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THE SELENIUM ANALOG OF METHIMAZOLE

MEASUREMENT OF ITS INHIBITORY EFFECT ON TYPE I 5′-DEIODINASE AND OF ITS ANTITHYROID ACTIVITY

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Abstract—Methimazole (MMI), unlike propylthiouracil (PTU) is a poor inhibitor of type I iodothyronine deiodinase (ID-1). Inhibition of the enzyme by PTU was attributed initially to formation of a mixed disulfide between PTU and a cysteine residue at the active site. Presumably, MMI was unable to form a stable mixed disulfide and thus did not inhibit the enzyme. However, it has been demonstrated recently that ID-1 is a selenium-containing enzyme, with selenocysteine, rather than cysteine, at the active site. This observation raised the possibility that the selenium analog of MMI, methyl selenoimidazole (MSeI), might be a better inhibitor of ID-1 than MMI itself, as formation of the Se—Se bond with the enzyme would be expected to occur more readily than formation of the S—SE bond. To test this possibility, we developed a procedure for the synthesis of MSeI and compared MSeI with MMI and PTU for inhibition of ID-1 and for antithyroid activity. For inhibition of ID-1, MMI and MSeI were tested at concentrations of 10–300 μM. No significant inhibition was observed with MMI. MSeI showed slight but significant inhibition only in the 100–300 μM range. PTU, on the other hand, showed marked inhibition at 1 μM. Thus, replacement of the sulfur in MMI with selenium only marginally increases its inhibitory effect on ID-1. As an inhibitor of ID-1, MSeI is much less than 1% as potent as PTU. MMI and MSeI were also compared for antithyroid activity, both *in vivo* and *in vitro*. As an inhibitor of the catalytic activity of thyroid peroxidase, MMI was 4–5 times more potent than MSeI in a guaiacol assay, but only twice as potent in an iodination assay. In *in vivo* experiments with rats, MMI was at least 50 times more potent than MSeI in inhibiting thyroidal organic iodine formation. The relatively low potency of MSeI *in vivo* suggests that it is much less well concentrated by the thyroid than is MMI.

Key words: thyroid; methimazole; selenium; 5′-deiodinase; antithyroid drugs

Methimazole (MMI)§ is an antithyroid drug, widely used in the treatment of hyperthyroidism (Graves' disease). Although its mechanism of action on the thyroid is similar to that of another clinically important antithyroid drug, PTU [1], MMI lacks the inhibitory effect of PTU on ID-1, an enzyme present in liver, kidney, thyroid and other tissues, which converts T_4 to the biologically active thyroid hormone T_3.

A mechanism for the inhibition of ID-1 by PTU was proposed by Leonard and Visser [2]. They obtained evidence for the formation of a mixed disulfide between PTU and a putative cysteine residue at the active site, resulting in irreversible inactivation of the enzyme.

More recently, it was demonstrated by Berry *et al.* [3, 4] that ID-1 is a selenium-containing enzyme, with selenocysteine rather than cysteine at the active site. This observation led them to propose the modified mechanism of inhibition of ID-1 by PTU shown in Fig. 1 [4]. This scheme, which is based on that originally proposed by Leonard and Visser, involves formation of an enzyme-Se—S-PTU adduct rather than the previously postulated mixed disulfide.

The scheme in Fig. 1 raises the possibility that the selenium analog of MMI, MSeI, might be a better inhibitor of ID-1 than MMI itself, as formation of the Se—Se bond would be expected to occur more readily than formation of the Se—S bond. To test this possibility, we developed a procedure for synthesizing MSeI, and we compared MSeI with MMI and PTU for inhibition of ID-1 in rat liver microsomes.

MSeI and MMI were also compared for their ability to inhibit organic iodine formation in rat thyroids *in vivo*, and for inhibition of TPO catalytic activity *in vitro*.

MATERIALS AND METHODS

Synthesis of the selenium analog of methimazole.

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§ Abbreviations: MMI, 1-methyl-2-mercaptoimidazole; MSeI, 1-methyl-2-selenoimidazole; PTU, 6-propyl-2-thiouracil; TPO, thyroid peroxidase; ID-1, 5′-iodothyronine deiodinase; T_4, thyroxine; T_3, 3′,3,5-triiodothyronine; and rT_3, 3′,5′,3-triiodothyronine.

