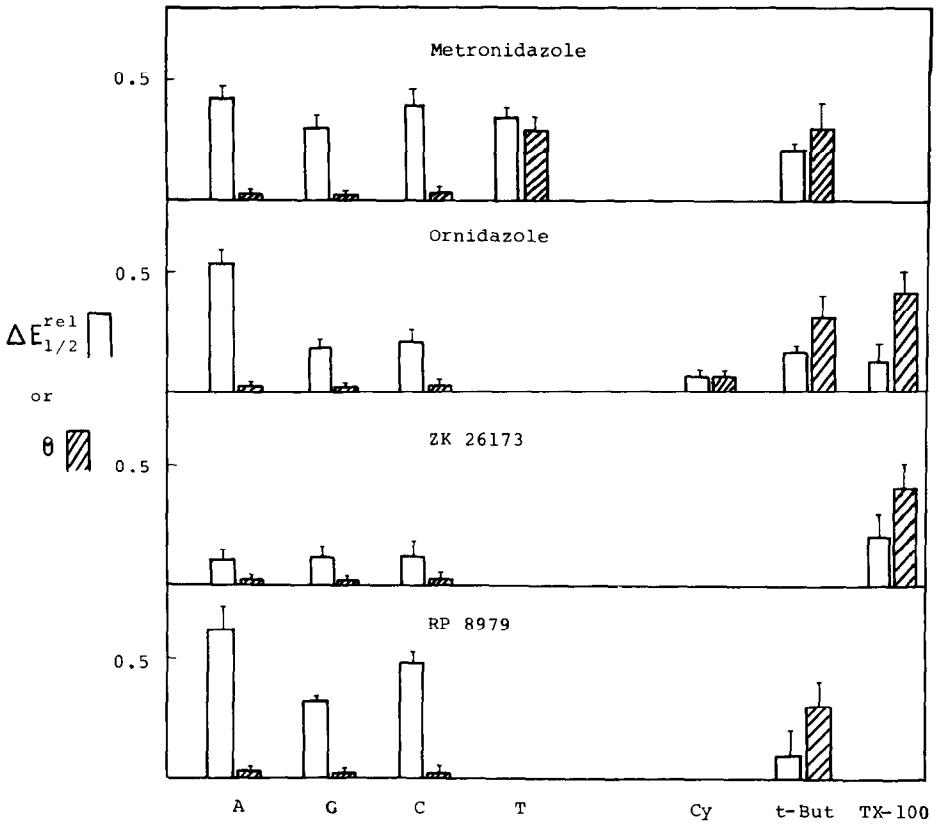

Fig. 3. Relative shift in half-wave potential ($\Delta E^{rel}_{1/2}$) and relative adsorption (θ) produced by the added product at a given concentration. Adenine (A) and guanine (G) : 1.00×10^{-5} M; cytosine (C) and thymine (T) : 1.00×10^{-4} M; cyclohexanol (Cy) : 1.0×10^{-3} M; *t*-butanol (t-But) : 6.0×10^{-2} M; Triton X-100 (TX-100) : 3.1×10^{-5} M.

Acknowledgements—P. J. Declerck was a Research Assistant of the National Fund for Scientific Research (Belgium).

Instituut voor Farmaceutische Wetenschappen
Laboratorium voor Analytische Chemie en Medicinale Fysicochemie
Katholieke Universiteit Leuven
Van Evenstraat 4
B-3000 Leuven, Belgium

P. J. DECLERCK*
C. J. DE RANTER

REFERENCES

1. M. Müller, *Scand. J. infect. Dis. Suppl.* **26**, 31 (1981).
2. I. J. Stratford, *Int. J. Rad. Oncol. Biol. Phys.* **8**, 391 (1982).
3. N. F. LaRusso, M. Tomasz, M. Müller and R. Lipman, *Molec. Pharmac.* **13**, 872 (1977).
4. N. F. LaRusso, M. Tomasz, D. Kaplan and M. Müller, *Antimicrob. Agents Chemother.* **13**, 19 (1978).
5. A. J. Varghese and G. F. Whitmore, *Cancer. Res.* **43**, 78 (1983).
6. P. J. Declerck, C. J. De Ranter and G. Volckaert, *FEBS Lett.* **164**, 145 (1983).
7. R. J. Knox, R. C. Knight and D. I. Edwards, *IRCS J. Med. Sci.* **8**, 190 (1980).
8. R. J. Knox, R. C. Knight and D. I. Edwards, *Int. J. Radiat. Biol.* **41**, 465 (1982).
9. M. Müller, *Surgery* **93**, 165 (1983).

