Evidence for direct interactions between methimazole and free radicals

J.J. Taylor, R.L. Willson* and P. Kendall-Taylor

Department of Medicine, Wellcome Research Laboratories, Royal Victoria Infirmary, University of Newcastle upon Tyne, Newcastle upon Tyne NEI 4LP and **Department of Biochemistry, Brunel University, Uxbridge UB8 3PH, Middlesex, England*

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Two experimental approaches have been used to investigate possible interactions of methimazole with hydroxyl ($OH^{\cdot}$) and iodine ($I_2^{\cdot-}$) free radicals. Methimazole protects sensitive enzymes against inactivation caused by $OH^{\cdot}$ and $I_2^{\cdot-}$. Pulse radiolysis reveals the presence of transient absorbing species formed as a result of the direct interaction between methimazole and these free radicals. Measurements of the absolute rate constants indicate that these reactions proceed very rapidly at very low drug concentrations. These experiments provide direct evidence that methimazole is a potent scavenger of $OH^{\cdot}$ and $I_2^{\cdot-}$ and they provide a possible explanation for the previously observed effects of this drug.

Thyroid *Free radical* *Iodine* *Antithyroid drug*

1. INTRODUCTION

Methimazole (MMI), the active metabolite of carbimazole, inhibits thyroid hormone biosynthesis by preventing the organification of iodide in the thyroid [1]. In addition to its antithyroid effect, there is considerable evidence that MMI also acts as an immunosuppressive agent in Graves' disease [2,3]. It has been suggested that MMI supresses the immune response by inhibiting the function of the accessory cells in the thyroid [4]. Biochemical events involving free radicals may be important in thyroid hormone biosynthesis [5] and also for macrophage/monocyte function [6]. MMI has been shown to reduce luminol-dependent chemiluminescence in antigen primed monocytes and it was suggested that MMI may inhibit the formation of oxygen radicals [7]. To explain the nature of the action of MMI on the thyroid, these studies were designed to demonstrate whether or not the drug acted as a scavenger of oxygen and iodine free radicals.

2. METHODS

2.1. *Enzyme inactivation studies*

Steady-state irradiations of enzyme solutions were performed using the Brunel University ^{60}cobalt source, calibrated using standard Fricke dosimetry. Enzyme solutions with or without methimazole (Sigma) were prepared immediately before, and assayed immediately after irradiation. Lysozyme activity was assayed by measuring the change in turbidity of a suspension of lyophilised *Micrococcus lysodeikticus* at 450 nm [8]. The activity of yeast alcohol dehydrogenase (yADH) was assayed by measuring the formation of NADH at 340 nm in a substrate solution containing NAD^+ [9].

2.2. *Pulse radiolysis studies*

Pulse radiolysis studies were carried out using the Brunel 4 MeV linear accelerator as in [10,11]. Briefly, solutions containing MMI were exposed to a single pulse of ionising radiation (1 krad as determined by thiocyanate dosimetry) and the products

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of the reactions of the radicals thus formed observed directly by fast detection spectroscopy.

2.3. *Reactions*

Irradiation of aqueous solutions yields hydroxyl radicals ($OH^{\cdot}$):

$$H_2O \longrightarrow OH^{\cdot} + e^-_{(aq.)} + H^+$$

When solutions are saturated with N_2O the yield of $OH^{\cdot}$ is doubled:

$$N_2O + e^-_{(aq.)} \xrightarrow{H_2O} OH^- + N_2 + OH^{\cdot}$$

In the presence of excess iodide ions (I^-), iodine radicals ($I_2^{\cdot -}$) are formed:

$$OH^{\cdot} + I^- \longrightarrow I^{\cdot} + OH^-$$
$$I^- + I^{\cdot} \longrightarrow I_2^{\cdot -}$$

When aqueous solutions containing formate are saturated with N_2O/O_2, $OH^{\cdot}$ are scavenged by formate ions and superoxide radicals ($O_2^{\cdot -}$) are formed upon reaction with O_2:

$$OH^{\cdot} + HCOO^- \longrightarrow COO^{\cdot -} + H_2O$$
$$COO^{\cdot -} + O_2 \longrightarrow O_2^{\cdot -} + CO_2$$

The interactions of MMI with $OH^{\cdot}$, $I_2^{\cdot -}$ and $O_2^{\cdot -}$ were thus studied.

3. RESULTS

3.1. *Protection of radical sensitive enzymes by MMI*

Hydroxyl radicals inactivate lysozyme in a dose-dependent manner. Addition of MMI (10^{-4} M) completely protects the enzyme against inactivation at all radiation doses studied (fig.1). Iodine radicals inactivate yADH in a dose-dependent manner. Addition of MMI (10^{-5} M) protects 60% of the original enzyme activity at 0.4 krad, at which dose the enzyme is completely inactivated in the absence of the drug (fig.2).