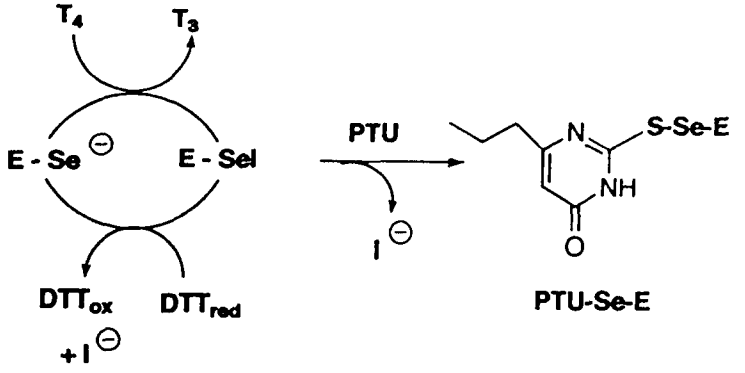

Fig. 1. Proposed mechanism for Type I iodothyronine deiodination and for inhibition by PTU.

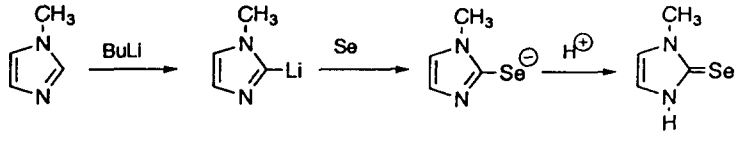

Fig. 2. Synthetic pathway for preparation of the selenium analog of MMI.

N-Methylimidazole was metallated with butyl lithium at −78°, and the resulting lithium derivative was treated with elemental selenium. The mixture was then acidified and extracted with chloroform. Concentration of the dried chloroform extract under reduced pressure afforded yellow–orange crystals of the selenium analog of MMI (Fig. 2). This product, melting point 133°, was characterized by proton and carbon NMR and infrared spectroscopy. Its spectroscopic properties were consistent with the proposed structure. The sample was homogeneous on thin-layer chromatography using silica gel. This result along with a clean C-13 NMR indicated that the sample (Prep I) as isolated was essentially pure. In another preparation (Prep II), the product was recrystallized from ethyl acetate-hexanes affording orange crystals melting at 142°. This sample gave a satisfactory elemental analysis. Its spectroscopic properties were identical in all respects with those obtained from the initial preparation. No byproducts or impurities could be detected in either sample by proton or carbon NMR spectroscopy. Details of the synthesis and characterization of the selenium analogue of MMI will be reported elsewhere.*

Prep I was used for most of the data presented in this paper. Prep II was used in the experiment of Fig. 4, and in one set of experiments (Series II) in Table 1. In comparable experiments performed with both Preps (data not shown), closely agreeing results were obtained.

TPO. Highly purified porcine TPO was prepared as previously described [5, 6]. The value for A_{412}/A_{280} was 0.48.

Measurement of ID-1 activity. This procedure was based on the method used in the laboratory of Dr. Reed Larsen, Brigham and Women's Hospital, Boston, MA.† The incubation system contained 0.5 μM ^{125}I-rT_3, 5 mM dithiothreitol, 25 μg/mL rat liver microsomal protein, and various concentrations of drug, in 65 mM phosphate buffer, pH 7.2, containing 2 mM EDTA (total volume, 200 μL). The reaction was started by addition of the microsomes, and after 20 min of incubation, with shaking, at 37°, organic ^{125}I was precipitated by successive additions of 100 μL of rabbit serum and 250 μL of cold 20% trichloroacetic acid. After the samples had been thoroughly mixed and centrifuged, [^{125}I]iodide production was measured by counting an aliquot of the supernatant, with correction for a blank sample incubated in the absence of microsomes. Results were calculated as picomoles rT_3 deiodinated per minute per milligram protein [7].

^{125}I-rT_3 was purchased from Dupont-NEN (Boston, MA). Stable rT_3 was obtained from Henning Berlin GmbH. To avoid high blanks in the measurement of deiodinase activity, the ^{125}I-rT_3 preparation was purified on a Sephadex LH-20 column, except when used within a few days after shipment. This procedure was suggested by Dr. Reed Larsen,† and involved application of the ^{125}I-rT_3, diluted with water, to the column, repeated washing with water, and elution with 70% ethanol.

Inhibition of TPO-catalyzed iodination by MMI, MSeI and PTU: concentration–inhibition curves. The incubation system contained 5 nM highly purified porcine TPO, 100 μM [^{125}I]iodide, 0.5 mg/mL BSA, various concentrations of drug, and 100 μM H_2O_2,

* Guziec LJ and Guziec FS Jr, *J Org Chem*, in press.

