, A NOVEL DRUG WITH THERAPEUTIC POTENTIAL AS ANTITHYROID, ON SOME BIOCHEMICAL AND HEMATOLOGIC PARAMETERS IN MICE AND RATS

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إن دواء أبوثيولين هو أحد الأدوية الحديثة ذات النشاط المضاد للدرقية . وقد تم تصميم هذا الدواء باستخدام علاقات التركيب الكيميائي - الفاعلية الدوائية التي هدفت إلى التقليل من الخواص المضادة للأكسدة للمركب وذلك بتحوير شطر الثيويورايلين اللاحلقي. إن التأثيرات المضادة للأكسدة للأدوية المتوفرة حالياً مثل دواء بروبيل ثيويوراسيل، ودواء ميثيمازول تكون مصحوبة بحدوث مرض فقد الخلايا المحببة وفقر الدم اللاتكوني. وفي هذه الدراسة، تم تقدير سمية دواء أبوثيولين التي تسبق الدراسة الإكلينيكية للدواء في الجرذان والفئران ، وتم مقارنتها مع مركبين مرجعيين هما دواء بروبيل ثيويوراسيل ودواء ميثيمازول. فبعد الإعطاء قصير المدى للدواء (7 أيام) للفئران ، تبين أن لدواء أبوثيولين تأثيراً ضئيلاً على المؤشرات البيوكيميائية ، وذلك بالرغم من الانخفاض المعنوي في البروتين الكلي والألبيومين. أما الدراسات طويلة المدى (30يوم) في الجرذان فقد كشفت عن تأثيرات معنوية لدواء أبوثيولين ودواء بروبيل ثيويوراسيل ودواء ميثيمازول على مستويات كل من الشوارد الكهربية في المصل والجلوكوز. ولم يكن لدواء أبوثيولين أي تأثير ضار على المؤشرات الهيماتولوجية. ومع ذلك فإن عدد الكريات البيضاء الكلي (في حالة دواء بروبيل ثيويوراسيل) ومستويات الكريات أليفة الأصباغ المتعادلة (في حالة بروبيل ثيويوراسيل وميثيمازول) قد انخفضت معنوياً بين مجموعات المعالجة الأخرى . وتدل نتائج هذه الدراسة على أن دواء أبوثيولين هو دواء واعد كمضاد للدرقية له مخاطر ضئيلة من حيث السمية الدموية المرتبطة بدواء بروبيل ثيويوراسيل ودواء ميثيمازول. ويحتاج هذا الأمر إلى المزيد من الدراسة لاستجلاء أمان وفاعلية استخدام هذا الدواء الجديد.

activity. Abouthiouline (ABL) was designed based on structure-activity relationships (E-state indexes) aimed at reducing the antioxidant properties of the compound by modification of acyclic thiourylene moiety. Antioxidant effects of currently available treatments such as propylthiouracil (PTU), methimazole (MTM) are associated with an incidence of agranulocytosis and aplastic anemia. In the present study, the preclinical toxicology of ABL was determined in mice and rats and compared with two reference compounds, namely, propylthiouracil, methimazole. Following short-term administration (7 days) to mice, ABL had minimal effects on biochemical parameters, although significant reductions in both total protein and albumin were noted. Long-term studies (30 days) in rats revealed significant effects of Abouthiouline, propylthiouracil and methimazole on serum electrolyte and glucose levels. Abouthiouline had no detrimental effects on hematologic parameters. However, total WBC count (propylthiouracil) and neutrophil levels (propylthiouracil and methimazole) were significantly decreased among other treatment groups. The results of this investigation suggest that Abouthiouline is a promising new antithyroid therapy with a reduced risk of hematologic toxicity that is associated with PTU and MTM. Further studies are warranted to assess the safety and efficacy of Abouthiouline.

Key words: Abouthiouline; Toxicology; Propylthiouracil; Methimazole; Preclinical

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Introduction

Antithyroid medications are used throughout the world in the treatment of hyperthyroidism resulting from Graves' disease (1,2). Antithyroid therapy is also indicated in patients with toxic adenoma or toxic multinodular goiter prior to chemical or surgical thyroidectomy (3). The most commonly prescribed antithyroid medications are the thionamides including methimazole (MTM) and propylthiouracil (PTU). Thionamide medications are generally administered for long periods of time. While the incidence of adverse effects with antithyroid therapy is low, these medications are usually associated with severe hematologic toxicity such as agranulocytosis and aplastic anemia (4-8). In a large-scale pharmacovigilance study conducted in the United Kingdom, data reported between 1963 and 2003 to the Committee on Safety of Medicines (Yellow Card Scheme) were analyzed to determine the relative frequency and spectrum of adverse drug reactions to carbimazole (the parent drug for MTM) and PTU (9). The study found that the number of prescriptions for thionamide drugs were 5.23 million prescriptions where 94% of which were for carbimazole. All thionamide medication studied induced significant adverse drug reactions, including agranulocytosis and neutopenia.

