References

[1] Anonymous. Hyperprolactinaemia: pituitary tumour or not? *Lancet* 1980;i:517-9.
[2] Gomez F, Reyes F, Faiman C. Nonpuerperal galactorrhea and hyperprolactinemia. *Am J Med* 1977;**62**:648-54.
[3] Rjosk HK, Fahlbusch R, vonWerder K. Spontaneous development of hyperprolactinemia. *Acta Endocrinol (Copenh)* 1982;**100**:333-6.
[4] Vezina JL, Sutton TJ. Prolactin-secreting pituitary microadenoma; roentgenologic diagnosis. *AJR* 1974;**120**:46-54.
[5] Swanson HA, du Boulay G. Borderline variants of the normal pituitary fossa. *Br J Radiol* 1975;**48**:366-9.
[6] Burrow GN, Wortzman G, Rewcastle NB, Holgate RC, Kovacs K. Microadenomas of the pituitary and abnormal sellar tomograms in an unselected autopsy series. *N Engl J Med* 1981;**304**:156-8.
[7] Jeske W. The effect of metoclopramide, TRH and L-dopa on prolactin secretion in pituitary adenoma and in functional galactorrhea syndrome. *Acta Endocrinol (Copenh)* 1979;**91**:385-96.
[8] MacGregor C, Maldonado D, Canales ES, Soria J, Zarate A. Prolactin responsiveness to TRH in amenorrheic women with and without galactorrhea. *Acta Obstet Gynecol Scand* 1977;**56**:333-6.
[9] Gonzalez-Villapando G, Szabo M, Frohman LA. Central nervous system mediated stimulation of prolactin secretion by cimetidine, a histamine H_2 receptor antagonist: impaired responsiveness in patients with prolactin-secreting tumors and idiopathic hyperprolactinemia. *J Clin Endocrinol Metab* 1980;**51**:1417-24.
[10] Pontiroli AE, Falsetti L, Voltolini AM, *et al.* Benserazide, a stimulator of prolactin release: a new test in the diagnosis of pituitary prolactin secreting tumors. *Acta Endocrinol (Copenh)* 1981;**78**:326-32.
[11] Muller EE, Genazzani AR, Murru S. Nomifensine: diagnostic test in hyperprolactinemic states. *J Clin Endocrinol Metab* 1978;**47**:1352-7.
[12] Fine SA, Frohman LA. Loss of central nervous system component of dopaminergic inhibition of prolactin secretion in patients with prolactin-secreting pituitary tumors. *J Clin Invest* 1978;**61**:973-80.
[13] Peters JR, Foord SM, Dieguez C, Rodriguez-Arnao MD, Hall R, Scanlon MF. Microprolactinoma and functional hyperprolactinemia: two clinical entities or two phases of the same disease? In: Molinatti GM, ed. *A clinical problem: microprolactinoma.* Amsterdam: Excerpta Medica, 1982:21-34. (ICS No 584.)
[14] Pontiroli AE, De Castro e Silva E, Mazzoleni F, *et al.* The effect of histamine and H_1 and H_2 receptors on prolactin and luteinizing hormone release in humans; sex difference and the role of stress. *J Clin Endocrinol Metab* 1981;**52**:924-8.
[15] Ferrari C, Reschini E, Peracchi M, Crosignani PG. Endocrine profile and therapeutic employment of a new prolactin-lowering drug, metergoline. *Gynecol Obstet Invest* 1980;**11**:1-11.
[16] Lancranjan I. Increasing use of dopamine agonists as the first choice therapy of prolactin-secreting adenomas. In: Molinatti GM, ed. *A clinical problem: microprolactinoma.* Amsterdam: Excerpta Medica, 1982:103-13. (ICS No 584.)
[17] Wiebe RH, Hammond CB, Borchert LG. Diagnosis of prolactin-secreting pituitary microadenoma. *Am J Obstet Gynecol* 1976;**126**:993-6.
[18] Pontiroli AE, Loda G, Roggia A, Scagliola P, Falsetti L. Benserazide and nomifensine in the diagnosis of prolactin-secreting pituitary adenomas. *Acta Endocrinol (Copenh)* 1982;**101**:171-9.
[19] Keye WR, Chang RJ, Jaffe RB. Hyperprolactinemic amenorrhea. *N Engl J Med* 1977;**297**:396-401.
[20] Serri O, Rasio E, Beauregard H, Hardy J, Somma M. Recurrence of hyperprolactinemia after selective transphenoidal adenomectomy in women with prolactinoma. *N Engl J Med* 1983;**309**:280-3.
[21] Tucker HSG, Grubb SR, Wigand JP, *et al.* Galactorrhea-amenorrhea syndrome; follow-up of forty five patients after pituitary tumor removal. *Ann Intern Med* 1981;**94**:302-7.
[22] Hokfelt T, Fuxe K. Effects of prolactin and ergot alkaloids on the tuberoinfundibular dopamine neurons. *Neuroendocrinology* 1972;**9**:100-72.
[23] Pontiroli AE, Pellicciotta G, De Castro e Silva E, *et al.* Interaction of dopaminergic and antiserotoninergic drugs in the control of prolactin and LH release in normal women. *Acta Endocrinol (Copenh)* 1980;**93**:271-6.