† Dr. Reed Larsen, personal communication. Cited with permission.

in 65 mM phosphate buffer, pH 7.0. The reaction was started with the H_2O_2, and after a 1-min incubation at 37° the reaction was stopped by inactivating the TPO with a large excess of MMI (final concn, 5 mM). The fraction of the total ^{125}I bound to protein was determined by paper chromatography in collidine-NH_4OH for 60 min [8]. This brief chromatography procedure is very convenient for separating ^{125}I-protein, which remains at the origin of the chromatogram, from unreacted [^{125}I]iodide, which moves close to the solvent front. Under the conditions described above, about 30% of the added ^{125}I was bound to protein in the control sample. With graded concentrations of drug, this value was progressively reduced, permitting construction of concentration–inhibition curves.

Inhibition of TPO-catalyzed guaiacol oxidation by MMI and MSeI. The incubation system contained 8 nM highly purified porcine TPO, 2 mM guaiacol, 0.5 mg/mL BSA, the desired concentration of drug, and 314 μM H_2O_2. The reaction was initiated directly in a 3-mL cuvette with 10 μL of 66 mM H_2O_2, added with a Calbiochem plumper. Absorbance was measured with a Gilford spectrophotometer, equipped with a digital absorbance meter and a data lister. The instrument was zeroed immediately before addition of H_2O_2. A stopwatch was started simultaneously with the addition of H_2O_2, and absorbance at 470 nm at 15 sec was recorded with the data lister.

Inhibition of organic iodine formation in thyroids of rats injected with MMI or MSeI. Two separate experiments were performed, each with 15 rats. In each experiment the rats were divided into 5 groups of 3. The rats in each group were injected i.p. with: (1) saline (controls), (2) MMI (1 μmol/100 g), (3) MMI (3 μmol/100 g), (4) MSeI (1 μmol/100 g), or (5) MSeI (3 μmol/100 g). Thirty minutes after the saline or drug injection, the rats were injected i.p. with 0.5 mL of 0.1 μM iodide containing 20–25 μCi of ^{125}I, and 1 hr after the ^{125}I injection the rats were anesthetized with ether and exsanguinated via the abdominal aorta; the thyroid glands were dissected and weighed. The thyroids from each rat were homogenized with a motor-driven pestle in an all-glass homogenizer in 500 μL of saline–Tris–MMI (0.11 M–0.04 M–0.05 M, pH 8.5). The homogenizer was surrounded by an ice bath during the grinding procedure. The homogenates were transferred to 1.5-mL plastic, conical centrifuge tubes and centrifuged in the cold room in a minifuge at 12,000 *g* for 3 min. Aliquots of both the original homogenate and the supernatant were counted, and it was observed that the supernatants contained 90–100% of the total ^{125}I in the gland. For determination of the fraction of the total ^{125}I in the gland that was organically bound, an aliquot (25 or 50 μL) of the supernatant was transferred to a filter paper strip for chromatography in collidine-NH_4OH. For each rat the percent of the injected dose of ^{125}I taken up by the gland (per 10 mg of tissue) was calculated, and this value was multiplied by the fraction of the ^{125}I that remained at the origin of the chromatogram (organically bound ^{125}I). This product represented the percent of the injected dose that was organically bound per 10 mg of thyroid tissue. For the control rats, the mean ± SD was 1.67 ± 0.19 in Expt. 1 and 1.60 ± 0.28 in Expt. 2. A similar calculation was made for each of the drug-injected rats, and expressed as a percent of the control.

Time course of inhibition of TPO-catalyzed iodination by MMI and MSeI. The incubation system (500 μL) contained 10 nM TPO, 100 μM $^{125}I^-$, 0.5 mg/mL BSA, 1 mg/mL glucose, 0.5 μg/mL glucose oxidase, and various concentrations of MMI or MSeI in 67 mM phosphate buffer, pH 7.0. The reaction was started at 37° by the addition of glucose oxidase. At intervals ranging from 2 to 45 min, 50 μL of the incubation mixture was withdrawn and added to a small tube containing 5 μL of 0.1 M MMI to stop the reaction. The tubes were kept in an ice bath, and after all the samples had been collected, 25 μL was transferred to a filter paper strip for chromatography in collidine-NH_4OH. The fraction of the total ^{125}I on the paper that remained at the origin was determined, and these values were plotted against time of incubation.

Test of inactivation of oxidized TPO by MMI and MSeI. A solution containing 12.5 pmol of TPO in phosphate buffer, pH 7.0 (approx. 25 nM), was treated with 50 pmol of H_2O_2. After 30 sec at 24°, various concentrations of MMI or MSeI were added to the oxidized TPO. The drug and oxidized TPO remained in contact for 1 min, and an aliquot of the mixture was then removed for guaiacol assay. The guaiacol assay mixture contained 33 mM guaiacol, 0.5 mg/mL BSA, and 314 μM H_2O_2. The value for A_{470} 1 min after addition of H_2O_2 was used as a measure of guaiacol activity. The guaiacol activity in the presence of drug was calculated as the percent of guaiacol activity in a control incubation mixture that contained H_2O_2 and an equivalent volume of buffer instead of drug.

