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An in-vitro test for the duration of action of insulin suspensions

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A method is described which measures the prolongation effects of commercial insulin suspensions by monitoring the rate of solution of an insulin suspension into a phosphate buffer, pH 7·4, at 37 °C. The method can rapidly categorize an insulin into the clinical classifications of either fast, intermediate or slow acting. It offers advantages of speed and sensitivity over the British Pharmacopoeial test for prolongation of insulin effect in fasted animals. Large differences between the dissolution rates of commercial samples of isophane insulin were observed which suggested a lack of bioequivalence.

Insulin preparations are often classified according to their clinical duration of action: fast acting, intermediate acting and slow acting (Larner 1980). Commercial injections of insulin suspensions are tested for their duration of action using the Prolongation of Insulin Effect as described in the British Pharmacopoeia 1980 (BP). This test compares the hypoglycaemic effects of an insulin suspension with those of a standard insulin solution following subcutaneous injection into fasting animals. However, the test is unreliable because the changes in the blood sugar levels in response to a given dose of insulin can vary markedly between animals, and the test does not incorporate a cross-over design. This variability is compounded by the inaccuracies involved in injecting a few μlitres of an insulin suspension subcutaneously. An alternative in-vitro procedure to monitor the dissolution characteristics of commercial insulin suspensions is described. The procedure involves continuously measuring the rate of dissolution of insulin particles into a phosphate buffer at 37 °C.

MATERIALS AND METHODS

In-vitro dissolution method

Two ml of insulin suspension, 100 U ml^{-1}, is placed in a 25 mm Millipore ultrafiltration cell containing a magnetic stirrer. The suspension is filtered through a 0·22 μm membrane filter under positive pressure and the trapped particles washed with 1 ml of cold distilled water, and adjusted to pH 7·0, to remove any preservatives. Sorensen's phosphate buffer, 0·067 M, pH 7·4, warmed to 37 °C, is pumped through the cell at a rate of 3 ml min^{-1} from a 200 ml reservoir stirred with a magnetic stirrer. The absorbance of the filtrate is continuously measured at 214 nm by a Varian spectrophotometer as it passes through a flow cell, and then back to the reservoir. The stirring rate in the ultrafiltration cell is 120 rev min^{-1}. Duplicate curves are obtained for each product. (If the phosphate buffer is replaced with 0·14% sodium acetate, pH 7·1, minimal dissolution occurs and there is no differentiation between formulations.)

In-vivo prolongation of insulin effect

The procedure of the BP was followed, using 9 rabbits per group. Each rabbit received insulin 0·5 U kg^{-1} and whole blood sugar levels were measured by a method based on that of Hoffman (1937), using a Technicon Auto-Analyser.

Products

Samples of commercial insulin injections, 100 U ml in 10 ml, manufactured and/or distributed by Boots, the Commonwealth Serum Laboratory, Nordisk, Novo, Weddel and Wellcome were used. The insulin formulations tested were the slow acting preparations, protamine zinc insulin and insulin zinc suspension crystalline; the intermediate acting insulins, isophane insulin and insulin zinc suspension mixed; and the fast acting insulin, insulin zinc suspension amorphous. All products complied with the relevant requirements of the BP 1980.

RESULTS

Dissolution curves were obtained for several types of insulin suspensions by continuously measuring the light absorbance caused by insulin dissolved in the filtrate, against elapsed time. Initial studies showed

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that the phosphate buffer volume did not limit the solubility of the insulin crystals, since the absorbance increased or decreased inversely with buffer volumes. Similarly, small changes (90–150 rev min^{-1}) in the stirring rate in the ultrafiltration cell did not alter the dissolution curve. Measurement of the protein content in the buffer against insulin standards using hplc confirmed that the absorbance was due to insulin. The precision of the dissolution test was estimated using a sample of insulin zinc suspension mixed. On each of six occasions, dissolution curves were obtained for three aliquots from a vial of the insulin sample, using the same stock solution of buffer. The absorbance values at 10 min from the start of each curve was used for calculations. The between- and within-day variability, expressed as a percent coefficient of variation for the absorbance values, were calculated as 11·1% and 9·8% respectively.

Fig. 1 shows the combined dissolution curves for several delayed action insulin formulations, characterized as either a slow, intermediate or fast acting type of insulin. The dissolution curves of all the formulations showed, within the first 5 min, an initial rise of absorbance caused by a relatively rapid release of insulin, followed by a plateau or linear response which was established when most of the insulin crystals had dissolved, as shown by the fast acting, insulin zinc suspension amorphous. The maximum absorbance for 200 U of an insulin solution was 0·850. All the other insulin suspensions gave shallow linear responses rather than a plateau, indicating that insulin was dissolving at a constant rate over the 20 min study period.