Agranulocytosis is a severe and life-threatening complication of thionamide therapy. It is presumably an autoimmune reaction to circulating anti-neutrophil antibodies and lymphocyte sensitization to antithyroid medications. The presence of a cyclic thionamide group in the structure of medications such as MTM and PTU may be responsible for the stimulation of anti-neutrophil antibodies that mediate agranulocytosis. Antibody production is thought to result from the antioxidant effects of these compounds (10,11).

Recent efforts have been directed towards the development of new antithyroid agents with a more favorable toxicity profile. A series of compounds have been synthesized using structure-activity relationships. The design of these compounds was based on atom level electrotopological state (E-state) indexes, a measure of atom electronic accessibility that is a useful tool in drug design of compounds with desired pharmacologic activity (12-14). E-state indexes of the thiourylene moiety have successfully been utilized to design antithyroid compounds with reduced antioxidant properties (15). One of these compounds is Abouthiouline [1-Cyclohexyl-3(3-quinolyl)-2-thiourea] (ABL) (Figure 1). A series of investigations have been published evaluating the antithyroid and antioxidant activities of ABL. Using the ^{125}I-thiocyanate discharge technique in rats, ABL demonstrated a significantly greater antithyroid efficacy compared with PTU (16). Additional studies of the chemiluminescence response and phagocytic activity of polymorphonuclear lymphocytes demonstrated that ABL had reduced antioxidant and phagocytic activity compared with PTU and MTM. Thus, it appears that ABL may represent a useful antithyroid medication with a reduced risk of toxicity.

In the present investigation, short-term and long-term effects of ABL were studied in mice and rats. The aim of these experiments was to compare the effects of ABL, PTU and MTM on biochemical and hematological parameters in these animal species.

Methodology

All animal experiments were conducted in accordance with the recommendations of Institute for Laboratory Animal Research (ILAR) "Guide for Care and Use of Laboratory Animals" (17), and the "Guide for the Care and Use of Laboratory Animals" approved by College of Pharmacy Animal Care and Use Center at King Saud University.

Assessment of short-term effects in mice:

Equimolar doses of test and reference compounds were utilized: ABL (17 $mg.kg^{-1}.day^{-1}$), PTU (10 $mg.kg^{-1}.day^{-1}$), MTM (6.7 $mg.kg^{-1}.day^{-1}$) and thyroxine (77 $\mu g.kg^{-1}.day^{-1}$). The compounds were freshly dispersed in 1% tween-80 aqueous solution prior to oral dosing.

Thirty male mice were divided into five study groups: control (n=10), ABL (n=5), PTU (n=5), MTM (n=5) and thyroxine (n=5). The control group received 0.1 $ml.10g^{-1}.day^{-1}$ of 0.1% Tween-80 aqueous vehicle. Each of the treatment groups were administered 0.1 $ml.10g^{-1}.day^{-1}$ of drug dispersion. Drug dosing (including control) was performed daily for seven days. At the end of the seventh day, blood samples were collected via the tail vein. Blood biochemical parameters were measured using an AxSym Analyzer (Abbott Laboratories, North Chicago, IL). Parameters included the following: glucose, urea, creatinine, sodium, potassium, chloride, uric acid, calcium, inorganic phosphorous,

magnesium, iron, total bilirubin, total protein, albumin, cholesterol, triglycerides, alkaline phosphatase, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase.

Table 1. Short-term Exposure Serum Biochemistry Data (Mean±SD) Compared with Control Following Daily Doses (7 days) of PTU (10 mg. $kg^{-1}.day^{-1}$), MTM (6.7 $mg.kg^{-1}.day^{-1}$) and ABL (17 mg. $kg^{-1}.day^{-1}$) in Mice.