3.2. *Direct interaction of $OH^{\cdot}$ and $I_2^{\cdot}$ with MMI*

Pulse radiolysis studies of N_2O-saturated solutions containing 10^{-4} M MMI revealed transient absorption spectra with three absorption maxima

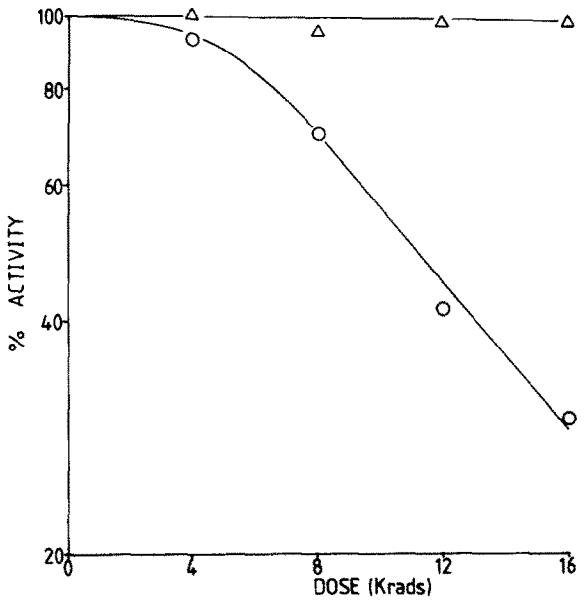

Fig.1. Effect of $OH^{\cdot}$ on the activity of a 50 μg/ml solution of lysozyme (○) and a similar solution containing 10^{-4} MMI (△).

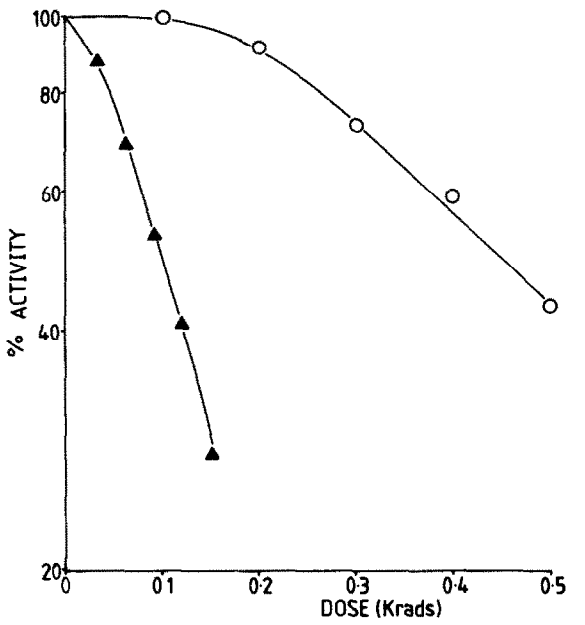

Fig.2. Effect of $I_2^{\cdot -}$ on the activity of a 100 μg/ml solution of yeast alcohol dehydrogenase (▲) and a similar solution containing 10^{-5} M MMI (○).

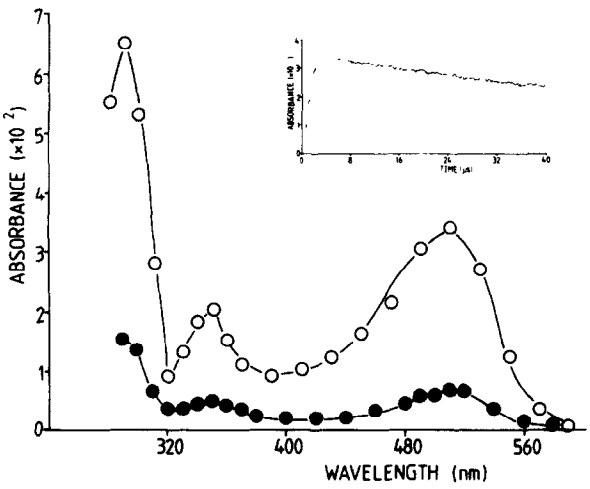

Fig.3. Absorption spectra of transients from the reactions of MMI (10^{-4} M) with OH· determined by pulse radiolysis of N_2O-saturated solutions. Spectra (normalised to a radiation dose of 1 krad) were recorded at 5 μs after pulse in the absence (○) and presence (●) of 10^{-2} M sodium formate. Inset: trace of absorption as a function of time at 510 nm.

(fig.3). These represent the products of the reaction of OH· with MMI since the parent compound has no absorption in this region (λmax = 250 nm) and addition of 10^{-3} M formate, which scavenges OH·, reduced the extinction of all three maxima by >80%. The absolute rate constant (K) for the reaction of OH· and MMI was measured using a competition technique [10] and was found to be 1.36×10^{10} $M^{-1} \cdot s^{-1}$. Transient absorption spectra of similar solutions containing 10^{-2} M KI revealed three absorption maxima (fig.4). The maximum at 360 nm is due to the iodine radical ($I_2^{\cdot -}$), the product of the primary reaction of OH· with the iodine ions present in excess in solution. The maxima at 280 nm and 510 nm are similar to those observed for the reaction of OH· and MMI in the previous spectrum, but they appear considerably later after pulse. This indicates that they correspond to the products of the reaction of $I_2^{\cdot -}$ and MMI. The absolute rate constant for the reaction of MMI and $I_2^{\cdot -}$ was measured directly and found to be 2.04×10^9 $M^{-1} \cdot s^{-1}$.