RESULTS

Effect of MMI and MSeI on ID-1 activity. MMI and MSeI were tested at 10, 30, 100 and 300 μM for inhibition of ID-1 activity. Results are shown in Table 1. Two series of experiments were performed, each consisting of four separate experiments. In each series a single microsomal preparation was used. In series I, MMI and MSeI were compared at 10, 30 and 100 μM, and in series II at 30, 100 and 300 μM. In series I, it appeared that MSeI was slightly inhibitory at 100 μM, in contrast to MMI. However, in series II, MSeI showed no significant inhibition at 100 μM. At 300 μM, however, significant inhibition ($P < 0.05$) was observed with MSeI, but not with MMI. In both series of experiments, PTU was very definitely inhibitory at 1 μM, as has been shown by previous investigators [2]. The difference in results between series I and series II may be related to the significantly higher control activity of the microsomal preparation in series II. In any case, it is clear that MSeI is only a very weak inhibitor of ID-1 and that high concentrations are required to demonstrate that replacement of the sulfur in MMI with selenium has an effect on inhibition of ID-1.

Inhibition of TPO-catalyzed iodination by MMI and MSeI. Concentration–inhibition curves are shown in Fig. 3 for the effects of MMI, MSeI and

Table 1. Inhibition of rat liver microsomal ID-1 by MSeI, MMI and PTU

	Series I				Series II				
	ID-1 (% of control activity)*					ID-1 (% of control activity)†			
	1 μM	10 μM	30 μM	100 μM		1 μM	30 μM	100 μM	300 μM
MSeI		95 ± 11	92 ± 6	78 ± 7	MSeI		97 ± 2	93 ± 4	72 ± 14
MMI		102 ± 10	99 ± 5	93 ± 10	MMI		96 ± 4	98 ± 4	95 ± 3
PTU	27 ± 5‡				PTU	47 ± 1‡			

See Materials and Methods for assay conditions. Values in each series are means ± SD of four experiments, unless noted otherwise.

* Control activity: 119–317 pmol rT_3 deiodinated per min per mg microsomal protein.

† Control activity: 321–831 pmol rT_3 deiodinated per min per mg microsomal protein.

‡ Single experiment with triplicate samples.

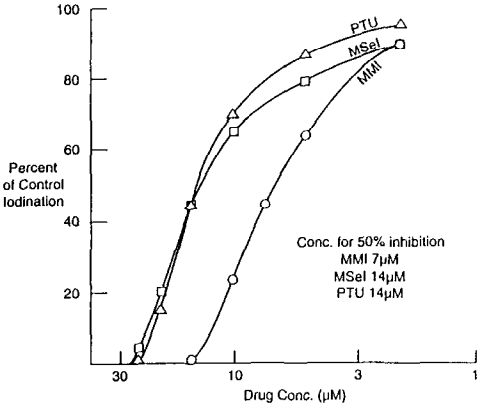

Fig. 3. Concentration–inhibition curves for inhibition of TPO-catalyzed iodination of BSA by MMI, MSeI and PTU. The incubation mixture contained 5 nM TPO, 100 μM [^{125}I]iodide, 0.5 mg/mL BSA, various concentrations of drug, and 100 μM H_2O_2 in phosphate buffer at pH 7.0 and 37°. The reaction was started with H_2O_2 and stopped after 1 min by addition of a large excess of MMI. In the control sample, 30 nmol I/mL were organically bound to BSA. Iodination in the presence of drug is plotted as percent of the control.

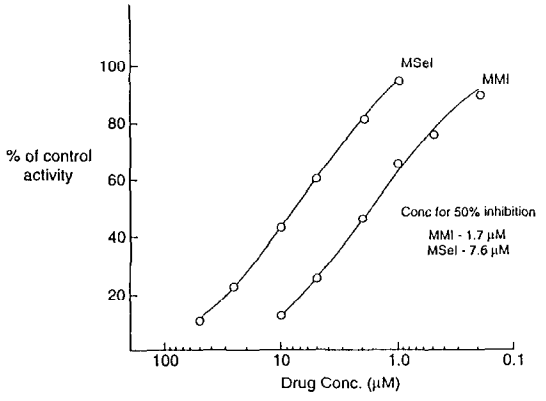

Fig. 4. Concentration–inhibition curves for inhibition of TPO-catalyzed guaiacol oxidation by MMI and MSeI. The incubation mixture contained 8 nM TPO, 2 mM guaiacol, 0.5 mg/mL BSA, various concentrations of drug, and 314 μM H_2O_2, in phosphate buffer, pH 7.0, at 24°. The reaction was started with H_2O_2 directly in a cuvette, and ΔA_{470} was measured at 15 sec. The control value for ΔA_{470} was 0.271. Values for ΔA_{470} in the presence of drug are plotted as percent of the control.