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Methimazole and generation of oxygen radicals by monocytes: potential role in immunosuppression

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Abstract

A study was conducted investigating the possibility that the immunosuppressive action of methimazole (the active metabolite of the antithyroid drug carbimazole) might be due to an effect on the production of oxygen radicals by monocytes. Techniques comprised measurement of luminol dependent chemoluminescence in monocytes and a spectrophotometric assay for production of hydrogen peroxide.

The results showed definite inhibition of formation of oxygen radicals by resting and stimulated monocytes, which may explain the immunosuppressive action of the drug in Graves' disease. The findings also suggest that the formation of oxygen radicals and the initiation of the immune response may be closely related.

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Introduction

Phagocytic and chemotactic stimuli such as opsonised zymosan and phorbol myristate acetate provoke a respiratory burst in macrophages and polymorphonuclear leucocytes characterised by an increase in oxygen consumption, activation of the hexose monophosphate shunt, and generation of oxygen radicals.[1] Formation of superoxide anion (O_2^-), the first of these radicals, is apparently catalysed by a membrane bound reduced form of nicotinamide adenine dinucleotide phosphate oxidase and a unique *b* cytochrome.[1 2] This is followed by dismutation of O_2^- to hydrogen peroxide (H_2O_2) and various further reactions which yield the highly reactive oxygen metabolites hydroxyl radical ($OH\cdot$), singlet oxygen ($^1O_2$), and hypochlorite anion (OCl^-).[3] Although the role of these active oxygen species in microbial killing and cytotoxicity is established,[3 4] their possible effects on other immune responses such as antibody production have received much less attention.

We recently showed that methimazole, the active metabolite of the antithyroid drug carbimazole, is an immunosuppressant which inhibits antibody production by an action on antigen presenting monocytes.[5] We have now investigated the possibility that this might be due to an effect on the production of oxygen radicals by these cells, since intracellular peroxidase is linked to the uptake of methimazole by cells[6] and inhibition of peroxidase within the thyroid follicular cell has been proposed as the primary mechanism of the drug's antithyroid activity.[7] In this

study luminol dependent chemoluminescence[8] and production of H_2O_2 were used to estimate the formation of oxygen radicals by monocytes in the presence and absence of methimazole.