* Present address: The Rockefeller University, 1230 York Avenue, New York, NY 10021, U.S.A.

Biochemical Pharmacology, Vol. 35, No. 1, pp. 61–64, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

Inhibition of hydrogen production in drug-resistant and susceptible *Trichomonas vaginalis* strains by a range of nitroimidazole derivatives

Metronidazole (Flagyl) is the first and most commonly used member of the class of nitroimidazole drugs which are specifically used in the therapy of anaerobic infections. Recently, clinical isolates of *Trichomonas vaginalis* exhibiting increased aerobic tolerance to the drug have been encountered. In several of these isolates oxygen has been shown to play an active part in resistance to the drug [1], and several of these strains (Table 1) have been shown to

Table 1. The apparent oxygen affinities and oxygen uptake rates of several isolates of *T. vaginalis*, compared with the inhibitory concentration of oxygen on hydrogen production as determined by membrane inlet mass-spectrometry [2]

Isolate	Apparent K_mO_2 (μM)	V_{max} (μM O_2 $min^{-1}/10^6$ cells)	Apparent K_iH_2 (μM O_2)
Susceptible:			
ATCC 30001	3.2 ± 2 (5)	2.5 ± 0.5 (5)	1.4
NYH 286	5.3 ± 2 (3)	2.0 ± 0.8 (3)	2.3
Resistant:			
Fall River	58 ± 9 (3)	3.5 ± 0.6 (3)	1.5
IR78	49 ± 9 (3)	2.5 ± 0.7 (3)	1.0
CDC85	33 ± 6 (3)	5.5 ± 0.5 (3)	1.4

possess oxidase activity with lowered affinity for O_2 than that of the susceptible strains [3]. Electron paramagnetic resonance measurements (Fig. 1) showed direct interaction of O_2 with the reduced products of the drug (Fig. 2) revealed as preferential quenching of the metronidazole radical in the resistant strain CDC-85 as compared with that in a susceptible strain held at identical O_2 partial pressure, presumably due to an increased intracellular O_2 concentration [4]. In this resistant isolate measurement of gas exchange by membrane-inlet mass spectrometry in stirred non-proliferating cell suspensions (Fig. 3) showed that a sixfold increase in the titre of metronidazole (Fig. 4) was required to effect a 50% inhibition of H_2 production as compared with that for susceptible strain ATCC 30001 [8].

The effect of a range of nitroimidazole drugs on H_2 production by a resistant isolate, CDC-85, and a susceptible strain, ATCC 30001, by use of membrane-inlet mass spectrometry of continuously stirred cell suspensions has been investigated [2]. Both 2'- and 5'-nitroimidazoles were used with one-electron reduction potentials ranging from −240 mV to −497 mV. The results obtained with the standard laboratory strain ATCC 30001 give a mean value of 0.15 mM for 50% inhibition of H_2 production for both the 2'- and 5'-nitroimidazoles tested and showed high K_i50

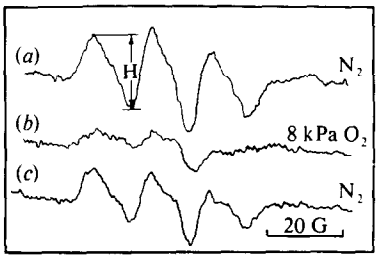

Fig. 2. Electron paramagnetic spectra of metronidazole radical anions in *T. vaginalis* strain 30001. (a), (b) and (c) were obtained as successive scans after attainment of anaerobiosis, exposure for 5 min to 8 kPa O_2 and switching back to a gas phase of N_2 respectively. ESR height (H) was measured at field positions indicated (chosen to give maximum change in anaerobic–aerobic transition). Instrument settings and conditions were: field set, 3265 G; scan rate, 200 G; modulation amplitude, 8 G; microwave power, 20 mW; microwave frequency, 9.117 GH_2; gain, 5×10^4; scan time, 16 min; time constant, 1 sec; cell concentration, 3×10^8 ml^{-1}. (Reproduced with permission from Lloyd and Pedersen [4].)