PTU on TPO-catalyzed iodination of BSA. MSeI was only 50% as potent as MMI in this assay system, but it was equally as potent as PTU. We have reported previously [9] that MMI is about twice as potent as PTU as an inhibitor of TPO-catalyzed iodination of goiter thyroglobulin.

Inhibition of TPO-catalyzed guaiacol oxidation by MMI and MSeI. These results are shown in the concentration–inhibition curves of Fig. 4. In the guaiacol assay system, MSeI was only about 20% as potent as MMI, compared to 50% in the iodination assay. Based on the concentration required for 50% inhibition, the inhibitory potency of MMI in the guaiacol assay was about 4 times greater than in the iodination assay.

Non-enzymatic oxidation of MSeI by H_2O_2. Since seleno compounds are more susceptible to oxidation than their sulfur analogs, the question arose whether non-enzymatic oxidation of MSeI by the excess H_2O_2 used in the guaiacol and iodination assays might interfere with the validity of the assay. To test this possibility, we measured the disappearance of 25 μM MSeI in the presence of 100 and 300 μM H_2O_2 by following the decrease in A_{257} (λ_{max} for MSeI). Decreases of 3.4 and 7.8%, respectively, were observed after 1 min. MMI under the same conditions showed no decrease even after 5 min. Although MSeI showed measurable non-enzymatic oxidation by H_2O_2, the slow rate of this reaction would not be expected to interfere appreciably with assays in which measurements were made 15 sec after addition of 300 μM H_2O_2 (guaiacol assay), or 1 min after addition of 100 μM H_2O_2 (iodination assay).

Time–course of inhibition of TPO-catalyzed iodination by MMI and MSeI. These results are shown in Fig. 5. The iodination system in this case

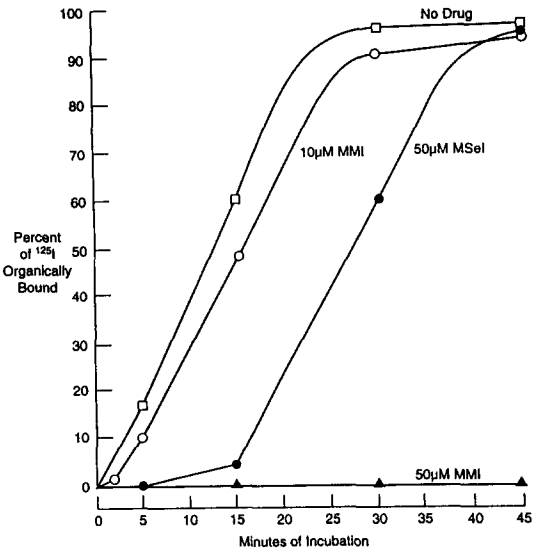

Fig. 5. Time course of inhibition of TPO-catalyzed iodination by MMI and MSeI. The incubation system contained 10 nM TPO, 100 μM $^{125}I^-$, 0.5 mg/mL BSA, various concentrations of drug, 1 mg/mL glucose, and 0.5 μg/mL glucose oxidase, in phosphate buffer, pH 7.0, at 37°. The reaction was started with glucose oxidase. Aliquots of the reaction mixture were removed at the indicated intervals and treated with a large excess of MMI to stop the reaction. Values are expressed as percent of added $^{125}I^-$ organically bound to BSA (equivalent to nmol I/mL bound to BSA, as the incubation mixture initially contained 100 nmol I^-/mL).

differed from that in Fig. 3 in that H_2O_2 was generated by glucose-glucose oxidase instead of being added as a bolus. Under these conditions, H_2O_2 generation is the rate-limiting step in iodination. In the presence of 10 μM MMI, iodination was strongly inhibited during the first 2 min of incubation but thereafter showed almost complete escape from inhibition. However, when the concentration of MMI was raised to 50 μM, iodination was inhibited completely throughout the 45-min incubation period. We have reported previously [10] that inhibition of TPO-catalyzed iodination by MMI (and PTU) can be reversible or irreversible, depending on the relative concentrations of drug and iodide. Whether inhibition is reversible or irreversible depends on the relative rates of drug metabolism by the enzyme and inactivation of enzyme by the drug. With a low concentration of drug relative to I^- (10 μM MMI in Fig. 5), drug metabolism is very rapid compared to enzyme inactivation. By 2 min most of the drug has been metabolized, whereas only a small fraction of the TPO is inactivated. However, with a higher concentration of drug relative to I^- (50 μM MMI in Fig. 5), inactivation of TPO is very rapid, and only a small fraction of the drug is metabolized.

