THE USE OF DNA REPAIR DEFICIENT MUTANTS TO DETECT ANTIANAEROBIC AGENTS

Sir:

Metronidazole and other nitroimidazoles are extensively used for the treatment of infection caused by anaerobic bacteria and protozoans.[1] The compounds used clinically are all synthetic and result from studies stimulated by the discovery of azomycin (2-nitroimidazole) in a *Streptomyces* broth.[2]

The nitroimidazoles are believed to act by forming an active intermediate under anaerobic conditions which damages cellular DNA.[3~6] The active species arises by reduction of the 5 nitro group of the imidazole probably involving ferridoxin.[7] Consistent with this is the observation that DNA repair deficient mutants of *Escherichia coli* are more susceptible to metronidazole than wild type.[8] We examined repair deficient strains of *E. coli* and *Bacillus subtilis* and confirmed this sensitivity and further showed that in media with low salt concentration these organisms were also sensitive aerobically. This is the first reported example of metronidazole acting aerobically. We therefore used this property to screen for natural products which might behave like metronidazole. Repair deficient strains have been used extensively in screening programs especially to look for natural products with potential antitumor activity. Since we wanted to eliminate such agents from our screen, we employed a biochemical induction assay[9] for detection of DNA damaging agents. We then tested the low number of leads which passed these tests for activity against a panel of anaerobes. The first novel compound we uncovered was a 4-diazo-3-methoxy-2,5-cyclohexadien-1-one compound (SQ 30,957) produced by *Penicillium funiculosum* and having marked antianaerobe activity.[10] We now show here that azomycin, previously reported only from *Streptomyces*,[2] has also been found in a *Pseudomonas* culture.

We found that by growing repair deficient strains, aerobically, in various agars the sensitivity to metronidazole was affected. For example on BBL seed agar (Cockysville, Md., U.S.A.) with 5 g/liter sodium chloride, the repair deficient strains *E. coli* SGB4 and *B. subtilis* SGB192, were insensitive to 30 μg/disc metronidazole, whereas on BBL seed agar without the salt, 19 mm and 25 mm zones of inhibition respectively, could be seen.

The MICs for nine antibiotics is shown in Table 1. The MIC values for wild type *E. coli* (SGB5) and *B. subtilis* (SGB163) are the same as or similar to those for repair deficient strains (SGB4 and 192) when the mode of action is unrelated to DNA damage, for example, chloramphenicol and benzylpenicillin.

In the case of 2-nitroimidazole and 4-nitroimidazoles, it would appear that these compounds

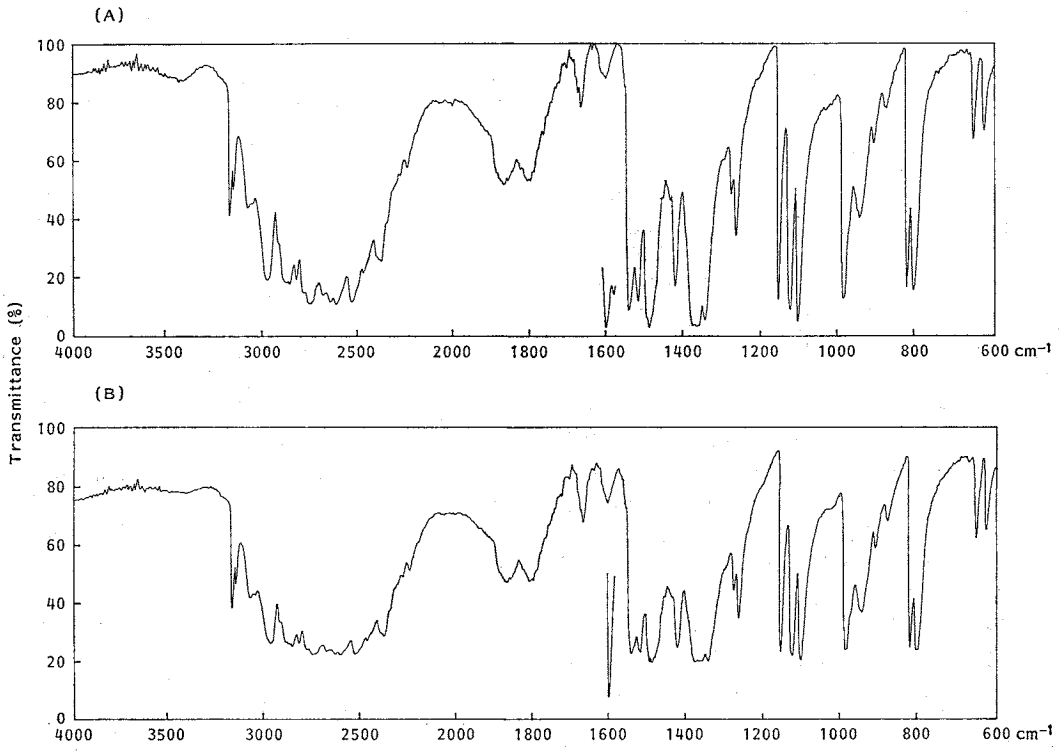
Table 1. The effect of nitroimidazoles and other agents on a Gram-positive and a Gram-negative rec$^+$/rec$^-$ pair grown aerobically.