Serum Parameters (SI units)	**Concentration of Parameters (Mean ± SD)**			
	Control (n=10)	**PTU** (n=5)	**MTM** (n=5)	**ABL** (n=5)
Glucose (mmol/L)	8.84±2.10	7.4±1.17	9.28±0.89	8.96±0.94
Urea (mmol/L)	10.1±1.47	10.52±1.61	8.90± 0.47	9.6±0.93
Creatinine (µmol/L)	33.3±3.72	32.2±3.31	30.5±1.80	28.8±3.31
Sodium (mmol/L)	151.8±2.36	153.6±1.4	153.0±0.71	154.4±0.49*
Potassium (mmol/L)	6.49±0.87	6.9±0.43	6.88±0.37	5.62±0.31
Chloride (mmol/L)	88.0±18.7	74.0±3.29	82.00±2.24	96.0±4.05
Uric Acid (µmol/L)	180.3±97	218.2±73.9	229.0±44.43	89.40±19.32
Calcium (mmol/L)	2.58±0.10	2.52±0.1	2.48±0.04	2.48±0.04
Inorg. Phosphorous (mmol/L)	3.35±0.25	3.44±0.54	3.33±0.23	3.28±0.28
Mg (mmol/L)	0.99±0.13	1.14±0.22	0.99±0.12	0.86±0.08
Fe (mmol/L)	46.1±12.57	49.8±8.61	61.75±12.17	42.80±6.62
Total bilirubin (µmol/L)	2.76±0.55	2.96±0.3	3.00±0.42	2.22±0.37
Total protein (g/L)	51.7±1.9	52.0±1.4	52.75±1.48	44.80±1.72*
Albumin (g/L)	28.1±1.45	26.6±1.02	29.00±0.00	25.20±0.98*
Cholesterol (mmol/L)	2.99±0.37	2.96±0.34	2.83±0.18	2.52±0.32
Triglycerides (mmol/L)	1.60±0.28	1.60±0.27	1.48±0.11	1.52±0.27
Alk. Phosphatase (U/L)	127.6±32.99	104.2±21.7	142.25±26.93	101.2±16.19
LDH (U/L)	963±381	917±141.2	813.5±100.64	728.4±75.76
ALT (U/L)	41.9±7.2	51.8±9.74	48.0±9.03	51.0±18.06
AST (U/L)	199.4±68.7	278±49.8	192.5±29.92	181.8±20.89
Creatine Kinase (U/L)	253±127.4	244.4±140	248.75±42.16	179.4±45.4

***** statistically significant compared with control ($p<0.05$)

Table 2. Long-term Exposure Serum Biochemistry Data (Mean±SD) for ABL, PTU and MTM Following Daily Doses (20 $mg.kg^{-1}.day^{-1}$) for 30 days in Rats.

Serum Parameters (SI units)	Concentration of Parameters (Mean ± SD)			
	Control (n=10)	**PTU** (n=5)	**MTM** (n=5)	**ABL** (n=5)
Glucose (mmol/L)	5.44±0.75	1.60±0.5*	3.44±0.71	2.32±0.61*
Urea (mmol/L)	5.90±0.53	11.2±1.2*	5.56±1.50*	7.14±0.70
Creatinine (µmol/L)	55.20±3.66	75.6±3*	55.6±1.74	54.80±3.66
Sodium (mmol/L)	144.6±0.8	143.2±2.1	143.6±0.49	144.2±1.33
Potassium (mmol/L)	5.16±0.25	3.0±0.5*	3.3±0.3*	2.54±0.53*
Chloride (mmol/L)	98.0±1.41	103±1.9*	98.2±0.4	91.0±2.97*
Uric Acid (micmol/l)	56.6±5.85	67.8±30	53.2±16.19	78.4±21.3
Calcium (mmol/L)	2.55±0.09	2.7±0.11	2.72±0.07	2.90±0.06*
Phosphorous (mmol/L)	2.50±0.11	2.1±0.34	2.36±0.14	2.60±0.18
Mg (mmol/L)	1.09±0.15	1.6±0.1*	1.6±0.06*	1.66±0.14*
Fe (mmol/L)	37±8.22	27.6±8.2	25±5.76	24.6±5.85
Total bilirubin (µmol/L)	2.16±0.81	4.1±0.4*	2.42±0.53	2.38±0.61
Total protein (g/L)	68.4±1.96	83.2± 2.2*	75.4±2.73*	76.20±1.72*
Albumin (g/L)	31.2±0.98	31±1.3	32.2±0.98	32.4±0.80
Cholesterol (mmol/L)	1.36±0.16	2.2±0.38*	2.44±0.54*	1.90±0.17
Triglycerides (mmol/L)	0.64±0.17	0.78±0.1	0.92±0.19*	0.82±0.17
Alk. Phosphatase (U/L)	150.2±35.7	178.6±30	158.6±11.41	172.2±23.4
LDH (U/L)	636±307	725±559.8	353±119	530.6±143.23
ALT (U/L)	45.6±8	64±8.5*	38.2±6.27	57.2±8.75
AST (U/L)	126.2±22.9	171±31.7*	96.6±4.63	147.4±9.16
Creatine Kinase (U/L)	516±231.7	518.6±249	648.2±477	439.8±57.31