Materials and methods

Monocytes (>70% pure) were prepared from peripheral blood as described,[5] suspended in HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulphonic acid) buffered Krebs medium (HEPES 25 mmol/l (600 mg/100 ml), NaCl 120·3 mmol/l (703 mg/100 ml), KCl 4·8 mmol/l (35·8 mg/100 ml), KH_2PO_4 1·2 mmol/l (16·3 mg/100 ml), $CaCl_2$ 1·3 mmol/l (14·4 mg/100 ml), $MgSO_4$ 1·2 mmol/l (14·4 mg/100 ml)), pH 7·4, and stored on ice. Fewer than 3% of the cells were neutrophils. Opsonised zymosan (20 g/l) was prepared by the method of Easmon *et al* using a 1/1 vol/vol dilution of human serum incubated at 37°C for 30 minutes followed by extensive washing.[9]

Monocyte luminol dependent chemoluminescence was measured using a purpose built luminometer with discriminator interfaced to a dual floppy disc computer. Between $0·5 \times 10^6$ and $1·0 \times 10^6$ cells were preincubated for five minutes at 37°C with luminol 0·02 mmol/l (350 μg/100 ml; 0·2% vol/vol dimethylsulphoxide) with or without methimazole 0·02-1·0 mmol/l (0·23-11·4 mg/100 ml) in 500 μl HEPES buffered Krebs medium. The sample was placed in the light tight housing of the luminometer at 37°C and the background (unstimulated) chemoluminescence measured as counts per 10 seconds. The stimulus (opsonised zymosan or phorbol myristate acetate) was then added in 500 μl HEPES buffered Krebs medium at 37°C and the rate of light emission over the next five minutes—measured as luminescence counts per 10 seconds—presented graphically by the computer. During a four hour experiment the background and peak height of the stimulated response of monocyte luminol dependent chemoluminescence decreased by mean values (SD) of 13·5 (4)% and 11·4 (5)% an hour respectively. Results were therefore expressed as a percentage of the expected values derived by interpolation from the time course.

The effect of methimazole on extracellular peroxidase and H_2O_2 production by monocytes was investigated by spectrophotometry.[10] After adherence for two hours to 96 well flat bottomed tissue culture plates, monocytes ($0·5 \times 10^6$ per well) were incubated at 37°C for one hour with phenol red 0·56 mmol/l (phenolsulphonpthalein; 21·0 mg/100 ml) in dextrose buffered saline containing horseradish peroxidase 19 U/ml with or without phorbol myristate acetate 200 nmol/l (12 ng/100 ml) as a stimulus. Estimation of the absorbance at 600 nm with an automatic eight channel spectrophotometer (Titertek) after the addition of 10 μl 1·0M NaOH gave an estimate of H_2O_2 production. The effect of methimazole on the horseradish peroxidase in the medium was estimated by adding H_2O_2 10 or 100 μmol/l (34 or 340 μg/100 ml) to wells without monocytes. All results were expressed as percentage change in the mean absorbance of triplicates from control values, the controls being (*a*) wells without cells or added H_2O_2 (for the effect on extracellular peroxidase) and (*b*) wells with cells but no added stimulus (for the effect on H_2O_2 production).

Results

After an initial lag of 30 seconds stimulation produced a rapid increase in monocyte luminol dependent chemoluminescence, with an initial peak at 60-70 seconds (fig 1). After a slight reduction in the rate of light emission a second phase occurred, reaching a maximum at 300 seconds. The amount of opsonised zymosan which produced a half maximal response (1 g/l) was estimated and used thereafter.

Preincubation of monocytes with methimazole at 37°C for five minutes before the addition of opsonised zymosan produced a decrease in background and stimulated chemoluminescence (fig 1), a preferential decrease in the size of the second phase of the response being seen when the percentage inhibition produced by the drug was calculated (fig 2). In the presence of methimazole 100 μmol/l (1·14 mg/100 ml) background chemoluminescence and the second phase of the response to stimulation were inhibited by mean values (SD) of 45 (1·7)% and 59 (1·7)% respectively. This inhibition could not be accounted for by any effect of the drug on cell viability estimated by staining with ethidium bromide-acridine orange, concentration of adenosine triphosphate in the cells, or release of lactate dehydrogenase (all within 10% of control values). Preincubation of monocytes with methimazole 20-400 μmol/l (0·23-4·5 mg/100 ml) before their stimulation with phorbol myristate acetate 200 nmol/l also inhibited monocyte luminol dependent chemoluminescence, methimazole 100 μmol/l producing a 50% inhibition of the maximum height of the chemoluminescent response to this stimulus.

