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Assessing the bioequivalence of analogues of endogenous substances ('endogenous drugs'): considerations to optimize study design

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The complexities of assessing the bioequivalence of endogenous drugs are recognised.
- The FDA have published guidelines regarding the evaluation of levothyroxine and potassium chloride bioequivalence, whilst other authors have documented specific strategies to counter biases inherent in biostudies of endogenous drugs.
- A consolidated consideration of valid methods to optimise the design of such studies is however lacking.

WHAT THIS STUDY ADDS

- This paper is to the author's knowledge the first summarising various key approaches used to assess the bioequivalence of endogenous drugs, and to propose a series of recommendations for studies in this field.

BACKGROUND

Assessment of the bioequivalence of generic versions of certain reference drugs is complicated by the presence of endogenous levels of said compounds which cannot be distinguished from externally derived compound levels following drug administration. If unaccounted for, the presence of endogenous compound biases towards equivalence in bioequivalence studies of these drugs. Bioequivalence assessments may be complicated further as disposition of the exogenous analogue can be subject to various endogenous processes resulting in nonlinear pharmacokinetics. To overcome these inherent biases a number of different strategies have been employed.

AIMS

To critically review methods used to overcome confounding biases in bioequivalence studies of 'endogenous' drugs.

METHODS

A literature search of the EMBASE and PubMed databases was performed.

RESULTS

The following strategies were identified: ablation/modulation of baseline endogenous substance levels; recruitment of 'substance-deficient' populations; restriction of dietary intake of the relevant substance; standardization of conditions with the potential to affect relevant homeostatic mechanisms; correction for baseline substance levels; and administration of supra-therapeutic drug doses.

CONCLUSIONS

On the basis of this review key study design concepts, intended to optimize the design of future bioequivalence studies of these so-called 'endogenous drugs', are described. The dual stable isotope method, which could be used in a specific context, is also discussed.

Introduction

Pharmaceutical companies that develop generic versions of oral solid dose products (e.g. tablets and capsules) are typically required to demonstrate bioequivalence to an approved 'reference' product [1]. However, assessment of the bioequivalence of certain generic and reference compounds is complicated by the presence of endogenous levels of said compounds (e.g. ions, vitamins, hormones, etc.) that cannot be distinguished from externally derived compound levels following drug administration. If unaccounted for, the presence of endogenous compound biases towards equivalence in biostudies of these so-called 'endogenous drugs', particularly where, after administration of the exogenous analogue, the endogenous baseline contributes the majority of total substance levels or where homeostatic mechanisms preclude an increase in substance levels within a particular matrix. Bioequivalence assessments may be complicated further as disposition of the exogenous analogue can be subject to various endogenous processes such as saturable enzyme processes, active or diffusional transport, feedback mechanisms and renal thresholds [2]. To overcome these inherent biases and facilitate valid bioequivalence assessments of endogenous drugs, several different strategies have been employed. The purpose of this review was to evaluate critically these various methods and thereby make an informed series of recommendations to guide the design of future studies in this field.

Methods

The EMBASE and PubMed databases were searched for the term 'bioequivalence' in conjunction with any of the following: 'thyroxine', 'testosterone', 'oestrogen', 'progesterone', 'corticosteroid', 'FSH', 'LH', 'TSH', 'TRH', 'ADH', 'GnRH', 'ACTH', 'CRH', 'parathyroid hormone', 'erythropoietin', 'ions', 'potassium', 'calcium', 'magnesium', 'iron', 'aluminium', 'zinc', 'sodium', 'catecholamine', 'adrenaline', 'dopamine', 'pancreatin', 'insulin', 'glucagon', 'vitamin', 'amino acid', 'glucose' and 'carnitine'. These terms were selected on the basis of their being endogenous substances for which approved exogenous analogues were known or likely to exist. Abstracts of all papers identified in this search were reviewed, and papers identified as being of potential interest were reviewed in full. Relevant US and EU regulatory guidelines were also reviewed. This appraisal was not intended to be an exhaustive summary of all relevant papers on the subject of endogenous drugs, but a distillation of key lessons learnt from pivotal publications in the field.