Compound	MIC (μg/ml)			
	Escherichia coli SGB4	*E. coli* SGB5	*Bacillus subtilis* SGB192	*B. subtilis* SGB163
Metronidazole	25	>200	6.3	>200
Tinidazole	20	>200	6.3	100
4-Nitroimidazole	100	100	100	100
2-Nitroimidazole	50	50	25	50
Mitomycin	6.3	50	0.2	6.3
Bleomycin	0.8	6.3	1.6	6.3
Nitrofurazone	12.5	50	1.6	12.5
Benzylpenicillin	50	50	6.3	6.3
Chloramphenicol	6.3	6.3	1.6	3.2

MICs were determined by 2-fold dilution series in a broth consisting of Peptone 6 g, casein digest 4 g, yeast extract 3 g, beef extract 1.5 g and glucose 1 g in 1 liter of water. Tubes containing 1 ml medium were inoculated with 25 μl of an overnight culture and grown statically at 37°C for 16 hours.

SGB5 and 163 are wild type *E. coli* and *B. subtilis* respectively; SGB4 is a *recA* deficient *E. coli* and SGB192 a *recE* deficient *B. subtilis*.

Fig. 1. IR spectra of azomycin (A) and lead compound (B).



also do not affect DNA since no significant differences in MIC values were seen between mutant and wild type. The increased sensitivity of the DNA repair deficient strains to 5-nitroimidazoles (metronidazole and tinidazole), bleomycin, mitomycin and nitrofurazone is seen in Table 1. We have used these organisms to search for antianaerobic agents in bacteria and actinomycetes isolated from nature. From approximately 230,000 bacteria, 45,000 actinomycetes and 1,500 fungi examined we found only one new source of nitroimidazole; a species of *Pseudomonas* was found to produce the 2-nitroimidazole, azomycin.

A culture of *Pseudomonas* sp. SC14725 was grown for 48 hours at 25°C in a shaken medium consisting of Bacto-tryptone 10 g, Bacto-yeast extract 5 g, and NaCl 5 g in a liter of water. Activity was extractable into ethyl acetate at pH 2 but not at pH 7 or 9. This extract was concentrated and applied to an Analtech silica gel TLC plate and developed with 10% methanol in chloroform. The active band was eluted and crystallized from methanol. The white crystals have a melting point of 280°C. The compound has a UV maximum at 315 nm ($E^{1\%}_{1cm}$ 557) in neutral or acidic pH and at 362 nm ($E^{1\%}_{1cm}$ 832) in basic pH.

The molecular weight, 113, was determined by chemical ionization (CI) and MS/MS mass spectrometry. The IR spectrum in KBr (Fig. 1) was superimposable with that of azomycin. In several other respects such as color tests, TLC Rf values and bioactivity, the compound was identical to azomycin.

We have shown here that metronidazole can act aerobically and that repair deficient bacterial strains can be used to screen for antianaerobic agents. The use of an aerobic test organism allows larger screening throughput than would normally be achieved with an anaerobe. It is clear from the literature and from our screening experience that nitroimidazoles are not common in nature; the only nitroimidazole we uncovered was the previously known azomycin, albeit from a novel source.

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