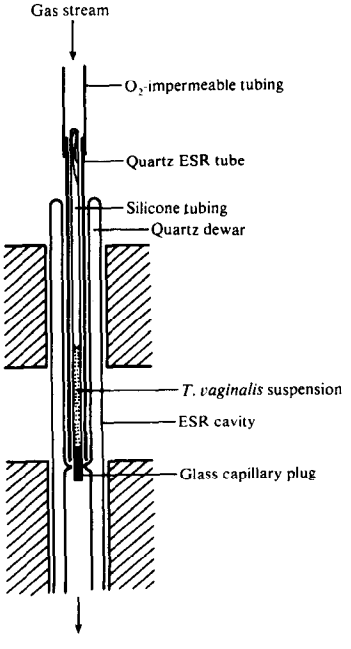

Fig. 1. System for obtaining electron paramagnetic resonance measurements in whole cell suspensions exposed to known partial pressures of O_2. (Reproduced with permission from Lloyd and Pedersen [4].)

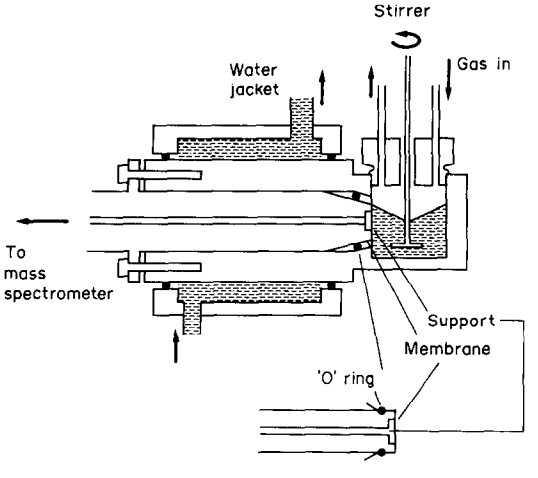

Fig. 3. The reaction vessel used for the continuous and simultaneous measurement of O_2, CO_2 and H_2 dissolved in cell suspensions of *T. vaginalis*. The mobile gas phase consists of purified N_2 into which known partial pressures of O_2 can be mixed. Gases diffuse through the 25 μm-thick Teflon membrane into the vacuum system of the mass spectrometer and are continuously measured at m/z values 32, 44 and 2 respectively. At constant stirring rates, gas uptake and production rates are calculated by determining gas exchange constants (determined in the absence of cells) [5–7].

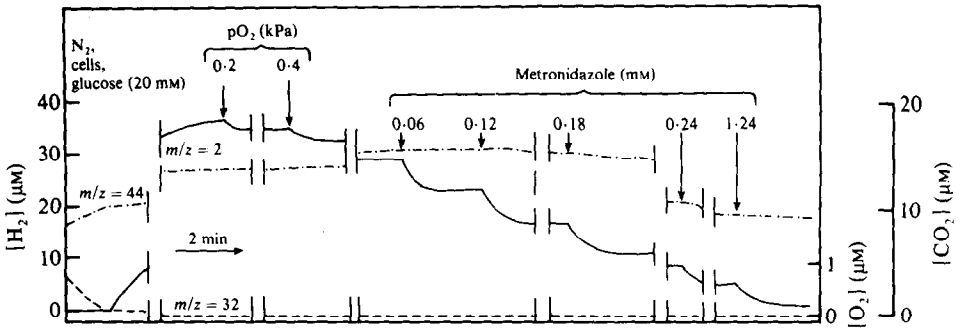

Fig. 4. Effects of O_2 and metronidazole on H_2 and CO_2 production in a washed cell suspension of *T. vaginalis* strain 30001. Cell density was 5×10^6 organisms/ml. Time lapses (indicated) less than 15 min. Dissolved O_2, H_2 and CO_2, m/z = 32, 2 and 44 respectively [8].

