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The use of critical flicker fusion frequency test for evaluating some central nervous effects of two indomethacin formulations

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The side effects of the non-steroidal anti-inflammatory agent indomethacin have been well documented (see for example: Wanka & Dixon 1964; Katz et al 1965; Boardman & Hart 1967). Central nervous side effects include headache, vertigo, light-headedness and disturbed sensorium while the common gastrointestinal effects are epigastric pain and peptic ulceration. The dosage form of the drug has a substantial effect on the incidence of the side effects. A reduced incidence of side effects with the conventional capsule dosage form compared with tablets has been widely reported (Ballabio & Caruso 1964; Michotte & Wanters 1964; Thompson 1964; Lövgren & Allender 1965; Smyth 1965).

We have examined the central effects of two formulations of indomethacin, a conventional capsule and a microencapsulated preparation of the drug compared with a placebo capsule by means of the Critical Flicker Fusion (CFF) frequency test (see Turner 1964) which is claimed to be useful in demonstrating changes in visual discrimination that occur after administration of centrally-acting drugs.

Materials and methods

The indomethacin raw material (wt median) particle size 6 μm as determined by Coulter Counter used in the preparation of the microcapsules and indomethacin conventional capsules (Indocid 25 mg used as received) were obtained from Merck Sharp & Dohme Ltd., Hoddesdon, Herts., U.K.

Microencapsulated indomethacin. Indomethacin microcapsules were prepared using a gelatin acacia complex coacervation procedure essentially as described by Nixon & Nouh (1978). The ratio of drug core to colloid coat was 1:5. The microcapsules were assayed by refluxing with methanol and the indomethacin content determined by comparing the u.v. absorbance of the filtered solution at 320 nm to the standard curve of indomethacin in methanol. Microcapsules equivalent to 25 mg of indomethacin content were hand filled into hard gelatin capsules. Placebo capsules contained lactose B.P. In vitro dissolution studies on the microencapsulated formulation showed no significant variance from the conventional formulation (Rowe 1980). In vivo plasma concentrations after a single capsule (25 mg) of either formulation to 6 healthy subjects showed no significant differences in the total amount of drug absorbed as measured by the area under the plasma concentration versus time curve. Neither was any significant difference observed between the peak plasma concentration or the time to reach this (Dr M. Aylward unpublished observations). Thus the two formulations may be considered bioequivalent.

Critical flicker fusion frequency (CFF) technique. The apparatus used to measure the CFF was the Flicker Fusion Monitor model 1199 (System 696LTD), in which the flicker frequency of the red light presented to the subject through an optically designed sight tube is measured. All measurements were carried out in a dark room after allowing the subject 10 min to accommodate. At any one time four determinations were made of the CFF, two with the frequency being increased (the ascending threshold) and two with the frequency being decreased (the descending threshold) after exposing subjects to an intermittent light of 20 or 50 Hz for 1 min.

The subject kept his eyes closed between determinations and the four thresholds were measured each time in the following order: (1) ascending, (2) descending after exposure to 20 Hz; (3) ascending; (4) descending after exposure to 50 Hz. The CFF value was taken as the mean value of the four thresholds. Each subject always used the same eye.

Eight male volunteers were given microencapsulated indomethacin, conventional indomethacin and placebo (2 capsules of each) in a random order 1 h after a standard light breakfast. No food was allowed for 1 h after but water was freely available; no alcohol or coffee was taken on the test day. CFF readings were taken at 1, 3, 5 and 7 h after the capsules had been taken. Subjects received each of the formulations at intervals of not less than 4 days.

Results and discussion

The means and standard deviations of the CFF values in the 8 subjects at various time intervals after the

Table 1. Mean and (standard deviation) CFF values in 8 subjects at various times after taking formulations of indomethacin (50 mg) compared with a placebo.

	CFF values (Hz)		
Time (h)	Microencapsulated Indomethacin	Indomethacin capsules	Placebo capsules
1·0	27·96 (0·70)	25·79 (0·69)	27·50 (1·10)
3·0	27·62 (0·71)	25·99 (0·48)	27·26 (1·04)
5·0	27·31 (1·13)	25·87 (0·63)	27·33 (1·06)
7·0	27·25 (0·89)	25·82 (0·72)	27·38 (1·14)

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administration of the two indomethacin formulations and placebo are shown in Table 1. Statistical analysis of the values using Student's *t*-test showed that at 1 and 3 h after administration there was a highly significant fall ($P < 0.001$) in the CFF value in the subjects receiving the conventional capsule formulation compared with an equivalent dose of microencapsulated indomethacin. A significant lowering of the CFF was also observed at these times with the conventional capsule compared with the placebo. At 5 and 7 h the CFF values for the conventional capsule were still significantly lower than ($P < 0.05$) those of the microencapsulated drug or placebo. There was no significant difference between the values for the microencapsulated indomethacin and placebo at any time. The degree of central nervous activity as measured by critical flicker fusion frequency is thus more pronounced in subjects receiving the conventional indomethacin formulation than an equivalent dose of the drug in a microencapsulated form. These results would seem to support the unpublished observations of Dr M. Aylward of an increased incidence of c.n.s. side effects with conventional indomethacin capsules compared with equivalent doses of microencapsulated indomethacin. As the two formulations are bioequivalent, the mechanism of the reduced incidence of c.n.s. activity encountered with the microencapsulated product is unknown but illustrates the effect of dosage form on the incidence of c.n.s. side effects encountered with this particular drug.

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The influence of calcium utilization on cardiac cyclic AMP

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Rapp & Berridge (1977) proposed that calcium and cyclic (c)AMP interact in several tissues by the use of two feedback loops which they called loop A and loop B. In loop A, an increase in cAMP content results in an increase in intracellular calcium. On the other hand an increase in intracellular calcium results in a decrease in cAMP content. Such a loop would be ideal for a tissue where cAMP might accommodate the rate of entry of calcium into a cell for subsequent utilization, i.e., muscle contraction or secretion. In loop B, these interactions would be reversed. An increase in cAMP would result in sequestration or removal of intracellular calcium. An increase in intracellular calcium would trigger an increase in cAMP synthesis. Such a system would be ideal for situations where cAMP resulted in relaxation. Rapp & Berridge suggested, however, that both smooth and cardiac muscle utilized a feedback loop similar to loop B.

Meisheri et al (1978) studying isoprenaline-induced relaxation of the oestrogen-primed uterus noted that, while increased extracellular calcium markedly inhibited the ability of isoprenaline to increase uterine cAMP, there was no effect upon basal levels of cAMP in this tissue. The data would imply that no feedback loop exits for the modulation of basal cAMP levels by calcium in the oestrogen-primed uterus. In addition, the influence of calcium on cAMP in this tissue would appear to be opposite from that which would be expected if, as according to Rapp & Berridge, cAMP were involved in relaxation.

The purpose of the present study has been to determine if altered calcium utilization in cardiac tissue could result in altered basal and/or drug-induced levels of cAMP content.

Methods

Guinea-pigs of either sex, 300–500 g, were treated with heparin (3·23 mg kg^{-1} s.c.) 30 min before being stunned by a blow to the head and exsanguinated. Thoracotomy was performed, the hearts were excised and immediately placed in oxygenated Chenoweth-Koelle solution (CKS) of the following composition (mM) NaCl, 120; KCl, 5·63; $CaCl_2$, 2·0; dextrose, 9·7; $MgCl_2$, 2·0; $NaHCO_3$, 25·0.

After separation from the ventricles, left atria were cut into two strips and mounted vertically in tissue chambers containing 50 ml CKS which was continuously oxygenated with 95% O_2, 5%CO_2. One

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