Results

Modulation of baseline endogenous levels

Ablation/suppression of endogenous compound secretion Ablation of endogenous compounds, if feasible, is the most straightforward method with which to assess the bioequivalence of test and reference endogenous drugs. This approach has been used to assess bioequivalence of growth hormone (GH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) preparations.

To assess the bioequivalence of two formulations of recombinant GH, Jacobsen and colleagues administered a continuous 120 μg h^{-1} somatostatin infusion to healthy volunteers, starting 2 h before recombinant GH administration in each period of their crossover study [3]. The infusion was continued for 22 h of the 24-h pharmacokinetic (PK) sampling period and suppressed endogenous GH levels, although not to zero in all patients.

Of note, in this study no correction for baseline levels was made in the assessment of bioequivalence, nor was an assessment made as to whether baseline levels had an influence on outcomes. Furthermore, patients with nonzero baseline levels were not excluded from randomization or the PK analysis. The authors assumed contribution of endogenous GH secretion to be negligible. It is also noteworthy that a slight increase in GH concentration was seen at 24 h, which is probably because the somatostatin infusion was discontinued 2 h prior to the last sample being drawn.

Lugan and colleagues assessed the bioequivalence of freeze-dried and liquid formulations of FSH [4] in healthy male and female volunteers. They suppressed endogenous FSH secretion by administration of a single depot dose of 3.6 mg goserelin. Patients were considered ineligible to receive treatment in either study period if their baseline endogenous FSH levels were above 4 IU l^{-1} in women and 2 IU l^{-1} in men. Adequate FSH suppression was achieved in 39 of 42 subjects. The influence of baseline FSH concentrations was assessed by ANCOVA. Although median baseline FSH levels were low (<2 IU l^{-1}), ANCOVA showed a significant relationship between baseline FSH concentrations and PK parameters. A correction for baseline FSH was therefore applied, although the authors do not detail what form this correction took. Despite the ANCOVA findings, bioequivalence was established with and without correction for baseline FSH levels. Very similar findings in terms of FSH suppression and statistical observations were reported by Picard and colleagues, who also utilized goserelin 3.6 mg to downregulate pituitary function in a study comparing the bioavailability of FSH administered as a 2 : 1 fixed dose FSH : LH combination *vs.* FSH administered alone in premenopausal women. The authors also conducted a parallel, similarly designed study of LH administered as a 2:1 fixed dose FSH : LH combination *vs.* LH administered alone. Of interest, LH suppression in response to 3.6 mg goserelin was notably less successful than FSH suppression, with a quarter of subjects ultimately excluded from the LH study on the basis of inadequate hormonal suppression, although a weakness of this study is that the two sites involved utilized different definitions of failure of pituitary downregulation (LH

>2.5 IU l^{-1} or FSH and LH >4 IU l^{-1}). As with FSH, however, LH was found to be a highly statistically significant covariate for PK parameters, although the authors do not detail whether correction for baseline LH levels altered bioequivalence conclusions [5].

Voortman *et al*. also assessed the bioequivalence of two formulations of FSH in 24 healthy female volunteers [6]. In their study suppression of endogenous FSH secretion was achieved by 6 weeks' treatment with a high-dose combined oral contraceptive pill (Lyndiol®, containing 50 µg ethinyloestradiol and 2.5 mg lynestrenol) with the intention being to achieve 'full pituitary suppression'. Only one of 24 subjects was subsequently excluded from the PK analysis as her FSH values were above the limit of quantification (0.25 IU l^{-1}) prior to recombinant FSH treatment in both study periods. Note that low-dose combined oral contraceptives do not ensure complete pituitary suppression in all subjects [7].