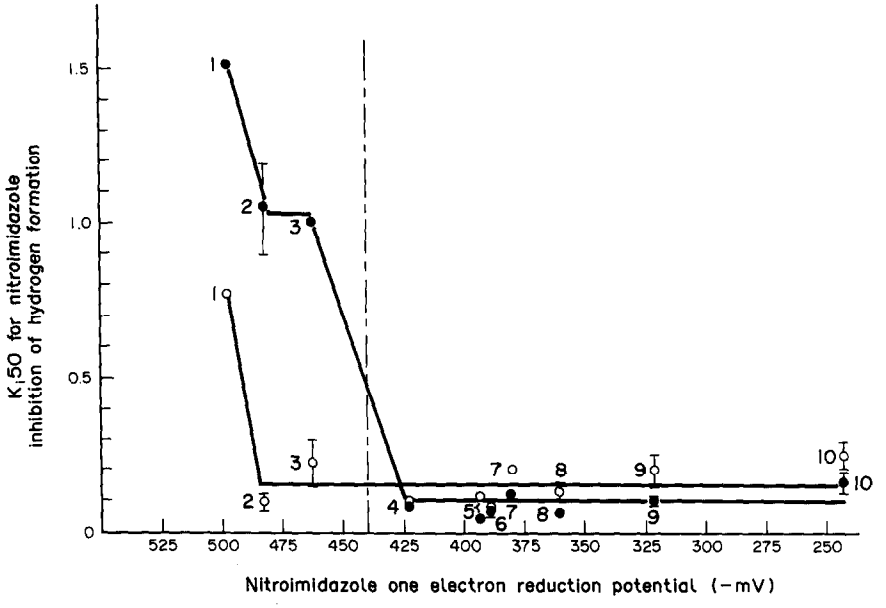

Fig. 5. Values for K_i50 for various nitroimidazoles for H_2 production in metronidazole-susceptible, 30001 (○), and resistant, CDC-85 (●), strains of *T. vaginalis*. Data obtained by membrane inlet mass spectrometry measurements of H_2 production as shown in Fig. 4. (1) Me 108, (2) metronidazole, (3) ornidazole, (4) L-6678, (5) L-8580, (6) misonidazole, (7) benznidazole, (8) ZK 28943, (9) RGW 806, (10) L-8711.

values only with those compounds with redox potentials lower than −485 mV (Fig. 5). The metronidazole resistant isolate CDC-85 had similar K_i50 values (0.1 mM) for those nitroimidazoles tested within the range −240 mV to −425 mV. Nitroimidazoles with potentials lower than −425 mV (5′-nitro derivatives) gave a ten-fold increase in the K_i50 values. In extracts of *T. vaginalis*, ferredoxin is necessary for the reduction of 5′-nitroimidazoles, but not for that of the 2′-nitroimidazoles (which can be reduced directly by pyruvate ferredoxin oxidoreductase [9]. We now further propose that the increased K_i50 values for compounds with redox potentials lower than −425 mV by the resistant isolate CDC-85 (rather than at −465 mV in 30001) may either indicate altered redox properties or a lowered content of ferredoxin in the resistant strain.

These results also support the hypothesis that damage to hydrogenosomal electron transport components by reduced metronidazole products [10] is an early event leading to loss of viability of the organism.

Acknowledgements—We are indebted to Dr. Peter Wardman, Dr. B. Goldstein and F. Hoffmann-La Roche & Co. Ltd. for provision of nitroimidazoles, and Dr. M. Müller for *T. vaginalis* strains.

Department of Microbiology D. LLOYD
University College N. YARLETT
Newport Road N. C. YARLETT
Cardiff, CF2 1TA, Wales, U.K.

REFERENCES

1. S. E. Milne, E. J. Stokes and P. M. Waterworth, *J. clin. Pathol.* **131**, 933 (1978).
2. N. Yarlett, N. C. Yarlett and D. Lloyd, *Molec. Biochem. Parasitol.*, submitted.
3. N. Yarlett, N. C. Yarlett and D. Lloyd, *VII Int. Cong. Protozool. Abst.* Prague (1985).

4. D. Lloyd and J. Z. Pedersen, *J. gen. Microbiol.* **131**, 87 (1985).
5. H. Degn, R. D. Cox and D. Lloyd, *Meth. Biochem. Anal.* **131**, 165 (1985).
6. D. Lloyd and R. I. Scott, *J. Microb. Methods* **1**, 313 (1983).
7. D. Lloyd, S. Bohátka and J. Szilágyi, *Biosensors* **1**, 179 (1985).
8. D. Lloyd and B. Kristensen, *J. gen. Microbiol.* **131**, 849 (1985).
9. N. Yarlett, R. Marczak, T. E. Gorrell and M. Müller, *Molec. Biochem. Parasitol.* **14**, 29 (1985).
10. A. Chapman, R. Cammack, D. Linstead and D. Lloyd, *J. gen. Microbiol.* **131**, in press.

