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Correspondence

Antibacterial activity of local anaesthetic agents

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Sir,

In their excellent overview on antibacterial activity of non-antibiotic drugs Cederlund & Mårdh (1993) did not mention local anaesthetics such as tetracaine, dibucaine, procaine and lidocaine, which have potent antimicrobial activity as detected by effects on cell growth rates and viability of *Escherichia coli* and *Candida albicans in vitro*. The effects of lidocaine on bacterial growth in a chemically defined medium has been compared with that of antibacterial agents such as ampicillin, chloramphenicol, puromycin and cationic surface-active agents (Abanzukwe, Fazley Bazaz & Salt, 1991). Although local anaesthetics are 1000 fold less toxic to both procaryotic and eucaryotic cells than active quaternary ammonium disinfectants, their antibacterial activity could be of concern for the yield of broncho-alveolar lavage (BAL) fluid bacteriology. Bronchoscopists instill 10–15 mL 2% lidocaine into the bronchial tree before performing BAL. If allowed to mix with 50 mL BAL fluid, 200–300 mg of lidocaine would attain a concentration of 4000–6000 mg/L, enough to induce membrane damage in bacterial organisms and possibly compromise their growth in culture. Fortunately removal of the local anaesthetic permits bacterial cell recovery and growth. There is, however, no study comparing the bacteriological yield of BAL with and without the use of local anaesthetics.

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References

Cederlund,. H. & Mårdh, P.-A. (1993). Antibacterial activities of non-antibiotic drugs. *Journal of Antimicrobial Chemotherapy* **32**, 355–65.

Abanzukwe, T. C., Fazley Bazas, B. S. & Salt, W. G. (1991). Further studies of the antimicrobial activity of beta-adrenergic blocking agents and local anaesthetics. In *Program and Abstracts of the Seventeenth International Congress of Chemotherapy, Berlin.* Abstract 726.

Evaluation of the *rpoB* gene in rifampicin-susceptible and -resistant *Mycobacterium avium* and *Mycobacterium intracellulare*

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Sir,

Mycobacteria of the *Mycobacterium avium* complex (MAC) are significant human and animal pathogens. Treatment options have been limited because of resistance to most antimicrobial agents. Such intrinsic resistance has been attributed to the characteristics of the cell wall structure of MAC, but the precise molecular bases have not been elucidated. In contrast, significant progress has been made in the understanding, at molecular level, of resistance to the main antituberculous drugs in *Mycobacterium tuberculosis*: resistance to isoniazid has been mapped to the gene for the catalase-peroxidase (Zhang *et al.*, 1992), rifampicin to the RNA polymerase subunit β (Telenti *et al.*, 1993a), fluoroquinolones to the gyrase A (Takiff, H., Salazar, L., Guerrero, C., Philipp, W., Huang, W. M., Kreiswirth, B. *et al.*, unpublished observations), streptomycin to the ribosomal S12 protein and 16S rRNA gene (Douglass & Steyn, 1993; Nair *et al.*, 1993). Recent work has identified mutations in a gene possibly involved in the mycolic acid metabolic pathway as an additional mechanism for isoniazid resistance in *M. tuberculosis* (Banerjee *et al.*, 1993).

We have evaluated the *rpoB* of several strains of *M. avium* and *Mycobacterium intracellulare* that displayed variable levels of susceptibility to rifampicin to clarify its role in resistance. For this purpose, the *rpoB* region where mutations have been identified in *M. tuberculosis* and *Mycobacterium leprae* (Honoré & Cole, 1993; Telenti *et al.*, 1993*a*)—the Rif locus—was amplified in *M. avium* serovar 1, and *M. intracellulare* serovar 15 reference strains by using primers previously described (Telenti *et al.*, 1993*a*). PCR fragments were sequenced directly or after cloning into a plasmid vector. In addition, the Rif locus was evaluated in 29 human MAC isolates displaying a rifampicin-susceptible phenotype (MIC < 1 mg/L, $n = 4$), moderate susceptibility (MIC 1–4 mg/L, $n = 6$), or resistance (MIC ≥ 4 mg/L, $n = 19$), by sequencing or by PCR single strand conformation polymorphism (Telenti *et al.*, 1993*b*).