* statistically significant compared with control ($p<0.05$)

Table 3. Long-term Exposure Hematology Data (Mean±SD) for ABL, PTU and MTM Following Daily Doses (20 mg.kg^{-1}.day^{-1}) for 30 days in Rats.

Blood Parameters (S.I. Units)	**Value of Parameter Mean±SD**			
	Control (n=10)	**PTU** (n=5)	**MTM** (n=5)	**ABL** (n=5)
White blood counts (×10^3/μL)	14.06±1.61	8.62±1.70	13.34±2.42	13.22±2.50
Red blood counts (×10^6/μL)	7.81±0.15	6.82± 0.52*	7.22±0.67	7.36±0.54
Hemoglobin (Hg) (g/dL)	14.58±0.32	13.44±0.53	13.72±0.92	14.2±0.70
Hematocrite (%)	45.78±1.09	43.68±2.64	44.22±3.08	53.12±2.77*
Mean cell volume (fl.)	58.64±2.27	64.22±3.98	61.40±2.08	72.54±6.46*
Mean cell Hg (pg.)	18.64±0.67	20.25±0.30	19.02±0.76	19.34±0.66
Mean cell Hg conc. (g/dL)	31.80±0.75	30.80±1.04	30.98±0.21	26.84± 2.01*
Red cell distribution width (%)	15.24±0.91	16.36±1.26	15.30±0.72	22.56±2.14*
Platelets (×10^3/μL)	951.4±30.05	714.2±121.9*	669.2±88.08*	953.4±261.29
Mean platelet volume (fl)	6.10±0.26	7.50±0.58*	7.12± 0.47*	6.72±0.42
Neutrophils (%)	28.20±7.60	21.0±12.99	5.60± 1.85*	39.0±15.13
Lymphocytes (%)	67.4±7.68	77.4±12.29	92.40±2.06*	59.0±15.17
Monocytes (%)	1.80±1.17	1.00±0.63*	1.00± 0.63*	0.60±0.49
Eosinophils (%)	2.60±1.02	0.60±0.49	1.00±0.89	1.40±1.85
Basophils (%)	0.0	0.0	0.0	0.0
Neutrophils Absolute value (×10^3/μL)	4.07±1.41	1.92±1.46	0.78±0.34*	5.01±1.96
Lymphocyte Absolute value (×10^3/μl)	9.36±0.54	6.57±1.25	12.30±2.13	7.98±2.88
Monocytes Absolute value (×10^3/μl)	0.27±0.18	0.08±0.06	0.13±0.10	0.09±0.07
Eosinophils Absolute value (×10^3/μl)	0.35±0.11	0.05±0.04*	0.13±0.11*	0.14±0.16
Basophils Absolute value (×10^3/μl)	0.0	0.0	0.0	0.0

* Statistically significant compared with control ($p<0.05$)

Assessment of long-term effects in rats:

The dose of test (ABL) and reference compounds (PTU, MTM) was 20 $mg.kg^{-1}.day^{-1}$. The compounds were freshly dispersed in 2% Cremophor EL/Saline solution prior to oral dosing. Twenty-five male Sprague-Dawley rats were divided into four study groups: control (n=10), ABL (n=5), PTU (n=5) and MTM (n=5). The control group received $0.1ml.10g^{-1}$. day^{-1} of 0.1% Cremophor EL/Saline aqueous vehicle. Each of the treatment groups were administered $0.1ml.10g^{-1}.day^{-1}$ of drug dispersion. Drug dosing (including control) was performed daily for 30 days. At the end of this period, blood samples were collected via the tail vein. Blood biochemical parameters were measured using an AxSym Analyzer as previously described. Hematological parameters were determined using a Cell-Dyne 1700 Analyzer (Abbott Laboratories, North Chicago, IL). The hematologic profile consisted of a CBC (WBC, RBC, HgB, HCT, MCV, MCH, MCHC, RDW and PLT), a three-part WBC differential (lymphocyte, monocyte, granulocyte) and histograms of RBC, WBC and platelets.