It is apparent from the results in Fig. 5 that the inhibition pattern with MSeI differed greatly from that with MMI. With 50 μM MSeI, iodination was inhibited only during the first 15 min of incubation. Thereafter, there was escape from inhibition, and iodination proceeded at a rate very close to that of the control. After 45 min of incubation in the presence of 50 μM MSeI, iodination reached essentially the same maximum value as that seen in the control. These results suggested that inactivation of TPO occurs much more readily with MMI than with MSeI. This is demonstrated more directly in the following section.

Inactivation of oxidized TPO by MMI and MSeI. Results are shown in Fig. 6 for the inactivation of oxidized TPO by various concentrations of MMI and MSeI. The TPO was first treated with H_2O_2 to produce an oxidized form of TPO. Drug was then added, and after 1 min of contact between drug and oxidized TPO, an aliquot of the reaction mixture was withdrawn for measurement of enzyme activity by the guaiacol assay. Enzyme activity was abolished almost completely by 1 μM MMI, whereas 100 μM MSeI had no significant effect on enzyme activity. It is apparent from these results that MMI is much more potent than MSeI as an inactivator of oxidized TPO.

Antithyroid effects of MMI and MSeI in vivo. Table 2 shows results of two separate experiments with rats injected with MMI or MSeI. Measurements were made of organic iodine formation in the thyroid, as described in Materials and Methods. At a dosage of 1 μmol/100 g body wt, MSeI displayed only a slight inhibitory effect, whereas MMI reduced organic iodine formation in the thyroid to about 1% of the control value. Even at 3 μmol/100 g body wt, MSeI inhibited organic iodine formation only about 50%. Based on the results in Table 2, it may be conservatively estimated that MMI is at least 50 times more potent than MSeI in inhibiting organic iodine formation in the thyroids of rats. This contrasts greatly with the *in vitro* results (Fig. 3), which showed MMI to be only twice as potent as MSeI as an inhibitor of TPO-catalyzed iodination.

One possible explanation for the great discrepancy between the *in vivo* and *in vitro* results is that MSeI might be cleared from the circulation much more rapidly than MMI. This possibility was examined by measuring the drug concentration in ultrafiltrates of serum at 5, 30, and 90 min after intravenous injection of 3 μmol/per 100 g body wt. As shown in Table 3, there was relatively little difference in clearance rate between the two drugs, making it unlikely that differences in peripheral metabolism could account for the discrepancy between the *in vivo* and *in vitro* results.

DISCUSSION

It was shown recently that ID-1 is a selenium-containing enzyme, with selenocysteine rather than cysteine at the active site [3]. As indicated in the introduction, this new information raised the possibility that the selenium analog of MMI (MSeI) might be a better inhibitor of ID-1 than the parent compound. MSeI was synthesized for the first time in this study and compared with MMI for inhibition of ID-1. It was also tested for inhibitory effects that are common to both MMI and PTU.

Contrary to our expectations, replacement of the

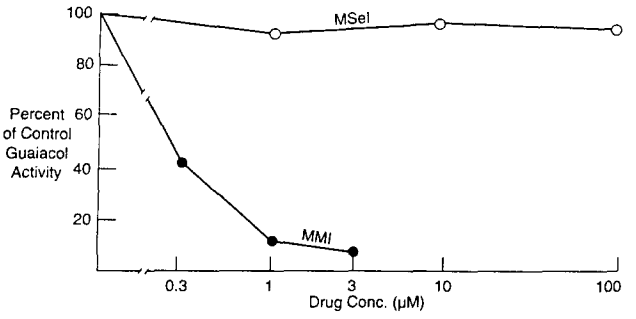

Fig. 6. Inactivation of oxidized TPO by MMI and MSeI. H_2O_2 (final concentration 100 nM) was added to a solution containing 25 nM TPO in phosphate buffer, pH 7.0. After 30 sec at 24°, MMI or MSeI was added at the indicated concentrations. One minute after the drug addition, an aliquot was removed for measurement of residual TPO activity by guaiacol assay. A buffer control was run with each drug sample. Values for ΔA_{470} for the buffer controls varied from 0.230 to 0.250. Results for each drug concentration (ΔA_{470}) are plotted as percent of the respective buffer control.

