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In vitro reductive activation of nitroimidazoles

Nitroimidazole drugs (e.g. metronidazole) are the current drugs of choice for the treatment of protozoal diseases (e.g. trichomoniasis) [1]. In addition they possess antibacterial and radiosensitizing activities [2]. The latter causes those nitroimidazoles to be of current interest as hypoxia-mediated drugs in cancer treatment. Although their activity ranges over diverse prokaryotic and eukaryotic cells, their activity remains a selective one. This selectivity originates from the anaerobic, or at least hypoxic, conditions of organisms or tissues that are susceptible to nitroimidazoles, and is related to the prerequisite for the biological activity, i.e. reduction of the nitro group. Previous experiments have shown that reductively activated nitroimidazoles interact with nucleic acids *in vitro* [3–8], and it is generally believed that DNA is the major target *in vivo* [9].

Although many experiments have been focused on the drug–target interaction there is still no conformity concerning the base-specificity of this interaction. Müller and co-workers [3, 4] showed that the covalent interaction with DNA was proportional to the G+C content. Varghese and Whitmore [5] observed a covalent adduct of reduced misonidazole with guanine and guanosine. Declerck *et al.* [6] reported a covalent reaction of reduced nitroimidazoles with DNA, specifically at the level of guanine. Degradation of DNA was never observed in all these experiments. Edwards and co-workers, however, observed degradation of DNA and suggested an interaction at the level of thymine [7, 8].

In the study here described we performed experiments in order to elucidate further the base-specificity of the interaction between reduced nitroimidazoles and DNA. Another technique, independent from previously described methods, has been used. We investigated the influence of the different DNA bases (adenine, guanine, thymine and cytosine) on the reduction of several nitroimidazoles. The parameter studied was the half-wave potential of the nitroimidazoles, which is a characteristic reduction feature.

Materials and methods

Materials. Metronidazole and ornidazole were gifts from Roche (Belgium); RP 8979 and RP 11193 were kindly provided by Rhône-Poulenc (France); Ronidazole and L 581,490 were gifts from Merck Sharp & Dohme (New Jersey); ZK 26173, CL 205,086, carnidazole and dimetridazole were kindly provided by Shering A.G. (West Germany), Lederle Laboratories (New York), Janssen Pharmaceutica (Belgium) and May & Baker (Essex), respectively. All other chemicals were of reagent grade or Merck "Suprapur" quality. Freshly quartz-distilled water was used to prepare all solutions.

Methods. Influence of the DNA bases on the polarographic behaviour of the nitroimidazoles was investigated by means of their influence on the half-wave potential of the nitroimidazoles. Half-wave potentials were determined by means of d.c. polarography and were measured relative to the saturated calomel electrode. In order to exclude the significant effect of other phenomena, i.e. adsorption, a.c. polarography was carried out to follow the adsorption process of the DNA bases at the working electrode (i.e. a dropping mercury electrode). All experiments were carried out in a Britton-Robinson buffer (pH 7.40)

Results and discussion

Adenine, guanine and cytosine induce a positive shift of the half-wave potential of all nitroimidazoles investigated. A typical concentration dependence is shown in Fig. 1. Adenine and cytosine cause an increasing positive shift reaching a limiting value. Guanine induces an increasing positive shift up to a concentration of 6×10^{-5} M, higher concentrations cause the positive shift to decrease and in some cases even to become negative. Thymine, however, induces negative shifts of the half-wave potentials.

It also appeared that, for a given DNA base, the values of the shifts were related to the nitroimidazole used. Therefore the effect of 1.00×10^{-5} M guanine on the half-wave

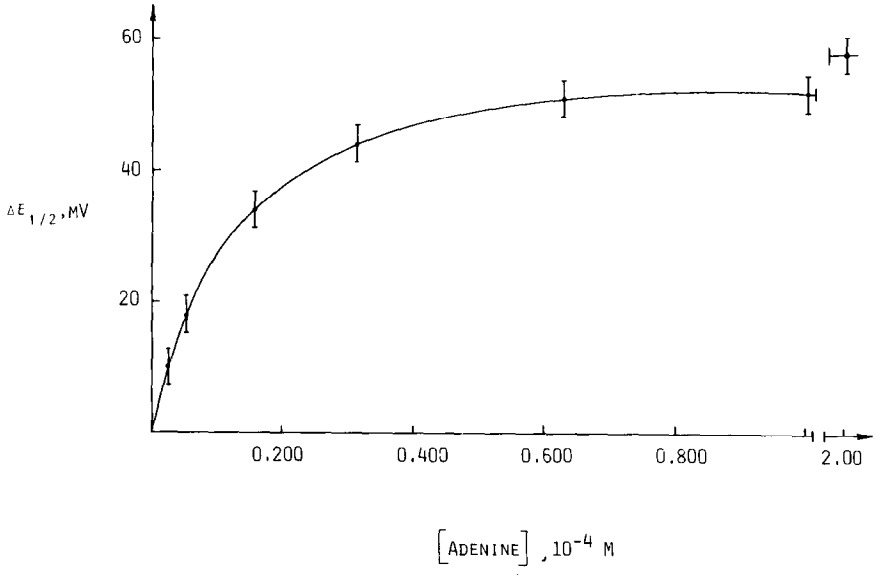

Fig. 1. Shift in half-wave potential ($\Delta E_{1/2}$) of metronidazole vs adenine concentration.