Fig. 2 shows the combined hypoglycaemic responses in rabbits produced by the same insulin products that were tested in the in-vitro dissolution test. Although the insulin formulations vary in the rate at which they induce changes in blood sugar concentration, the only statistically significant difference between the curves for the insulin suspensions in the test for prolongation of insulin effect at 6 h was the retardation produced by protamine zinc insulin, which was greater than for the other formulations (Student–Newman–Keul's test, $P < 0{\cdot}05$). The rabbit test gives variable results. For example the between test coefficient of variation for the mean blood sugar concentration 6 h after injection of

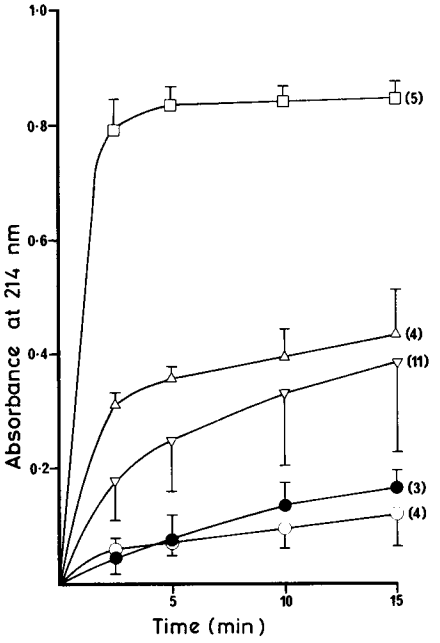

FIG. 1. The combined absorbance curves for each of several insulin formulations. The test measured the dissolution rate of 200 U of the insulin sample into 200 ml of phosphate buffer, pH 7·4, at 37 °C. The standard deviation for each mean is shown and the number of commercial insulin products is given in the brackets. The formulations are: insulin zinc suspension amorphous (□), insulin zinc suspension mixed (△), isophane insulin (▽), protamine zinc insulin (●), and insulin zinc suspension crystalline (○).

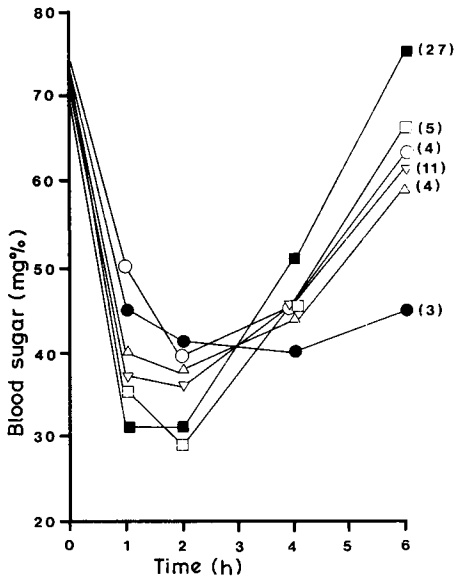

FIG. 2. Hypoglycaemic responses of fasted rabbits to delayed acting insulin formulations. The number of products (or, for the standard insulin solution, the number of tests) is given in the brackets. Each product was tested in 9 rabbits using insulin 5 U kg^{-1}. The formulations are: insulin zinc suspension amorphous (□), insulin zinc suspension mixed (△), isophane insulin (▽), protamine zinc insulin (●) and insulin zinc suspension crystalline (○). A solution of the 4th International Standard of Insulin (■).

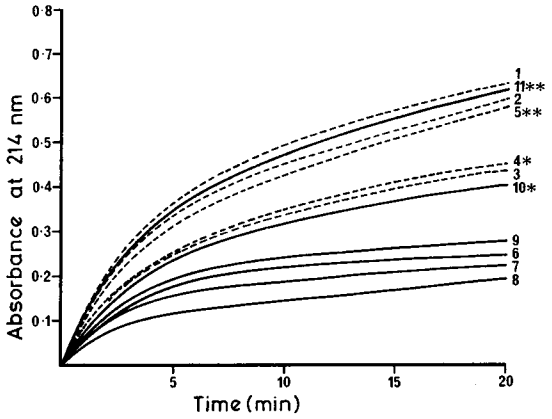

FIG. 3. Absorbance curves for 11 samples of isophane insulin. The dotted lines are for products of porcine origin and the solid lines are for products of bovine origin. The numbering of the samples is the same as that in Table 1 and the asterisks indicate products of the same manufacturer.

Table 1. Analytical data for commercial isophane insulin injections grouped according to the species source of the insulin. The asterisks indicate products of the same manufacturer.

Insulin product	Mean particle size (μm)	Particle shape	Zinc content (μg/100 U)	Dissolution (abs. at 10 min)
	5–60†	Rod shaped†	40†	
Porcine				
1	12	splintered rods	21·0	0·49
2	12	splintered rods	20·8	0·45
3	12	splintered rods	18·0	0·34
*4	12	splintered rods	28·6	0·35
**5	12	splintered rods	22·3	0·42
Bovine				
6	23	regular rods	29·0	0·22
7	30	regular rods	30·5	0·18
8	20	regular rods	26·9	0·14
9	14	regular rods	38.0	0·24
*10	13	splintered rods	28·0	0·31
**11	12	regular rods	22·5	0·47

† Requirement of the BP.

standard insulin solution was 10·2%. This estimate was based on 27 tests each using 9 rabbits.