Table 2. Inhibition of organic iodine formation in thyroids of rats injected with MMI or MSeI

Injected dose (μmol/100 g body wt)	Organically bound ^{125}I in thyroid (% of control)			
	Expt. 1		Expt. 2	
	MMI	MSeI	MMI	MSeI
1	1.3 ± 1.0(3)	97 ± 21(3)	1.1 ± 0.4(3)	75 ± 9.1(3)
3	0.2 ± 0.06(3)	42,49(2)	0.2 ± 0.007(3)	57 ± 35(3)

Values are means ± SD. Control values for percent of injected dose organically bound per 10 mg thyroid tissue were 1.67 ± 0.19% in Expt. 1 and 1.60 ± 0.28% in Expt. 2.

Table 3. Serum concentrations of MMI and MSeI at intervals after injection into rats

	Drug in serum (μM)		
	5 min	30 min	90 min
MMI	35 ± 3	28 ± 1	25 ± 0.6
MSeI	40 ± 3	26 ± 3	22 ± 2

Rats under pentobarbital anesthesia were injected via the femoral vein with 3 μmol of MMI or MSeI per 100 g body weight, and blood samples were withdrawn from the subclavian vein at 5, 30 and 90 min. Ultrafiltrates of serum were analyzed by HPLC. Values are means ± SD, N = 3.

sulfur in MMI with selenium has only a slight effect on inhibition of ID-1. Only at concentrations in the range of 100–300 μM was MSeI slightly more inhibitory than MMI. As reported by previous investigators [11], we observed that MMI itself showed no significant inhibitory effect. PTU, on the other hand, displayed marked inhibitory activity at 1 μM, in agreement with previously reported results [2].

While the present study was in progress, Visser *et al.* [12] reported that the selenium analog of PTU is about twice as potent as PTU as an inhibitor of ID-1 activity. This is a greater effect of sulfur replacement with selenium than we observed in the present study with MMI. We have subsequently prepared the selenium analog of PTU, and, in contrast to the results of Visser *et al.*, we observed that it was about equally as potent as PTU in inhibiting ID-1 activity. These results will be reported separately.*

MMI and MSeI were also compared as inhibitors of TPO catalytic activity. Concentration–inhibition curves were prepared for TPO-catalyzed iodination of BSA (Fig. 3) and for TPO-catalyzed oxidation of guaiacol (Fig. 4). In the iodination assay, MSeI was 50% as potent as MMI. Based on our previous studies [13], it is likely that the mechanism of inhibition involves competition between the drugs and tyrosyl residues in BSA for an oxidized species of I (I^+ or HOI). The active I species can act either to iodinate the tyrosyl residues or to oxidize the drug (probably to the disulfide or diselenide).

In the guaiacol assay, MSeI was only about 20% as potent an inhibitor as MMI. We have reported previously [14] that MMI competes with guaiacol for a common site on oxidized TPO. On this basis, we would conclude that MSeI competes with guaiacol for this site less favorably than does MMI.

Studies of the time–course of iodination in the

* Taurog A, Dorris ML, Hu W-X and Guziec FS Jr, Manuscript submitted for publication.

presence of MMI and MSeI (Fig. 5) suggested that MSeI, unlike MMI, cannot act as an irreversible inhibitor of TPO. As mentioned above (see Results), irreversible inhibition occurs under conditions that favor rapid inactivation of the TPO by the drug. However, as shown in Fig. 6, MSeI differs from MMI in that it does not react with oxidized TPO to inactivate the enzyme. As suggested previously for TPO [14] and as shown more definitively for lactoperoxidase (LPO) [15], rapid inactivation of the peroxidase by MMI involves a suicide reaction between the heme and an oxidized form of the drug. Presumably, therefore, the analogous reaction with MSeI is much slower than that with MMI. MSeI, therefore, acts only as a competitive inhibitor of peroxidase-catalyzed iodination. In this respect it is similar to carbimazole [10] and *N*, *N'*-dimethylbenzimidazole-2-thione [16].

Experiments were also performed in the present study comparing the antithyroid action of MMI and MSeI *in vivo* in rats. These were acute experiments in which inhibition of organification of ^{125}I in the thyroid was measured (Table 2). MMI was at least 50 times more potent than MSeI as an inhibitor of organic iodine formation in the thyroid. This contrasts greatly with the *in vitro* results, which showed that MMI was only twice as potent as MSeI as an inhibitor of TPO-catalyzed iodination (Fig. 3). As one plausible explanation for this discrepancy, we considered the possibility that MSeI was cleared much more rapidly from the circulation than was MMI. However, this possibility was excluded by the results shown in Table 2. Another possible explanation is that MMI is more readily concentrated by the thyroid than is MSeI. We [17] and others [18] have shown that MMI and PTU are concentrated by the thyroids of rats. We have also obtained evidence that concentration of the drug by the thyroid is essential for antithyroid activity [19]. Thus, although definitive proof is lacking, we suggest that MSeI is only poorly concentrated by the thyroid, and that this accounts for its relatively weak antithyroid activity in rats.