potential of a series of nitroimidazoles was investigated. This is shown in Fig. 2. From these results the differences are obvious and are clearly related to the half-wave potential. Most probably, this relationship reflects the relative stability of the generated reactive intermediates: the nitro compound with the most negative half-wave potential is forming the most stable intermediates.

In order to demonstrate that the positive shifts observed in the presence of adenine, guanine and cytosine are not due to adsorption, whereas the negative shifts caused by thymine are indeed due to adsorption, the influence of some purely adsorbing products (cyclohexanol, *t*-butanol and Triton X-100) on the half-wave potential of the nitroimidazoles has been investigated. In addition, the adsorption behaviour of the added DNA bases has been studied. These results are shown in Fig. 3. This represents the relative effect (either induced shift or adsorption) caused by a certain product at a given concentration. From the adsorption studies it was obvious that the purely adsorbing products cause negative shifts (as is also observed in the presence of thymine) and that both effects, i.e. adsorption and shift induction, occur in the same concentration range. For adenine, guanine and cytosine positive shifts occur as mentioned above, and although these products also exhibit some adsorption, the latter only happens at relatively high concentrations. So, the observed positive shifts occur at a concentration range in which the adsorption can be neglected. We also observed that in the case of guanine the decline in positive shift coincides with the appearance of the adsorption.

From this set of results it can be stated clearly that the shifts induced by thymine are caused by its adsorption. On the other hand the positive shifts caused by adenine, guanine and cytosine are obviously not due to adsorption but are the consequence of a chemical reaction between the DNA base and the generated reactive nitroimidazole derivatives. This interaction is especially pronounced in the case of adenine and guanine (Fig. 3).

Due to the apparent instability of the reactive intermediates, we still lack the knowledge concerning their chemical nature. It should be mentioned, however, that other polarographic techniques (e.g. linear sweep voltametry) exist, which possibly can distinguish between the different intermediates formed during nitroimidazole reduction. In this respect, experiments similar to those described here, but using other polarographic methods, seem to be very promising for revealing which of the reduced nitroimidazole derivatives is responsible for the observed interactions.

In summary, the present study shows that mainly adenine and guanine are susceptible for interaction with reductively activated nitroimidazoles. So these data support our previous results [6] and those of other investigators [3–5]. Moreover, the results also demonstrate that reaction at the level of thymine, although suggested by another group [7, 8], is unlikely to occur. Further studies are required to elucidate the nature of the reduced active intermediate responsible for interaction with DNA.