Methimazole produced a dose dependent inhibition of horseradish peroxidase, leading to a mean change of 57 (SD 8)% in absorbance when exogenous H_2O_2 was added in the presence of methimazole 100 μmol/l (fig 3). When monocytes were stimulated with phorbol

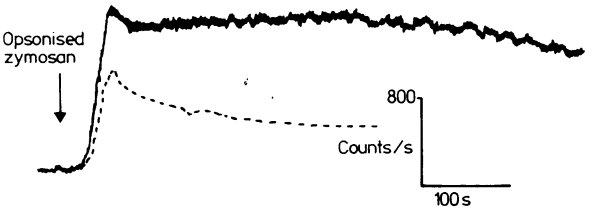

FIG 1—Chemoluminescent response of 1×10^6 monocytes after addition of opsonised zymosan in absence (heavy line) and presence (dotted line) of 100 μmol methimazole/l (1·14 mg/100 ml).

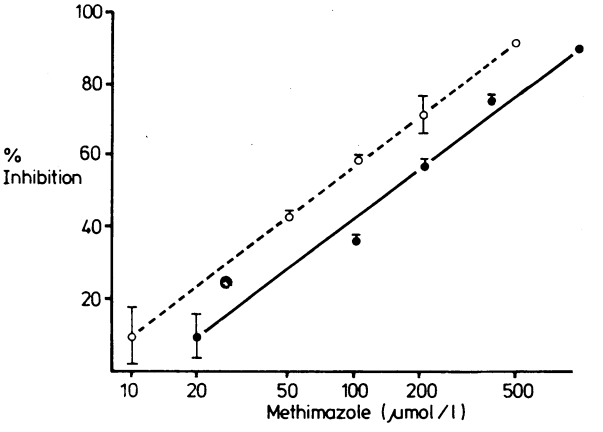

FIG 2—Percentage inhibition of background (●) and second phase (300 seconds after stimulation) (○) chemoluminescent responses of 1×10^6 monocytes stimulated with opsonised zymosan in presence of increasing concentrations of methimazole. Bars represent SEM.

Conversion: SI to traditional units—Methimazole: 1 μmol/l ≈ 11·4 μg/100 ml.

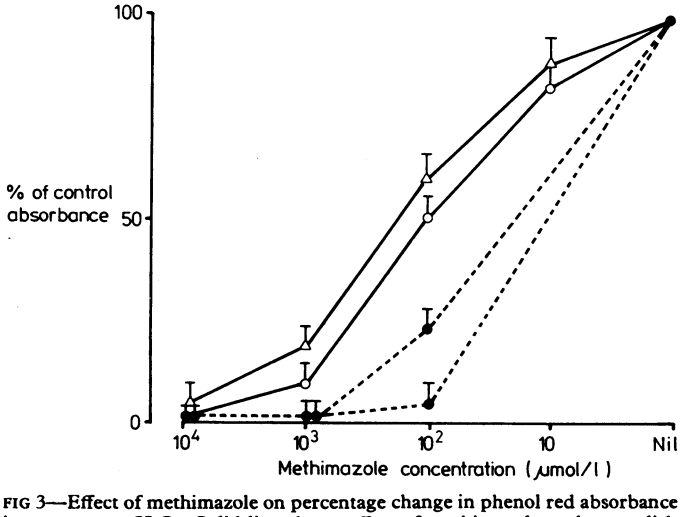

FIG 3—Effect of methimazole on percentage change in phenol red absorbance in response to H_2O_2. Solid line shows effect of methimazole on horseradish peroxidase alone after addition of 100 μmol (△) and 10 μmol (○) H_2O_2/l (340 and 34 μg/100 ml). Dotted line shows effect of methimazole on phorbol myristate acetate stimulated H_2O_2 production by monocytes from two subjects. Bars represent 1SD.

