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Comparative bioavailability studies on brands of diazepam tablets

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Summary

The chemical and biological equivalence of two brands of diazepam tablets marketed in Nigeria were compared to that of Valium (Roche) tablets. The tablets used were Relanium (Polfa) and Tropium (Biode).

Bioequivalence studies of the brands were carried out on nine healthy volunteers by monitoring the cumulative diazepam excreted as oxazepam (both free and conjugated) in urine over 48 h. The bioequivalence of Relanium was found not to be significantly different from that of Valium while that of Tropium was significantly different from that of Valium ($P < 0.05$).

The results of the investigation showed that the drug products were all chemical equivalents but not biological equivalents.

Résumé

L'équivalence chimique et biologique de deux types du comprimé Diazepam vendus au Nigéria a été comparée avec celle du comprimé Valium (Roche). Les deux comprimés employés ont été Relanium (Polfa) et Tropium (Biode).

La bioéquivalence a été étudiée par (une) expérimentation sur neuf volontaires pleins de santé en observant le Diazepam cumulatif libéré comme Oxazepam (pur et conjugué) dans l'urine pendant 48 h. La bioéquivalence de Relanium a été observée de n'être pas significativement différente de celle de Valium, alors que celle de Tropium a été significativement différente de celle de Valium ($P < 0.05$).

Le résultat de l'investigation a montré que les produits des drogues ont été tous les équivalents chimiques, mais non pas biologiques.

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Introduction

Koch-Weser (1974) has recently reemphasized the problem of inequivalence in pharmaceutical products from multiple sources. In the benzodiazepine group of drugs, the only report of problems of inequivalence of drug products was that on chlordiazepoxide by Foldes, Campbell and Wohlman (1970). Klebovich and Verecz-key (1978) and Berlin *et al.* (1982) in their reports on the bioavailability of diazepam preparations showed equivalency of the preparations tested. We report here the results of our investigation on the comparative bioavailability of some other brands of diazepam tablets (hitherto untested) marketed in Nigeria.

Two brands of diazepam tablets were compared with the innovator's product, Valium® (Roche). One of the two brands was Relanium (Polfa) and the other was Tropium (Biode). All the preparations were assayed according to the British Pharmacopoeia (BP) (1980).

The results of the *in vitro* dissolution rate tests were compared with those of the *in vivo* bioavailability studies which employed the cumulative urinary excretion of oxazepam obtained from volunteers after a single oral dose (10 mg) of diazepam. Kaplan *et al.* (1973) reported that oxazepam, in free and conjugated form, is the major urinary metabolite of diazepam, hence the justification to monitor the total oxazepam excreted as an estimate of the diazepam absorbed from a preparation of the drug. In the determination of oxazepam in urine by high pressure liquid chromatography (hplc) a modification of the methods of Kabra,

Stevens and Marton (1978) and Brodie, Chasseaud and Taylor (1978) was adopted.

Materials and methods

Assay

All the tablet brands were assayed by the spectrophotometric method described in the BP (1980). (The diazepam tablets used were kindly supplied by the Pharmacy section of the University of Ife Health Centre, Ile-Ife, Nigeria.) Twenty tablets were powdered and used for the analysis. In the method the absorbance of a filtered solution of a known weight of the powdered sample was read at 284 nm in the Carl Zeiss spectrophotometer. The diazepam content of the sample was calculated using the value of A (1%, 1 cm) of 446 as given by the BP.

Dissolution rate test

The test was carried out in Erweka Tablet Dissolution Rate Tester. One tablet was placed in the basket which was then dipped in 0.1 M HCl at 37° ± 0.5°C. The basket was rotated at 100 rpm and 5 ml samples were withdrawn for analysis at predetermined intervals for 1 h. After filtering, a 3 ml aliquot of the sample was diluted to 100 ml and the absorbance was read at 284 nm in the spectrophotometer with 0.1 M HCl as reference. The concentration of the drug was determined from a calibration curve plotted using Diazepam AS solution in 0.1 M HCl.

Bioavailability studies

Analysis of the urine samples for oxazepam was carried out on a Varian Model 5000 hplc containing a C-18 reverse phase column and coupled to a variable wavelength uv-visible detector and a chromatographic data system (CDS 111L). The solvent system was methanol: 0.5% w/v ammonium acetate (4:1) at a flow rate of 2 ml/min and detection was at 284 nm.

The study was carried out on nine apparently healthy volunteers aged between 20 and 30 years and weighing between 65 and 78 kg. A completely randomized cross-over design was used, whereby each of the volunteers took all the three products at different stages of the cross-over. About 2 weeks was allowed between each stage to allow for essentially complete elimination of the drug from the body.

The drug (10 mg) was administered after breakfast. Urine samples were collected before the administration and thereafter every 2 h up to 12 h, then at 24 and 48 h. A 2 ml aliquot of each sample was incubated for 24 h with β-glucuronidase after adjusting to pH 5.0. Borate buffer (2 ml) was added before extracting with ether (3 × 5 ml).

The ethereal layers were combined and evaporated to dryness. 0.25 ml of fluorazepam HCl solution (0.5% w/v) in methanol was added as internal standard before evaporating again to dryness. The residue was redissolved in 50 µl of methanol and 4 µl injected into the liquid chromatograph.

The oxazepam content of the urine was calculated from a calibration curve prepared by spiking blank urine samples with varying amounts of a solution (0.01% w/v) of oxazepam in methanol.

Results and discussion

Assay

The percentage contents of diazepam were found to lie between 96 and 102% in the three brands under investigation (Table 1). This agrees with the requirements of the BP (92.5–107.5%), thus indicating that they are chemical or pharmaceutical equivalents.