Statistical Analysis:

The effects of ABL and reference compounds on biochemical and hematological parameters were analyzed by one-way analysis of variance (ANOVA). Dunnett's test was utilized to identify significant differences between individual treatment groups and controls. The statistical level of significance was taken as 0.05 and the results were expressed as mean±SD.

Results

Short-term effects in mice:

The results of acute toxicity studies in mice are presented in Table 1. Reported in the table are the biochemical parameters for control studies together with the compounds tested. Overall, there were few treatment effects on serum chemistry. Mice treated with thyroxine and ABL had a slightly higher sodium level, although this finding appears to be clinically insignificant. Additionally, both compounds significantly reduced uric acid. ABL administration was associated with significant reductions in total protein and albumin concentrations.

Long-term effects in rats:

The effects of long term exposure (30 days) of ABL, PTU and MTM on serum biochemical parameters in rats are presented in Table 2. In contrast to the acute toxicity studies (Table 1), a number of effects were noted. Both PTU and ABL were associated with significant reductions in serum glucose. Each compound studied produced varying degrees of electrolyte imbalance (K, Cl, Mg), and total protein levels were significantly increased. On the other hand, PTU administration significantly increased creatinine, bilirubin, ALT and AST levels.

Table 3 provides a summary of hematologic findings. ABL administration was associated with changes in Hct, mean cell volume and mean cell width. No other changes in hematology were noted with ABL. PTU caused a significant reduction in total WBC count. Neutrophil concentrations were significantly reduced by both PTU (absolute value) and MTM (both absolute value and %total).

Discussion

ABL is a novel antithyroid compound specifically designed to reduce the incidence of severe hematologic toxicity that is associated with medications that are currently used in the treatment of Graves' disease (PTU and MTM). Previous studies have demonstrated the pharmacologic activity of ABL (15,16). The goal of this investigation was to assess the tolerability of ABL as compared to PTU and MTM. Acute toxicity studies in mice established that ABL compared favorably with the reference compounds. Although serum protein and albumin levels were decreased by ABL, the reason for this effect is unclear. Reduced protein concentrations can sometimes be a manifestation of liver toxicity, although this seems unlikely given the short duration of the study and the fact that other indices of hepatobiliary function (e.g., ALT, AST, bilirubin, LDH) did not change. Furthermore, these changes did not occur following long-term administration to rats.

ABL also appeared to perform well following chronic administration. Although the compound appeared to cause electrolye imbalance and hypoglycemia, similar observations were observed with PTU and MTM. The clinical significance of this observation is yet to be evaluated, although the

magnitude of this imbalance is unlikely to be dose limiting. There was evidence of drug-induced kidney and liver damage in rats treated with PTU. These findings were not observed with ABL and MTM.

ABL did not appear to cause any disturbances in hematological values. There was no evidence associating ABL with the most severe side effect of present antithyroid therapy, agranulocytosis (characterized by neutropenia). Conversely, both MTM and PTU were associated with changes in WBC (PTU) and neutrophil concentrations (PTU and MTM). This finding is consistent with the findings of other investigators (18, 19), where they found that the side effects of the conventional antithyroid drugs, including agranulocytosis, significantly occurred in all dose levels regardless of treatment duration. On the other hand, a recent study by Pearce (9) observed that adverse drug reactions appeared more frequently with PTU than with carbimazole (MTM is an active metabolite of carbimazole) reflecting the higher toxicity of PTU compared with carbimazole in humans. Our long-term study results in rats revealed that MTM had a more pronounced effect on neutophils and monocytes than that of PTU.

It has been shown that the fatalities attributed to the conventional antithyroid therapy (PTU, carbimazole and consequently MTM) accounted to almost half of the fatalities due to neutophil dyscrasia (agranulocytosis and neutopenia) especially in older patients (9). Other studies with thionamide drugs have documented that these side effects are commoner in patients receiving large doses of MTM (20). In summary, the results of the present preclinical toxicity study indicated that ABL compared favorably with the existing conventional antithyroid medications. Serum chemistry data from mice and rats indicated similar effects of ABL, PTU and MTM on electrolyte and glucose levels. In contrast to the reference compounds, there were no detrimental effects of ABL on hematologic parameters (i.e., WBC and neutrophils). These results suggest that ABL is a promising new antithyroid therapy with a reduced risk of the blood dyscrasias (agranulocytosis and aplastic anemia) that have been reported with commonly prescribed medications. Additional studies are warranted to further investigate the safety and efficacy of this compound.