Biochemical Pharmacology, Vol. 35, No. 1, p. 64, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

The role of the gut flora in the reduction of sulphoxide containing drugs

Sulphinpyrazone and sulindac each contain a sulphoxide moiety which can undergo both oxidation and reduction to the sulphone and sulphide (thioether) analogues. The sulphide metabolites are more potent in their activities (anti-platelet-aggregatory and anti-inflammatory respectively) than the parent drugs. Due to the longer half-lives the sulphides are probably responsible for much of the therapeutic effect seen during chronic administration [1, 2].

Both the liver and gut microflora are possible sites for formation of these sulphide metabolites. Studies on the fate of sulphinpyrazone in rats [3] and rabbits [4] showed that the gut flora was the principal and possibly the sole site of reduction *in vivo*. The peak plasma concentration of the sulphide occurred about 8 hr after a single oral dose of sulphinpyrazone. In contrast the liver of rabbits shows extensive reduction of sulindac *in vitro* [4], whilst *in vivo* peak plasma concentration of the sulphide occurred soon after that of the parent compound [5].

The role of the gut flora in the reduction of these drugs in man has been determined by comparison of the plasma concentration–time curves after a single oral dose in normal volunteers and in ileostomy patients (who have undergone surgical removal of the lower bowel). The remaining intestine of such patients is not sterile but contains greatly reduced numbers of strict anaerobes. The absorption of sulphinpyrazone assessed by peak concentration and the area under the plasma concentration–time curve (AUC) was normal in ileostomy patients. However, the sulphide metabolite, which in normal subjects reached a peak concentration (1.6 μg/ml) about 15 hr after dosing was almost undetectable (0.08 μg/ml) [6]. The 25-fold difference in AUC for the sulphide *in vivo* showed a strong correlation ($P < 0.001$) to the extent of reduction by samples of faeces and ileostomy effluent. These data indicate that the gut flora is the sole site of sulphinpyrazone reduction in man. In contrast the sulphide metabolite of sulindac showed a similar initial peak plasma concentration and time to peak (about 3 hr) in both subject groups. This suggests that sulindac is reduced rapidly by the liver to the sulphide metabolite. In normal subjects the sulphide showed a long half-life, and the AUC from 12 hr after dosing to infinity represented about 55% of the total AUC. In ileostomy patients the levels of sulphide in plasma decreased rapidly so that none was detectable at 24 hr and the AUC 12-α was tenfold less than that in normal volunteers. Ileostomy effluent showed a limited ability to reduce sulindac compared with normal faeces [7]. These data suggest that the gut flora contribute significantly to the formation of sulindac sulphide in man, probably by the reduction of sulindac which is excreted in the bile [8].

Thus the reduction of sulphoxides may be due to the liver and/or the gut flora. The relative importance of these two sites is dependent on the substrate and the delivery of the substrate to the hind gut flora.

Clinical Pharmacology Group
University of Southampton
Medical and Biological Sciences Building
Bassett Crescent East
Southampton SO9 3TU, U.K.

A. G. RENWICK
H. A. STRONG
C. F. GEORGE

REFERENCES

1. B. Rosenkranz, C. Fischer, P. Jakobsen, A. Kirstein-Pedersen and J. C. Frolich, *Eur. J. clin. Pharmac.* **24**, 231 (1983).
2. D. E. Duggan, L. E. Hare, C. A. Ditzler, B. W. Lei, and K. C. Kwan, *Clin. Pharmac. Ther.* **21**, 326 (1977).
3. A. G. Renwick, S. P. Evans, T. W. Sweatman, J. Cumberland and C. F. George, *Biochem. Pharmac.* **31**, 2649 (1982).
4. H. A. Strong, A. G. Renwick and C. F. George, *Xenobiotica* **14**, 815 (1984).
5. D. E. Duggan, K. F. Hooke, R. M. Noll, H. B. Hucker and C. G. Van Arman, *Biochem. Pharmac.* **27**, 2311 (1978).
6. H. A. Strong, J. Oates, J. Sembi, A. G. Renwick and C. F. George, *J. Pharmac. exp. Ther.* **230**, 726 (1984).
7. H. A. Strong, N. J. Warner, A. G. Renwick and C. F. George, *Clin. Pharmac. Ther.*, in press.
8. M. R. Dobrinska, D. E. Furst, T. Spiegel, W. C. Vincek, R. Tompkins, D. E. Duggan, R. O. Davies and H. E. Paulus, *Biopharm. Drug Dispos.* **4**, 347 (1983).