Recruitment of populations deficient in the endogenous substance under test This is not always feasible for practical and/or ethical reasons. Furthermore, heterogeneity in the extent of deficiency among patients may make such a population less well defined and the study less well controlled than when using healthy volunteers [3], while recruitment of patients on existing replacement therapy may be complicated by variable baseline control. Mayor and colleagues attempted to evaluate the bioavailability of four levothyroxine products in a study of known hypothyroid patients who were considered to be euthyroid on a stable dose of levothyroxine replacement therapy. However, of 24 subjects 11 had at least one thyroid laboratory value outside the normal range at screening [8]. As a result of such difficulties, other methods are now considered preferable to assess the comparative bioavailability of products such as GH and levothyroxine.

Despite the difficulties described above, the use of patients with low baseline levels of endogenous substance is well established in the case of bioequivalence studies of oestradiol hormone replacement therapy. Studies typically enrol postmenopausal or bilaterally oophorectomized female subjects [9–11]. Menopause is generally confirmed by baseline oestradiol values <20 pg ml^{-1} and FSH >30–40 mIU ml^{-1}. This is the target population, but has the specific advantage that endogenous levels of oestradiol and oestrone (the principal metabolite of oestradiol) decrease significantly during menopause, thereby minimizing the impact upon subsequent bioavailability comparisons. Given low baseline values, peak oestradiol and particularly oestrone values (oestradiol is rapidly converted to oestrone) in these studies are substantially in excess of baseline values and remain so for 48–72 h in most subjects. In Zimmerman's study of two oestradiol valerate-containing preparations, basal oestradiol values were <20 pg ml^{-1} with postdose C_{max} values of approximately 40 pg ml^{-1}, while basal free oestrone values were generally <10 pg ml^{-1} with postdose C_{max} values of approximately 170 pg ml^{-1} [9].

Dietary restriction of endogenous compounds Dietary restriction may be employed where relevant to limit the confounding influence upon bioequivalence studies of high dietary intake. Sahajwalla and colleagues adopted such an approach in their study of three dosage forms of L-carnitine [12]. L-carnitine is a carrier molecule in fatty acid metabolism and is indicated in the treatment of primary or secondary carnitine deficiency. In this study subjects were given diets low in L-carnitine that generally contained <100 µmol day^{-1} and samples of food were frozen and analysed for free and total L-carnitine content. As a result of dietary restriction the relative contribution of L-carnitine from the test products was approximately 120–200-fold that from the diet, although endogenous substance levels still contributed approximately 60–65% of total exposure, hence a baseline-corrected analysis was employed. Unfortunately, the authors did not include a control group on a 'normal' diet to allow assessment of whether this would confound assessment of bioequivalence. The comparison of only a single dose of all three compounds is a further limitation of Sahajwalla's study.

Standardization of input/output/production of endogenous substances

Standardization of diet and environmental conditions is routine in bioequivalence studies, but assumes even greater importance in studies of endogenous drugs where dietary intake, variable bodily losses and homeostasis are relevant.

Bioequivalence studies of potassium chloride supplements are a good example of these principles and the US Food and Drug Administration (FDA) have issued detailed guidance highlighting the routes of potentially significant potassium losses and the potential importance of homeostatic mechanisms (Na^+-K^+ exchange in the kidney, and the renin–angiotensin–aldosterone axis) [13]: the standardized diet should contain known quantities of potassium, sodium and fluid intake; subjects should be housed in a climate-controlled environment to avoid excessive sweating, hence potassium loss, and subjects should be encouraged to refrain from unnecessary physical activity; information regarding excessive sweating or diarrhoea should be actively sought; a test for faecal occult blood should be performed on each dosing day; and subjects should remain upright for at least 3 h following dosing.

Correction for baseline levels of endogenous compounds

Ablation of endogenous compound levels is not feasible in most situations, so that correction for baseline compound levels is generally required in the PK analysis to facilitate an assessment of the bioequivalence of 'endogenous' drugs.