Conversion: SI to traditional units—Methimazole: 1 μmol/l ≈ 11·4 μg/100 ml.

myristate acetate, however, the inhibition produced by methimazole was greater than could be accounted for by its effects on the peroxidase in the medium: 100 μmol of the drug/l produced a decrease of 74 (5)% and 94 (4)% of the control value in two experiments (fig 3).

Discussion

These results show that methimazole at concentrations found in the thyroid glands of patients with Graves' disease treated with carbimazole[11] inhibits production of oxygen radicals by monocytes. The precise mechanism by which methimazole inhibits resting and stimulated monocyte luminol dependent chemoluminescence requires further investigation. This inhibition could not be explained by effects on cell viability, metabolism, stimulatory capacity, quenching of luminol excited state, or decreased light transmission. Similarly the trivial neutrophil contamination and shape of the luminol dependent chemoluminescence response curves indicated that these effects were truly on monocytes, although neutrophil chemoluminescence in response to chemotactic peptide is also inhibited by methimazole (data not shown). Methimazole may produce these effects by oxygen radical scavenging or by inhibiting production of oxygen radicals, which is in part catalysed by a peroxidase.[12] Spectrophotometric studies showed that the drug may inhibit peroxidase in a cell free system (which is the proposed mode of its action in the thyroid cell) and also reduce production of H_2O_2 by monocytes. Support for an oxygen scavenging role comes from studies in which methimazole scavenged OH· radicals in sympathetic nerve terminals.[13] The inhibition of monocyte luminol dependent chemoluminescence may therefore be the result of both mechanisms, the net result of which is a decreased concentration of at least one of the oxygen radicals.

We found an effect of methimazole within minutes of its addition to monocytes. This time scale was appropriate to the action of the drug on antigen priming of monocytes, which subsequently leads to diminished antibody production,[5] and we have shown elsewhere that methimazole has no effect on antigen uptake by itself.[14] The luminol dependent chemoluminescence response appears to be associated with production of oxygen radicals either outside the cell or within the phagosome,[15] so that the ambient external rather than internal concentration of the drug may be critical in the inhibition which we observed. This is supported by the rapidity of the effect. Methimazole, however, would be expected to be taken up by monocytes. These cells contain peroxidase, which is important in accumulation of the drug,[6] and polymorphonuclear leucocytes (whose luminol dependent chemoluminescence response is also inhibited by the drug) take up methimazole actively during phagocytosis.[16] A direct immunosuppressive effect of methimazole on lymphocytes has been excluded,[5] and these cells do not take up the drug,[17] probably owing to their lack of peroxidase.[6] We therefore propose that methimazole inhibits production of oxygen radicals by monocytes (via scavenging or peroxidase catalysed synthesis or both) and that this phenomenon may be related to the immunosuppressive action of the drug in vivo and in vitro. There are many possible mechanisms by which interference with generation of oxygen radicals might subsequently modulate the immune response, including effects on the generation of monokines, catabolism of antigen, and cytotoxic potential.[3] Besides their clinical implications, the results suggest a role for oxygen radicals in triggering immune responses, which requires further elucidation.