Dissolution rate test

The results illustrated in Fig. 1 revealed the following dissolution times for the brands. Valium and Relanium tablets have $t_{50\%}$ of 7.0 and 9.0 min and $t_{90\%}$ of 21.0 and 27.0 min respectively, whilst in the case of Tropium, the $t_{50\%}$ was 26.0 min and the $t_{90\%}$ was greater than 60 min (the period of the dissolution rate test). There was no significant difference in the mean of the percentage dissolution after 60 min for Valium (100% ± 0.4%) and Relanium (96.11% ± 0.6) ($P < 0.05$) whereas the percentage dissolution of Tropium (62.2% ± 0.5) was significantly different from that of Valium ($P <$

Table 1. Results of assay and bioavailability studies on diazepam tablets

Brand	Batch number	% content	Cumulative % excreted after 48 h	Maximum excretion rate V_{max} (μg/min)
Valium	B 046094 T	96.71 ± *2.70	66.96 ± †1.70	10.80 ± †4.79
Relanium	810777	97.90 ± 2.58	69.48 ± 3.32	8.03 ± 2.69
Tropium	1567	102.39 ± 2.20	42.57 ± 1.66	6.51 ± 1.53

*± sd, $n = 3$.
† ± sd, $n = 9$.

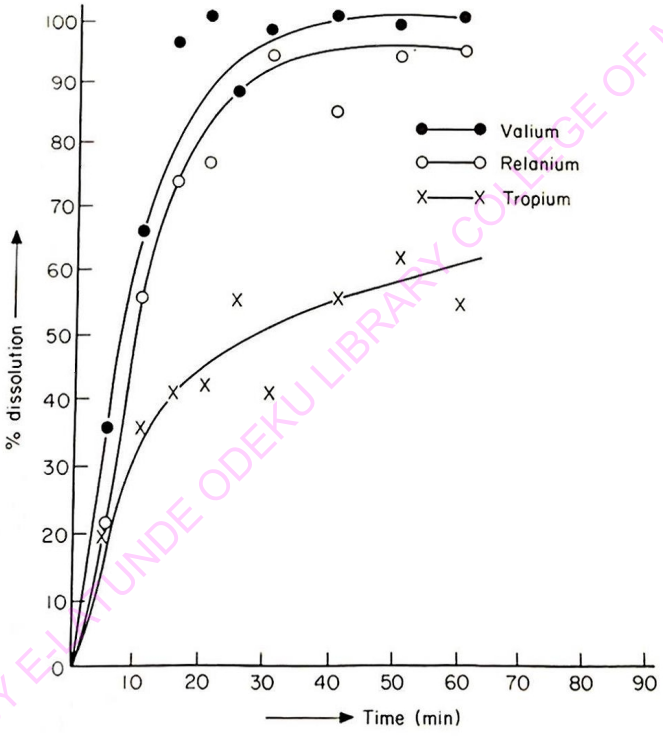

Fig 1. Average dissolution rate profile of the three brands of diazepam.

0.05). This suggests inequivalence on the part of Tropium compared to Valium.

Bioavailability studies

The results of the bioavailability trials are as shown in Table 1 and illustrated in Fig. 2. These results showed a lower bioavailability of Tropium compared to Valium. The cumulative dose excreted by the nine subjects after administration of Relanium, 69.48 ± 3.32, in 48 h was not significantly different from that after administration of Valium, 66.96% ± 1.70 ($P < 0.05$). However, with Tropium, the cumulative excretion over the same period was found to be 42.57% ± 1.66, which is significantly different from that of Valium ($P < 0.05$). The maximum

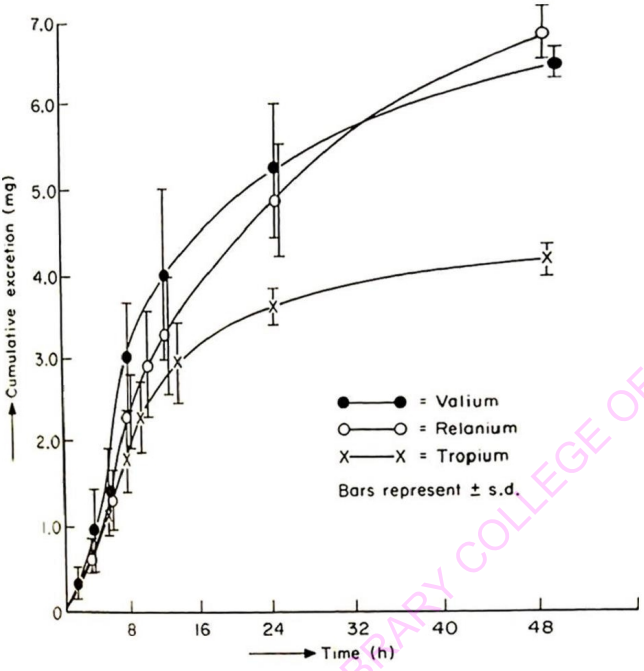

Fig. 2. Cumulative oxazepam excreted after administration of the three brands of diazepam to nine volunteers.

excretion rate of oxazepam following administration of Valium was 10.80 ± 4.79 μg/min and this was found not to be significantly different ($P < 0.05$) from that following Relanium, 8.03 ± 2.69 but significantly different ($P < 0.05$) from that following Tropium, 6.51 ± 1.53 (Table 1).

The results obtained in this study, have shown that inequivalency does occur in diazepam preparations contrary to previous reports in the literature. This is, however, not surprising since another benzodiazepine, chlordiazepoxide, has been found to have inequivalence problems.

The agreement in our results of the dissolution rate tests and the urinary excretion of the drug suggests that dissolution rate test may serve as an indicator of the bioavailability of diazepam preparations.

It is suggested that substitution of any diazepam tablets for Valium should at least be based on results of a comparative *in vitro* dissolution rate test.

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