The most frequent correction method is simply to subtract the baseline endogenous compound level from all subsequent post-treatment values [9, 14, 15]. The baseline level is usually taken as the mean of three or so levels in the 24 h prior to dosing. Although this method is generally accepted, a potential limitation is that administration of the exogenous analogue may affect production of the endogenous substance – hence consistent subtraction of the baseline value from all post-treatment matrix values may result in overcorrection. In a collection of eight studies by Walter-Sack and colleagues evaluating the bioequivalence of eight tablet strengths of levothyroxine (from 25 to 200 μg) *vs.* reference liquid solution, the authors noted that the prespecified (standard) method of baseline correction increased the random error and may have overcorrected for baseline values [16]. Of particular note in Walter-Sack's studies, residual standard deviation was least when an adjustment for log baseline total thyroxine (TT4) was performed, with TT4 used as a covariate. Log TT4 explained 35% of the log AUC variation and 17% of the log C_{max} variation. Addition of log TT4 to the statistical model substantially reduced dependencies on season, age and thyroid volume. The authors surmised that although addition of an additive term to the log model was not a precise mathematical description of the relationship between baseline TT4 and total AUC, it appeared to work well as an approximation of the feedback process, whereas simple subtraction of baseline levels did not.

More sophisticated versions of simple baseline subtraction have also been employed, such as measurement of endogenous substance levels at time points prior to drug administration, which correspond to those of postdrug administration assays [17], thereby establishing a variable diurnal baseline. However, it has not been well established that such correction increases the sensitivity of the bioequivalence analysis.

Corrected endogenous substance values post dosing of less than zero may be derived following subtractive correction and imply overcorrection associated with a variable baseline (the estimation of baseline as an average of three or so measurements is intended to limit this risk) or may imply a baseline decreased by administration of the exogenous analogue. A routine approach to handling below zero values in the PK analysis is to count them as zero [9]. Nonzero substance values that are measured after a prior zero value may also be seen, and may again imply a naturally variable baseline or an overshooting endogenous response [9]. In such instances it is usually appropriate to disregard nonzero levels after an initial zero value assuming the nonzero values are minor. Large nonzero values in this context may suggest that an alternative study design/alternative method for deriving the baseline is required.

One particular situation that warrants caution with respect to baseline correction is where lengthy washouts/treatment periods may be employed, e.g. for substances with a long half-life such as thyroxine ($t_{1/2}$ of 6–9 days), in which case potential seasonal variations should be considered. In Walter-Sack's studies, significant period effects for TT4 were noted that could be explained almost entirely by seasonal effects. These findings are in keeping with those of other authors, which have shown highest TT4 values in winter, lowest TT4 values in summer [18–20] and seasonal variation of thyroid size in healthy men [21]. Behall and colleagues also noted seasonal effects for glucose, glucagon and insulin levels [18].

Administration of supratherapeutic doses of endogenous drugs

Intuitively, baseline correction is less critical if endogenous levels comprise a lesser proportion of the total levels post drug administration. Thus a standard method in biostudies of endogenous drugs is administration of supratherapeutic doses to minimize the confounding effects of endogenous levels, assuming safety concerns do not exist. This method largely overcomes the difficulties that arise when negative values are generated following baseline correction that preclude calculation of bioequivalence ratios.

The best described example relates to levothyroxine. Endogenous levels of levothyroxine constitute a significant portion of total blood levels after therapeutic dosing. FDA guidelines therefore advocate single doses of 600 μg levothyroxine be administered in a volunteer crossover study [22] (single doses as high as 3 mg are reported to be safe [16]), which compares to a usual maintenance dose in patients of 100–200 μg daily. Doses of 400–450 μg in a volunteer study yield concentrations close to baseline, thereby precluding accurate assessment of differences between products [14], which is supported by the findings of Blakesley: using standard baseline subtractive correction C_{max} for a 450-μg dose of levothyroxine was actually lower than for a 400-μg dose of the same formulation, and 90% confidence intervals for C_{max} ratio excluded the true ratio of 1.125 [14, 17]. Note that in contrast, in a study submitted to the FDA using the same baseline adjustment method a comparison of 500 μg and 600 μg levothyroxine yielded an observed ratio of almost exactly 1.2 (600/500) for both AUC and C_{max} [14]. It is also noted that the coefficient of variation (CV) based on the assay alone approximately doubles if a 300-μg *vs.* a 600-μg levothyroxine dose is used, providing further support for using high doses in this context [14].