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REFERENCES

1. Taurog A, Hormone synthesis: Thyroid iodine metabolism. In: *Werner and Ingbar's The Thyroid: A Fundamental and Clinical Text* (Eds. Braverman LE and Utiger RD), 6th Edn., pp. 51–97. J.B. Lippincott, Philadelphia, 1991.
2. Leonard JL and Visser TJ, Biochemistry of deiodination. In: *Thyroid Hormone Metabolism* (Ed. Hennemann G), pp. 189–229. Marcel Dekker, New York, 1986.
3. Berry MJ, Banu L and Larsen PR, Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. *Nature* **349**: 438–440, 1991.
4. Berry MJ and Larsen PR, The role of selenium in thyroid hormone action. *Endoc Rev* **13**: 207–219, 1992.
5. Rawitch AB, Taurog A, Chernoff SB and Dorris ML, Hog thyroid peroxidase: Physical, chemical, and catalytic properties of the highly purified enzyme. *Arch Biochem Biophys* **194**: 244–257, 1979.
6. Yokoyama N and Taurog A, Porcine thyroid peroxidase: Relationship between the native enzyme and an active, highly purified tryptic fragment. *Mol Endocrinol* **2**: 838–844, 1988.
7. Pazos-Moura CC, Moura EG, Dorris ML, Rehnmark S, Melendez L, Silva E and Taurog A, Effect of iodine deficiency and cold exposure on thyroxine 5'-deiodinase activity in various rat tissues. *Am J Physiol* **260**: E175–E182, 1991.
8. Lamas L, Dorris ML and Taurog A, Evidence for a catalytic role for thyroid peroxidase in the conversion of diiodotyrosine to thyroxine. *Endocrinology* **90**: 1417–1426, 1972.
9. Nakashima T and Taurog A, Rapid conversion of carbimazole to methimazole in serum: Evidence for an enzymatic mechanism. *Clin Endocrinol (Oxf)* **10**: 637–648, 1979.
10. Engler H, Taurog A, Luthy C and Dorris ML, Reversible and irreversible inhibition of thyroid peroxidase-catalyzed iodination by thioureylene drugs. *Endocrinology* **112**: 86–95, 1983.
11. Visser TJ, Van Overmeeren E, Fekkes D, Docter R and Hennemann G, Inhibition of iodothyronine 5'-deiodinase by thioureylenes; Structure–activity relationships. *FEBS Lett* **103**: 314–318, 1979.
12. Visser TJ, Kaptein E and Aboul-Enein HY, Selenouracil derivatives are potent inhibitors of the selenoenzyme Type I iodothyronine deiodinase. *Biochem Biophys Res Commun* **189**: 1362–1367, 1992.
13. Taurog A, Dorris ML and Guziec FS Jr, Metabolism of ^{35}S- and ^{14}C-labeled 1-methyl-2-mercaptoimidazole *in vitro* and *in vivo*. *Endocrinology* **124**: 30–39, 1989.
14. Engler H, Taurog A and Nakashima T, Mechanism of inactivation of thyroid peroxidase by thioureylene drugs. *Biochem Pharmacol* **31**: 3801–3806, 1982.
15. Doerge DR, Mechanism-based inhibition of lactoperoxidase by thiocarbamide goitrogens. *Biochemistry* **25**: 4724–4728, 1986.
16. Doerge DR, Decker CJ and Takazawa RS, Chemical and enzymatic oxidation of benzimidazoline-2-thiones: A dichotomy in the mechanism of peroxidase inhibition. *Biochemistry* **32**: 58–65, 1993.
17. Nakashima T, Taurog A and Riesco G, Mechanism of action of thioureylene antithyroid drugs: Factors affecting intrathyroidal metabolism of propylthiouracil and methimazole in rats. *Endocrinology* **103**: 2187–2197, 1978.
18. Marchant B and Alexander WD, The thyroid accumulation, oxidation and metabolic fate of ^{35}S-methimazole in the rat. *Endocrinology* **91**: 747–756, 1972.
19. Nogimori T, Braverman LE, Taurog A, Fang S-L, Wright G and Emerson C, A new class of propylthiouracil analogs: Comparison of 5'-deiodinase inhibition and antithyroid activity. *Endocrinology* **118**: 1598–1605, 1986.