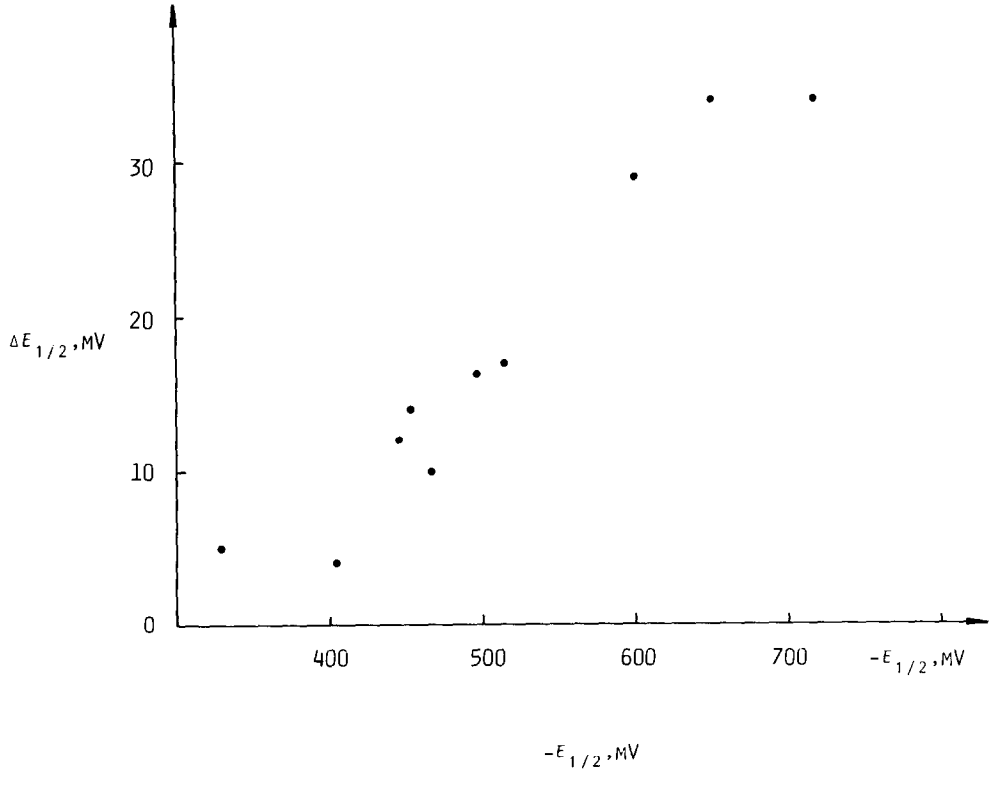

Fig. 2. Determination of the shift in half-wave potential ($\Delta E_{1/2}$) for different nitroimidazoles; [guanine] = 1.00×10^{-5} M. The obtained value is plotted against the half-wave potential of the respective nitroimidazole in the absence of guanine. These half-wave potentials are −329 mV, −404 mV, −445 mV, −453 mV, −466 mV, −496 mV, −517 mV, −600 mV, −648 mV and −717 mV for ZK 26173, CL 205,086, ronidazole, carnidazole, ornidazole, metronidazole, dimetridazole, RP 8979, L 581,490 and RP 11193, respectively.

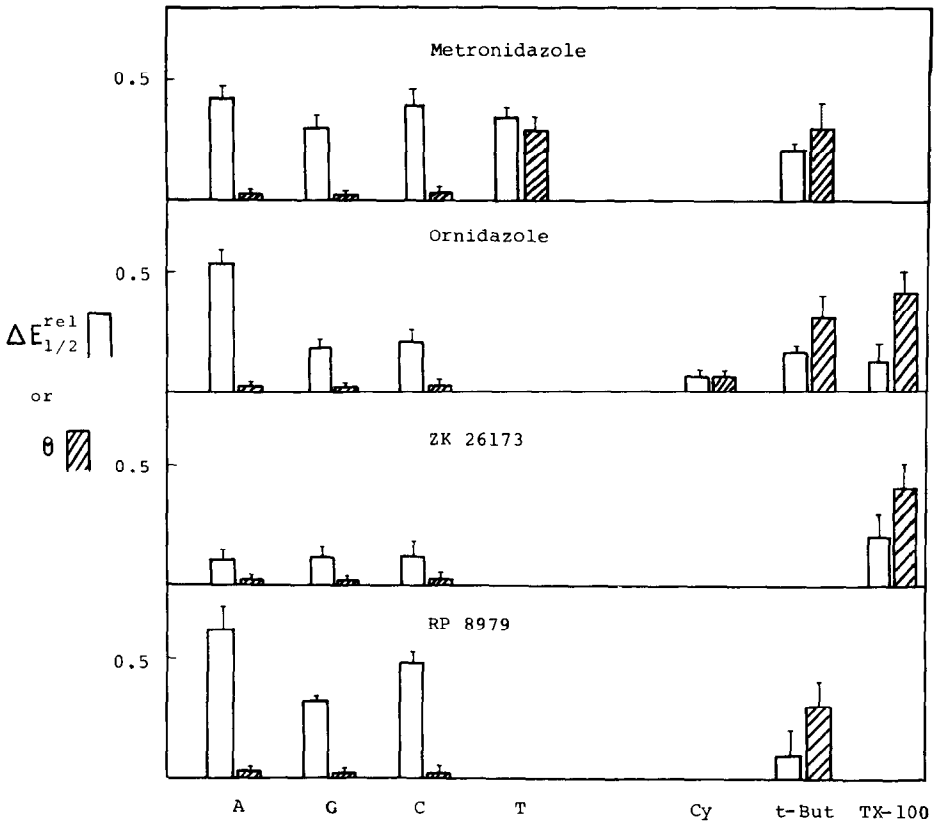

Fig. 3. Relative shift in half-wave potential ($\Delta E^{rel}_{1/2}$) and relative adsorption (θ) produced by the added product at a given concentration. Adenine (A) and guanine (G) : 1.00×10^{-5} M; cytosine (C) and thymine (T) : 1.00×10^{-4} M; cyclohexanol (Cy) : 1.0×10^{-3} M; *t*-butanol (t-But)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

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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).

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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