Correspondence

Br Heart J 1982; 48: 604

Immunological probes in cardiovascular disease

Sir,

Dr Haber's recent paper (*Br Heart J* 1982; 47: 1-10) describes the use of Fab (digoxin-specific Fab fragments of sheep IgG/Wellcome) in the treatment of digoxin toxicity. We have used the drug in 13 patients who we felt would benefit from the rapid reversal of the effects of digoxin. Clinical and electrocardiographic improvement occurred within four hours in all toxic patients treated, with the exception of a neonate who was asystolic for 10 minutes before Fab was given. There were no overt immunological or haemodynamic side effects.

We feel that attention should be drawn to the results (Table) obtained with various radioimmunoassays

Table Plasma digoxin levels ($\mu\text{g/l}$)

	Assay method		
	A	B	C
Before Fab	4.5	5.1	4.6
60 minutes after Fab	1.5	ND	25

ND, not detected.

A: In-house, PEG.

B: Becton-Dickinson Immunodiagnosics Kit.

C: Corning Medical and Scientific Kit.

before and after Fab treatment. The assays gave acceptably similar results for samples before Fab, but we found a wide variation in values after treatment. Interpretation of the data from all three assays is difficult, but method B, involving a charcoal separation, probably reflects digoxin in excess of the binding capacity of Fab, while method C, which employs a solid phase antibody, probably indicates the excess of digoxin binding sites. The polyethylene glycol (PEG) assay results are the most difficult to interpret as they give an unreliable measure of the lowering of digoxin levels as a result of variable Fab interference in the assay.

We suggest that a charcoal based assay may give the simplest, most readily available indication of the circulating bioavailable digoxin, avoiding lengthy equilibrium dialysis procedures. Studies are in progress to validate the use of simple assay techniques to measure the concentration of bound and unbound digoxin and total Fab binding capacity, and to characterise Fab pharmacokinetics in man.

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This letter was shown to Dr Haber who replies as follows:

Sir,

The question raised by Jennings and his colleagues is a difficult one to resolve. None of the methods that they have used to determine free digoxin concentration is entirely reliable. As they correctly point out, assay methods based on an immobilised antibody yield false results because all the labelled digoxin is bound to excess Fab from the patient's plasma. Any assay method based on precipitation of antibody will give uncertain results, because the conditions for precipitation of Fab and antibody differ and are dependent on the method chosen. The charcoal method for absorbing free digoxin is likely to be more reliable, but only if the affinity of the Fab used in therapy is similar or higher than the affinity of the assay antibody. If the Fab is of significantly lower affinity, then there will be transfer of labelled digoxin from Fab to charcoal, resulting in falsely raised free digoxin concentrations.^{1,2} The only entirely reliable method is equilibrium dialysis, though this is admittedly tedious, time consuming, and not really applicable to the routine clinical laboratory.

References

- 1 Skubitz KM, Smith TW. Determination of antibody-hapten association kinetics: a simplified experimental approach. *J Immunol* 1975; 114: 1369-74.
- 2 Smith TW, Skubitz KM. Kinetics of interactions between antibodies and haptens. *Biochemistry* 1975; 14: 1496-502.

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