Table. Alignment of the *rpoB* Rif locus of rifampicin susceptible *M. tuberculosis* H37rv with sequences from *M. avium* and *M. intracellulare* reference strains and 15 clinical isolates (grouped as 'types' according to sequence homology) exhibiting susceptible, intermediate resistant, and rifampicin resistant phenotypes. With the exception of the *M. intracellulare* isolate 'D', all isolates had full amino acid homology with *M. tuberculosis* H37rv. Shown in bold are the mutations described in rifampicin-resistant *M. tuberculosis*

M. tuberculosis	AAC	ATC	CGG	CCG	GTG	GTC	GCC	GCG	ATC	AAG	GAG	TTC	TTC	GGC	ACC	AGC	CAG	CTG	AGC	CAA	TCC	ATG	GAC	CAG
494	Asn	Ile	Arg	Pro	Val	Val	Ala	Ala	Ile	Lys	Glu	Phe	Phe	Gly	Thr	Ser	Gln	**Leu**	Ser	**Gln**	Phe	Met	**Asp**	Gln
M. avium	AAC	ATC	CGT	CCC	GTC	GTG	GCG	GCG	ATC	AAG	GAG	TTC	TTC	GGC	ACC	AGC	CAG	CTG	TCC	CAG	TTC	ATG	GAC	CAG
type A ($n = 3$)	...	...	...	...	...	. .	...	...	...	.	..	...	...	...	...	...	...	...	...	...	...	...	...	..
type B ($n = 4$)	. .	..	...	..A	...	...	...	...	...	...	...	. .	...	...	..	...	...	...	..	...	...	...	. .	...
type C ($n = 1$)	..	...	...	.	...	...	..C	...	...	..	...	...	..	..	...	...	...	..C	...	. .	...	..	...	...
M. intracellulare	.	. .	. G	.G	..	..C	. C	.	..	...	...	. .	...	...	...	...	...	..	AG.	. .	. .	. .	. .	..
type A ($n = 1$)	.	...	..G	..G	..	..C	..C	..	...	...	...	.	...	...	..	..	. .	...	AG.	..	...	..	. .	..
type B ($n = 3$)	...	...	..G	..G	...	..C	..C	...	.	...	...	...	...	...	...	...	...	...	..G	...	...	...	...	...
type C ($n = 2$)	...	...	.G	..G	...	..C	. C	...	...	...	.	. .	...	. .	..	...	...	. .	..G	. .	...	...	..	. .
type D ($n = 1$)	.G.	...	..G	..G	...	..C	..C	...	...	...	...	...	...	...	..	...	...	. .	AG.	...	...	..	.	...
	Ser																							
M. tuberculosis	AAC	AAC	CCG	CTG	TCG	GGG	TTG	ACC	CAC	AAG	CGC	CGA	CTG	TCG	GCG	CTG	GGG	CCC	GGC	GGT	CTG	TCA	CGT	GAG
518	**Asn**	Asn	Pro	Leu	**Ser**	Gly	Leu	Thr	**His**	Lys	Arg	Arg	Leu	**Ser**	Ala	**Leu**	Gly	Pro	Gly	Gly	Leu	Ser	Arg	Glu
M. avium	AAC	AAC	CCG	CTG	TCG	GGG	CTC	ACC	CAC	AAG	CGC	CGC	CTG	TCG	GCG	CTG	GGC	CCG	GGT	GGT	CTG	TCC	CGG	GAG
type A ($n = 3$)	...	..	..	...	...	..	..	...	...	. .	...	...	...	...	...	...	...	...	...	..	. .	...	...	..
type B ($n = 4$)	...	...	...	..	...	...	..	...	...	. .	...	...	...	...	...	...	...	...	...	...	...	...	...	...
type C ($n = 1$)	...	..	...	..	..	.T	..G	...	...	..	..	...	...	..	...	...	. .	...	...	...	...	...	..T	...
M. intracellulare	.	..	...	...	..C	.T	..G	...	..	..	...	...	.C	...	...	..	...	..C	..C	...	...	...	.T	...
type A ($n = 1$)	...	...	. .	...	..C	..T	.G	...	...	..	..	...	..C	..	...	...	..	..C	..C	..	...	...	..T	...
type B ($n = 3$)	...	. .	. .	...	..C	. T	..G	...	. .	...	...	...	..C	...	..	. .	...	. C	. C	...	...	...	..T	...
type C ($n = 2$)	...	. .	...	...	..C	..	..G	...	...	..	..T	..T	. C	...	...	. .	...	..C	...	...	...	...	..T	. .
type D ($n = 1$)	...	..	..	...	...	. .	. G	...	...	..	..T	..T	...	...	. .	..	..G	..C	. .	...	...	..	..T	...