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References

1 Babior BM. Oxygen-dependent microbial killing by phagocytes. *N Engl J Med* 1978;**298**:659-69.
2 Segal AW, Jones DT. The subcellular distribution and some properties of the cytochrome b component of the microbial oxidase system of human neutrophils. *Biochem J* 1979;**182**:181-8.
3 Fantone JC, Ward PA. Role of oxygen-derived free radicals and metabolites in leucocyte-dependent inflammatory reactions. *Am J Pathol* 1982;**107**:397-418.
4 Nathan CF. Secretion of oxygen intermediates: role in effector functions of activated macrophages. *Fed Proc* 1982;**41**:2206-11.
5 Weetman AP, McGregor AM, Hall R. Methimazole inhibits thyroid autoantibody production by an action on accessory cells. *Clinical Immunology and Immunopathology* 1983;**28**:39-45.
6 Connell JMC, McCruden DC, Small M, Ferguson MM, Alexander WD. Accumulation of thioureylene antithyroid drugs in mouse salivary gland. *J Endocrinol* 1983;**96**:91-6.
7 Engler H, Taurog A, Luthy O, Dowis ML. Reversible and irreversible inhibition of thyroid peroxidase-catalyzed iodination by thioureylene drugs. *Endocrinology* 1983;**112**:86-95.
8 Campbell AK, Holt ME, Patel A. Chemiluminescence in medical biochemistry. *Recent Advances in Clinical Biochemistry* (in press).
9 Easmon CSF, Cole PJ, Williams AJ, Hastings M. The measurement of opsonic and phagocytic function by luminol dependent chemiluminescence. *Immunology* 1980;**41**:67-74.
10 Pick E, Mizel D. Rapid microassays for the measurement of superoxide and hydrogen peroxide production by macrophages in culture using an automatic enzyme immunoassay reader. *J Immunol Methods* 1981;**46**:211-26.
11 McGregor AM, Petersen MM, McLachlan SM, Rooke P, Rees Smith B, Hall R. Carbimazole and the autoimmune response in Graves' disease. *N Engl J Med* 1980;**303**:302-7.
12 Rosen H, Klebanoff SJ. Chemiluminescence and superoxide production by myeloperoxidase deficient leukocytes. *J Clin Invest* 1976;**58**:50-60.
13 Cohen G, Heikkila RE, Allis B, *et al.* Destruction of sympathetic nerve terminals by 6-hydroxydopamine: protection by 1-phenyl-3(2 thiazolyl)-2-thiourea, diethyl-dithiocarbamate, methimazole, cysteamine, ethanol and n-butanol. *J Pharmacol Exp Ther* 1976;**199**:336-52.
14 Weetman AP, Holt ME, Campbell AK, McGregor AM. The immunosuppressive action of methimazole. *Ann Endocrinol* 1983;**44**:72A.
15 Hallett MB, Campbell AK. Two distinct mechanisms for stimulation of oxygen-radical production by polymorphonuclear leucocytes. *Biochem J* (in press).
16 Lam DCC, Lindsay RH. Accumulation of 2-(^{14}C) propylthiouracil in human polymorphonuclear leukocytes. *Biochem Pharmacol* 1979;**28**:2289-96.
17 Shewring GS, Lazarus JH. The accumulation of (^{35}S) methimazole by human and rat lymphocytes. *Acta Endocrinol (Copenh)* 1983;**102**:68-70.

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ONE HUNDRED YEARS AGO The *Liverpool Echo*, of the 15th instant, contains a clever article upon the abuse of medical charity. The writer admits the existence of the abuse but urges that the working classes are driven to the hospitals by the high scale of fees demanded by the medical men. "Why," he asks, "should doctors all live in big houses, drive carriages, and live expensively?" But if the writer really thinks that all medical men live expensively, he is very much mistaken; and if he will study the scale of fees by which most practitioners work, we are sure he will agree with us, that there is not much room for cutting down medical charges, if the business of a medical man is still to be reckoned among the learned professions. It is not in this direction that we must look for the remedy of the abuse of medical charity. And, as this course is out of the question, we must turn as the writer suggests, to the establishment of reliable provident dispensaries. Herein lies the true solution of the difficulty. Let Liverpool follow the example of Manchester, and set on foot a comprehensive system of provident dispensaries, only taking care to avoid those faults which have been pointed out in the Manchester Provident Dispensaries Association. Thus it is that, by endeavouring to create an improved provident dispensary, we shall best remove the abuse of medical charity, and bring good medical attendance within the reach of the working classes. (*British Medical Journal* 1884;i:179.)