The individual dissolution curves for several brands of each form of insulin, except isophane insulin, lay within a narrow range. The greater variability between products for the dissolution of isophane insulin was investigated by examining the species source of the products and various physical parameters of the formulation. Fig. 3 shows the dissolution curves obtained for 11 samples of isophane insulin. In general, isophane insulin of porcine origin showed a faster rate of dissolution than that of bovine origin. This trend was also found in the in-vivo test for prolongation of insulin effect, with the combined hypoglycaemic response for isophane insulin of bovine origin being significantly smaller at 1 and 2 h than that for porcine products (t-test, $P < 0·05$). However, the rates of dissolution of bovine and porcine isophane insulin products were similar when the particles were similar. Table 1 provides a comparison of the isophane insulin preparations tested with regard to particle size and shape, zinc content and absorbance value from the dissolution curve at 10 min. The porcine insulin preparations all contained irregular, sometimes clumped, splinter shaped particles with a mean size of 12 μm, whereas the bovine insulin particles were generally regular, rod shaped particles, of similar size within each sample, but varying from 12–30 μm in length between brands. For each of two manufacturers who produce both a bovine and a porcine isophane insulin (products 4 and 10 and 5 and 11 in Table 1 and Fig. 3), the dissolution rates of their products from the two species were similar, as were the appearance and zinc content of the particles.

Variation between the samples of isophane insulin was also evident from the results of the test for prolongation of insulin effect in rabbits. The eleven samples gave mean blood sugar levels ranging from 27 to 55 mg% 1 h post injection and the ranges at 2, 4 and 6 h post injection were 26 to 44, 39 to 50 and 59 to 68 mg%, respectively.

DISCUSSION

The animal method in the BP for measuring the duration of action of insulin suspensions is based on the hypoglycaemic responses after intracutaneous injection of insulin into fasting rabbits or guinea-pigs (BP 1980). Although clinically the various insulin formulations have different times of onset, peak effect and duration of effect, the results of this in-vivo technique are too variable to detect these differences. Consequently, the various degrees of retardation produced by different formulations are similar over most of the 6 h period of observation and the retardation of each formulation can only be measured when compared with the effect produced by an injection of soluble insulin. Moreover, the animal test does not reflect the clinically observed differences for all the formulations. For example, the long acting, insulin zinc suspension crystalline cannot be distinguished from intermediate acting insulins.

The in-vitro dissolution method offers advantages of speed and sensitivity over the in-vivo method. Also, different types of insulin could be readily grouped into the clinical categories of fast, intermediate or slow acting by the in-vitro dissolution

method and either compared directly with a similar insulin reference material or identified in absolute absorbance terms. Thus, 200 U of slow acting insulins could be identified as producing an optical absorbance, at 10 min, of less than 0·2 absorbance units, while the absorbances of 200 U of fast acting insulins exceed 0·8 absorbance units.

The only insulin formulation to show marked variation between products in the in-vitro dissolution test was isophane insulin, some brands of which gave dissolution curves that were closer to those for long acting insulins than for the intermediate types. Since the solubility of the insulin crystals determines the shape of the dissolution curve, factors that can affect the solubility of insulins were examined. These are the species source of the insulin, particle appearance and zinc content (Schlichtkrull et al 1975). Kasama et al (1980) proposed that porcine insulin is more soluble than bovine insulin due to conformational differences between the two. A faster dissolution rate for porcine insulin suspensions would therefore be expected. However, the results of the present study suggest that the particle appearance and not the species source is the most important factor in the dissolution rate of isophane insulin, since bovine and porcine insulin gave similar dissolution rates if their mean particle sizes and shapes were similar. Schlichtkrull et al (1975) showed that the solubility of insulin decreases as the zinc concentration increases. However, at neutral pH and at the concentrations normally present in isophane insulin, zinc ions have little effect on the solubility of the particles. Schlichtkrull et al (1975) found that zinc concentrations above 30 μg/100 U of insulin were needed to reduce the solubility of insulin, which may explain why sample 9, with a high zinc content, gave a slow dissolution rate despite a relatively small particle size.

The results demonstrate that the generic brands of isophane insulin produced by different manufacturers can vary markedly in the dissolution characteristics due largely to differences in particle size and shape. Differences between the isophane products were also indicated by the range for the samples of the mean hypoglycaemic responses in rabbits. The consequence is that, although complying with the BP 1980 requirements for isophane insulin, the different brands may not be bioequivalent. The in-vitro dissolution test offers a means by which the dissolution characteristics of an insulin suspension can be conveniently monitored during both product development and routine manufacture.

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