Use of matrices other than blood/plasma/serum

Homeostatic mechanisms maintain levels of an endogenous substance within a physiological range and prevent changes in substance levels that may be harmful. In certain instances intake of even large quantities of an exogenous analogue is rapidly countered by homeostatic equilibria and results in a negligible increase in blood levels, complicating the assessment of bioequivalence. Several examples exist, such as a number of ions (potassium, calcium, mag-

nesium, iron and aluminium), glutamine, L-carnitine and some vitamins (D_2, D_3 and B_{12}). Folic acid is a notable exception, exogenous administration producing a large increase in exposure (~95% of total AUC with a 15-mg dose is due to the drug) [23].

Where drugs are cleared by urinary excretion and are not subject to hepatic metabolism/biliary excretion, measurement of urinary excretion offers an alternative means by which to assess the bioequivalence of different formulations using the parameters cumulative urinary excretion (CUE) and maximal rate of urinary excretion (R_{max}). It should be noted that saturable renal thresholds may result in nonlinear urinary excretion. Potassium chloride supplements are the best known example where urinary bioequivalence is routinely assessed, and the FDA have issued a detailed guidance document in this regard [13].

Use of surrogate/pharmacodynamic parameters to compare bioavailability

Mayor *et al*.'s study of four levothyroxine products is useful in considering the issues associated with using a surrogate parameter to assess bioequivalence [8]. Thyroid-stimulating hormone (TSH) has been proposed as an alternative variable to levothyroxine in bioequivalence studies of the latter, based on the fact that attaining TSH levels in the normal reference range is the biochemical target of levothyroxine treatment and is used to guide levothyroxine replacement dosage [14]. However, as Mayor and other authors have demonstrated [8, 24], the variability associated with TSH, as a secondary effect, is extremely high (CV of up to ~200% reported in these studies), while even greater variability was noted for total tri-iodothyronine [8].

Discussion and conclusions

The appropriate design for a bioequivalence study of endogenous drugs should be considered on a case-by-case basis. The key concepts for consideration are discussed below.

1. Control of baseline endogenous levels should be attempted where feasible. However, instances where endogenous levels may be pharmacologically ablated, or where homogeneous populations deficient in the relevant substance and appropriate for study enrolment exist, are limited. Several endocrine hormones appear to be the most suitable candidates for pharmacological ablation/suppression of endogenous compound levels in order to assess the bioequivalence of exogenous analogues. Of the studies discussed above, the methodology employed by Voortman and colleagues, i.e. excluding patients with nonzero baseline levels from randomization or PK analysis, is considered optimal and obviates the needs for further correction. Sponsors attempting to ablate/modulate baseline substance levels should therefore prospectively define randomization criteria whereby subjects are excluded from treatment if their baseline levels are above a defined threshold. Assumptions, such as those made Jacobsen, of negligible endogenous secretion during the study despite nonzero baseline values should be avoided, particularly since it is simple to recruit a larger sample of patients and exclude from randomization those in whom full suppression in all study periods cannot be achieved. It is also important that the robustness of endogenous hormone suppression for the full duration of the requisite study period has been well established prior to, or is confirmed as part of, any pivotal bioequivalence study.
2. Dietary intake of endogenous substances should be strictly controlled/standardized where it has the potential to confound bioequivalence assessment (e.g. in biostudies of ions or vitamins).
3. Homeostatic mechanisms regulating levels of an endogenous substance and mechanisms of substance loss should be routinely considered, and the biostudy design should take these into account to limit potential confounding influences.
4. Baseline correction should be routinely employed, except in rare cases where substantial increases over baseline endogenous levels may limit the need for this, e.g. in biostudies of folic acid tablets. Concerns as to overcorrection with the standard baseline subtraction method, whilst valid, are mitigated by the crossover design of typical bioequivalence studies. Hence use of this approach is reasonable although its limitations are recognized. The alternative method of incorporating the relevant baseline covariate into the statistical model may offer a more precise estimate of comparative bioavailability, although, as this is not a standard approach, its validity needs to be demonstrated on a case-by-case basis. Where large diurnal variations in endogenous levels occur, point-by-point correction could be considered, although the advantages of this methodology over standard single-point subtractive correction have not been established. However, the baseline diurnal profile should be based on at least two and preferably more series of measurements. For a particular substance where a method of baseline correction has been documented as being appropriate, it is acceptable for that method to be re-employed in future studies. For endogenous substances where there is a dearth of published data to guide practice it would be appropriate if at least two prospectively defined corrective methods were used. The relative merits of each should then be discussed in the analysis of results.
5. Administration of supratherapeutic doses of endogenous drugs should be considered on a routine basis where safe and where saturable processes, e.g. enteral absorption, do not prelude measurable rises in a particular matrix.