No transient absorption spectra were observed when N_2O/O_2-saturated solutions containing MMI and formate were irradiated providing no evidence for a reaction between $O_2^{\cdot -}$ and MMI.

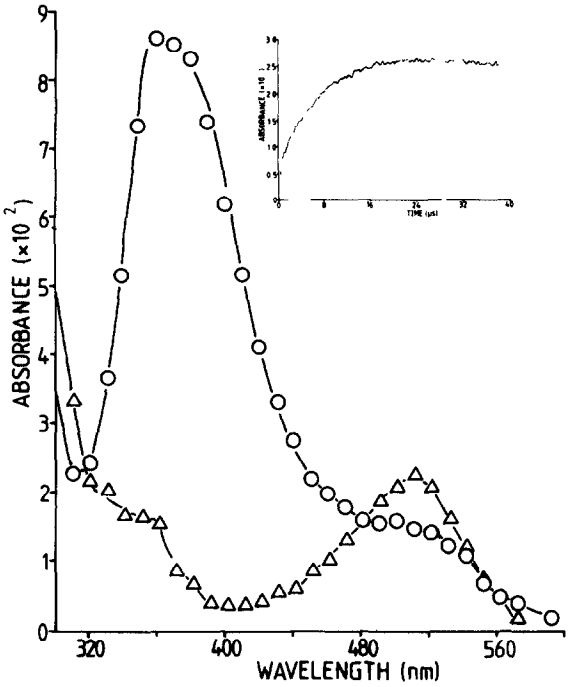

Fig.4. Absorption spectra of transients from the reactions of MMI (10^{-4} M) with $I_2^{\cdot -}$ determined by pulse radiolysis of N_2O-saturated solutions. Spectra (normalised to a radiation dose of 1 krad) were recorded at 3 μs (○) and 80 μs (△) after pulse. Inset: trace of absorption as a function of time at 510 nm.

4. DISCUSSION

We have demonstrated that MMI, the active metabolite of the antithyroid drug methimazole, can scavenge OH· and $I_2^{\cdot -}$ protecting sensitive enzymes against inactivation by these radicals. Pulse radiolysis facilitated the direct observations of the products of these reactions. Measurements of the absolute rate constants for the reaction of MMI with OH· and $I_2^{\cdot -}$ indicate that these reactions are likely to proceed very rapidly at intrathyroidal levels of MMI achieved during treatment for hyperthyroidism [12]. These findings therefore suggest that MMI is capable of acting as a free radical scavenger in the thyroid.

The products of the reaction of MMI and OH· and $I_2^{\cdot -}$ are likely to include the drug radical cation:

$$OH^{\cdot} + MMI \longrightarrow OH^- + MMI^{\cdot +}$$

and $$I_2^{\cdot} + MMI \longrightarrow 2I^- + MMI^{\cdot +}$$

This may be analogous to the reactions between free radicals and the phenothiazine drugs which share structural similarities with MMI. Phenothiazines act as free radical scavengers in vitro and in vivo [13] and react with $OH^{\cdot}$ and halogen radicals to give radical cations which have absorption maxima at 510 nm [14].

Methimazole has been shown to protect against damage by $OH^{\cdot}$ in sympathetic nerve terminals in vitro, presumably by radical scavenging [15]. Recent data demonstrating that hydroxyl radical scavengers inhibit natural killer cell cytotoxicity in vitro have indicated that compounds with this property may be capable of altering the functions of cells involved in the immune response [16]. The finding that MMI inhibits luminol-dependent chemiluminesence in antigen-primed monocytes has led to the supposition that this drug acts upon these cells by direct inhibition of peroxidase enzymes and/or scavenging of oxygen radicals [7]. Our data demonstrate that scavenging of $OH^{\cdot}$ by concentrations of MMI used in that study does indeed take place.

Iodine free radicals may be formed during the iodination of tyrosine residues by the thyroid peroxidase system [4]. The interaction of MMI with the components of thyroid hormone biosynthesis is complex and the finding that MMI readily scavenges radicals may only be a fundamental mechanism involved in the interactions between iodine, thyroid peroxidase and MMI. It is worthy of note, however, that recent evidence suggests that the extent of inhibition of thyroid hormone biosynthesis by MMI is dependent on the ratio of drug to iodide available to the thyroid peroxidase system [17].

The data presented here expose a fundamental property of MMI which may provide a biochemical basis for the actions of this drug on the thyroid follicular cells and on the antigen presenting cells during treatment for Graves' hyperthyroidism.

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