References

1. Chiovato L, Santini F, Pinchera A. Treatment of hyperthyroidism. Thyroid Int 1995; **2**:1-22
2. Roti E, Minelli R, Gardini E. Controversies in the treatment of thyrotoxicosis. Adv Endocrinol Metab 1994; **5**: 429-460.
3. Klein I, Becker DV, Levey GS 1994 Treatment of hyperthyroid disease. Ann Intern Med **121**:281-288
4. Bartalena L, Bogazzi F, Martino E 1996 Adverse effects of thyroid hormone preparations and antithyroid drugs. Drug Safety **15**: 53-63
5. Tajiri J, Noguchi S, Murakami T, Murakam T. Antithyroid drug–induced agranulocytosis: the usefulness of routine white blood cell count monitoring. Arch Intern Med 1990; **150**: 621-624
6. Tajiri J, Noguchi S, Okamura S, Morita M, Tamaru M, Murakami N, Niho Y. Granulocyte colony-stimulating factor treatment of antithyroid drug-induced granulocytopenia. Arch Intern Med 1993; **153**: 509-514
7. Retsagi G, Kelly JP, Kaufman DW. Risk of agranulocytosis and aplastic anemia in relation to use of antithyroid drugs: international agranulocytosis and aplastic anemia study. BMJ 1988; **297:**262-265
8. Biswas N, Ahn Y, Goldman JM, Schwartz JM. Case report: aplastic anemia associated with antithyroid drugs. Am J Med Sci 1991; **301**:1353-1362
9. Pearce SHS. Spontaneous reporting of adverse reactions to carbimazole and propylthiouracil in the UK. Clin Endocrinol 2004; **61**: 589-594
10. Wall JR, Fang SL, Kurocki T, Lagbar SH, Braverman LE. In vitro immunoactivity of propylthiouracil, methimazole and carbizole in patients with Graves' disease: a possible cause of antithyroid drug-induced agranulocytosis. J Clin Endocrinol Metab 1984; **58**:868-872
11. Wilson R, McKillop JH, Travers M, Smith J, Smith E, Thomson JA. The effect of antithyroid drugs on intracellular mediators. Acta Endocrinol Copenh 1990; **122**: 605-609
12. Hall LH, Mohney B, Kier LB. The electrotopological state: an atom index for QSAR. Quant. Struct. Act Relat 1991; **10**: 43-52
13. Kier LB, Hall LH**.** An atom description in QSAR models: development and use of an atom level index. In: Testa B (Ed.) Advances in Drug Design, Academic Press Ltd, New York, 1992, pp. 1-38
14. Hall LH, Kier LB. Binding of salicylamides: QSAR analysis with electrotopological state indexes. Med. Chem Res 1992; **2**: 497-502
15. Abou-Shaaban RRA, Al-Khamees HA, Abou-Auda HS, Simonelli AP. Atom level electrotopological-state indexes in QSAR: designing and testing antithyroid agents. Pharm Res 1996; **13**: 129-136
16. Abou-Shaaban RRA, Al-Khamees HA, Abou-Auda HS, Simonelli AP. Development of new antithyroid compounds with reduced antioxidant property. Saudi Pharm J 1995; **3**: 180-195
17. Institute for Laboratory Animal Research. National Research Council. Guide for the Care and Use of Laboratory Animals. Washington DC, National Academy Press, 1996
18. Grebe SKG, Feek CM, Ford HC, Fagerström JN, Cordwell DP, Delahunt JW, Toomath RJ. A randomized trial of short-

term treatment of Graves' disease with high-dose carbimazole plus thyroxine versus low-dose carbimazole. Clin Endocrinol 1998; **48**: 585-592

19. Maugendre D, Gatel A, Massart C, Guilhem I, Lorcy Y, Lescouarch J, Herry JY, Allannic H. Antithyroid drug and Graves' disease – prospective randomized assessment of long-term treatment. Clin Endocinol 1999; 50: 127-132
20. Meyer-GeBner M, Benker G, Lederbogen S, Olbricht T, Reinwein D. Antithyroid drug-induced agranulocytosis: clinical experience with ten patients treated at one institution and review of the literature. J Endocrinol Invest 1994; **17**: 29-36