In *M. avium* and *M. intracellulare* reference strains, the *rpoB* region homologous to the Rif locus of *M. tuberculosis* exhibited differences at nucleotide level but a full amino acid identity with rifampicin-susceptible *M. tuberculosis* (Table). Sequence information from clinical isolates demonstrated significant sequence heterogeneity, in particular among strains identified as *M. intracellulare*. However, with only one exception, all retained the amino acid sequence corresponding to a rifampicin-susceptible *M. tuberculosis* (Table). One isolate presented an Asn → Ser in codon 494, a mutation not reported previously in rifampicin-resistant *M. tuberculosis* (Telenti *et al.*, 1993*a*), or *M. leprae* (Honoré & Cole, 1992). This isolate did not display high level resistance—it had no growth at 4 mg/L of rifampicin; thus, the contribution of the mutation to the resistant phenotype was deemed doubtful.

In summary, our data confirm at the molecular level that the most frequent mechanism of resistance to rifampicin among clinical isolates of *M. avium* and *M. intracellulare* does not involve alterations of the RNA polymerase subunit β. Thus, alternative mechanisms of resistance are responsible for the intrinsic resistance to rifampicin in MAC. The frequency with which these isolates exhibit resistance to multiple structurally unrelated antimicrobial agents, and the existence of intermediate and high-level resistance phenotypes are most consistent with changes in drug uptake or with efflux mechanisms. A significant permeability barrier to rifampicin, that could be reduced with Tween, has been described in a type strain of *M. intracellulare* and *Mycobacterium smegmatis* shown to possess a rifampicin-susceptible RNA polymerase (Hui, Gordon & Kajioka, 1977). The genetic determinants of permeability, and the possibility for additional mechanisms of resistance to antimicrobial agents in MAC, including the acquisition of exogenous genetic elements encoding for drug resistance, have not yet been established. This information will be important in the development of more active drugs against MAC.

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References

Banerjee A., Dubnau, E., Quemard, A. & Jacobs, W. R. (1993). Cloning of a novel gene conferring resistance to isoniazid in mycobacteria: a possible target for isoniazid. In *Abstracts of the Ninety-Third General Meeting of the American Society for Microbiology, Atlanta, 1993.* Abstract U-15, p. 171. American Society for Microbiology, Washington, DC.

Douglass, J. & Steyn, LM. (1993). A ribosomal gene mutation in streptomycin-resistant *Mycobacterium tuberculosis* isolates. *Journal of Infectious Diseases* **167,** 1505–6.

Honoré, N. & Cole, S. T. (1993). The molecular basis of rifampicin resistance in *Mycobacterium leprae. Antimicrobial Agents and Chemotherapy* **37,** 414–8.

Hui, J., Gordon, N & Kajioka, R. (1977). Permeability barrier to rifampin in mycobacteria. *Antimicrobial Agents and Chemotherapy* **11,** 773–9.

Nair, J., Rouse, D., Bai, G. & Morris, S. (1993) Genetic analyses of streptomycin resistance in single and multiple drug resistant strains of *Mycobacterium tuberculosis.* In *Abstracts of the Ninety-Third General Meeting of the American Society of Microbiology, Atlanta, 1993.* Abstract U-14, p. 171. American Society for Microbiology, Washington, DC.

Telenti, A., Imboden, P., Marchesi, F., Lowrie D., Cole, S., Colston, M.J. *et al.* (1993a). Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis. Lancet* **341,** 647–50.

Telenti, A., Imboden, P., Marchesi, F., Schmidheini, T. & Bodmer, T. (1993b). Direct, automated detection of rifampicin-resistant *M. tuberculosis* by PCR and single strand conformation polymorphism. *Antimicrobial Agents and Chemotherapy.* **37,** 2054–8.

Zhang, Y., Heym B., Allen, B., Young, D. & Cole, S. (1992). The catalase-peroxidase gene and isoniazide resistance of *Mycobacterium tuberculosis. Nature* **358,** 591–3.

Outer membrane protein profiles of *Xanthomonas maltophilia* isolates displaying temperature-dependent susceptibility to gentamicin

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Sir,

Xanthomonas maltophilia is increasingly isolated from hospitalized patients, particularly immunocompromised individuals receiving broad-spectrum antibiotics. Treatment is difficult because of its inherent resistance to many antibiotics. Wheat,