LETTERS TO THE EDITOR

Co-amoxiclav levels in bronchial mucosa

We are rather puzzled by the findings of Gould *et al* (October 1994;**49**:999–1001) in that they found increased concentrations of both amoxycillin and clavulanic acid in bronchial mucosal tissue when compared with serum levels. This is at variance with numerous other studies which have looked at the penetration of β-lactam antibiotics into this site and have found penetration to be about 40–70%.[1 2] There are sound theoretical reasons why a drug which remains extracellular should produce levels in biopsy tissue of around 40% of simultaneous serum levels.[3] The authors have misquoted details from reference 13 where, although similar serum levels were found for amoxycillin and clavulanate, the median levels in bronchial mucosa (3·5 mg/kg and 0·6 mg/kg respectively) were much lower. In a recent study which we have undertaken we found no such concentration of co-amoxiclav in bronchial biopsy tissue.[4]

These findings therefore stimulate questions about the methodology used to measure concentrations of both drugs and also the calculations used to analyse the data.

With regard to methodology, the authors mention that the tissue samples were transported very quickly to the laboratory but they do not mention that samples were transported in a humidifier. In a report by Cars *et al*[5] the importance of transporting tissue in a humidifier was stressed to avoid the overestimation of drug concentrations in tissues because of the loss of moisture. This may be even more significant in this study where very small pieces of tissue with a large surface area were exposed to the air.

Although mean weights of the biopsy samples are given for each of the drug regimens, no standard deviation or range is given. It would have been very useful to have this information so that actual assayed concentrations could be calculated and the number of samples with levels very close to the lower limit of detection ascertained and thus the reliability of the data at the lower dosing regimens assessed. The mean weights of the biopsy samples are much lower than in other published work, and this may be a significant source of error when attempting to measure antibiotic concentrations at the lower limit of detection of the assay.

The paper lacks a reference to the calculation used to determine the concentration of drug in the mucosal tissue. An error in this calculation would obviously have a major impact on the final result.

We would wish to highlight a technical error. The paper states the addition of the internal standard, salicylamide, before derivatisation. It is generally accepted that this should be added after derivatisation as salicylamide may interfere with the derivatisation stage.

As the findings of this study are so different from those of many other studies on β-lactams, serious concern about the methodology used in this study must be raised.

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4 Cook PJ, Andrews JM, Woodcock J, Wise R, Honeybourne D. Concentration of amoxycillin and clavulanate in lung compartments in adults without pulmonary infection. *Thorax* 1994; **49**:1134–8.
5 Cars O, Orgen S. A microtechnique for the determination of antibiotics in muscle. *J Antimicrob Chemother* 1981;**8**:39–48.

AUTHORS' REPLY We wish to reply to the various points raised as follows. There may be theoretical reasons why β-lactams should produce levels in mucosal tissue biopsy samples of around 40% of simultaneous serum levels, but Honeybourne *et al* do not quote their own work which showed apparent concentration of cefuroxime (penetration of up to 900% with a mean of 204%).[1] Similarly, other workers (some quoted in our paper) have demonstrated high relative levels of β-lactams in mucosa and sputum. Honeybourne *et al* have found intracellular penetration of clavulanic acid (and also amoxycillin) which could explain our high levels. Tissue penetration also varies according to physicochemical properties of a particular drug and the time sampled in relation to drug dosing. With reference to this, our biopsy samples were taken longer after drug dosing than those sampled by Honeybourne *et al*. We are sorry if Honeybourne *et al* feel that we misquoted their work (reference 13). In fact, the wording in our manuscript did say that concentration was only found in some patients but this was removed by the Editor. Nevertheless, apparent concentration of amoxycillin and clavulanic acid was observed by Honeybourne *et al* in some biopsies, and in another paper they observed mucosal levels of amoxycillin to be 75% of serum levels.[2] We note that Honeybourne *et al* used bioassays while we used an HPLC technique. We are not the only authors to observe higher mucosal levels of antibiotic by HPLC than bioassay; this may be another significant factor,[3] perhaps relating to bound/intracellular drug.

The samples were transported in a closed container filled with ice. We feel this would have provided effective protection against desiccation.

The range of biopsy weights for the five dosing regimens were: (i) 1–3 mg, (ii) 0·5–3·4 mg, (iii) 0·5–2·1 mg, (iv) 0·5–2·2 mg, (v) 0·5–4 mg. Most patients had two sets of biopsies (left and right) which were analysed separately and the results meaned. Correlation was good between results from paired biopsy samples. Only two patients had a sample with a level at the lower limit of detection where there was not a paired biopsy sample available of a greater weight.

The concentration of antimicrobial drug in the tissues was calculated using the formula given in a paper by Honeybourne *et al*.[2]

We do not think there is a technical error in our HPLC assay. There is a school of thought among people brought up on GLC analysis that the purpose of the internal standard is to control variability of injection volume into the chromatograph. This may be the case in GLC assays which do not involve an extraction step. In liquid chromatography injection precision is good and the sole purpose of the internal standard is therefore to control the variability of the extraction procedure. The addition of salicylamide before extraction is thus mandatory. Whether this is added before derivatisation, at the same time, or afterwards is irrelevant since the imidazole reagent is added in excess compared with the clavulanic acid and the salicylamide will react with this excess, if it is going to, whenever it is added to the clavulanic acid before extraction. In our original paper[4] we checked the recovery of clavulanic acid as 84·8 (4·6)% (n = 10). This means that we spiked samples, applied our method, and recovered less (not more) than we spiked. This is normal and certainly does not indicate derivatisation of the internal standard. We see a clearly defined peak for salicylamide which is at the same retention as underivatised salicylamide. We showed linearity of calibration over two ranges of clavulanic acid with different concentrations of salicylamide.

Some further observations are perhaps of interest. Firstly, our serum levels are much lower than those recently reported by Honeybourne confirming a later phase of drug distribution and suggesting there is not a problem with the assay. Indeed, our tissue levels of clavulanic acid for the 750 mg dose are very similar to those of Honeybourne *et al*; the problem seems to be in the relative level to the serum concentration. Secondly, and inexplicably, we found the lowest relative levels after the 750 mg dose (the dose used by Honeybourne *et al*). Indeed, the only two patients with unrecordable clavulanic acid levels were in this group. We did enquire of SmithKline Beecham whether there was any difference in tablet formulation that might have explained these lower levels but they were unaware of any. Finally, the small weight of our samples suggests that little submucosal tissue will have been sampled, which may well have affected the measured concentrations.

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