If a predictable separation in exposure following administration of different doses of a particular endogenous substance has not been previously established, this must be done, either in a pilot study or as part of the principal biostudy using different doses of the reference formulation, in order to ensure that any dose used for the pivotal bioequivalence comparison is known to provide assay sensitivity. For example 500 *vs.* 600 μg of levothyroxine has been shown to have the expected baseline corrected ratio of almost exactly 1.2 for both AUC and C_{max}, whereas doses of 400 and 450 μg of levothyroxine did not demonstrate expected PK ratios compared with 600 μg [14].

6 Use of matrices other than those that are blood-derived should be considered, where homeostatic mechanisms preclude significant rises in blood substance levels.

7 Where the most sensitive variable/matrix to assess bioequivalence has not been determined and where a rational choice may exist (e.g. total/free/conjugated substance levels or blood/urine), consideration should be given to a self-reference study of several doses of the reference product in order to determine which parameter/matrix may be optimal to assess bioequivalence.

8 Use of surrogate parameters has not been successfully employed in bioequivalence studies of endogenous drugs to date.

9 Nonlinearity is likely to be a frequent issue in bioequivalence studies of endogenous drugs given homeostatic mechanisms, saturable processes, interchangeable substance pools, etc. Therefore, in addition to the concepts described above, the dose most likely to distinguish potency differences should be considered in designing a biostudy to compare such drugs.

10 Consideration should also be given to the dual stable isotope technique to demonstrate bioequivalence between test and reference formulations, a method that has been described for various 'non-endogenous' drugs [25–30]. However, this method, which involves the substitution of chemical elements within the drug molecule by stable isotopes that are not subject to radioactive decay such as ^{2}H, ^{13}C or ^{15}N, is only feasible where isotopes can be incorporated into the chemical structure of both test and reference formulations during the manufacturing process. In practical terms this will be the case only where a company alters its own drug formulation. However, where the reference formulation is manufactured by a competitor company (by far the commonest scenario in bioequivalence studies) the competitor's manufacturing methods would not be in the public domain and only the finished product would be commercially available, thereby precluding use of the dual stable isotope technique. This has restricted the use of the stable isotope method in bioequivalence studies and, indeed, no studies of endogenous drugs using this methodology were identified during the course of this review. However, since companies do intermittently amend their drug formulations the potential of this method is real. To undertake successfully a bioequivalence study using this technique, the formulations generated should be of sufficiently different mass to be distinguished from one another and from the endogenous substance by mass spectrometry [29], it should be confirmed that there is no 'isotope effect', i.e. labelling must not affect formulation PK or metabolism [29, 30] and simultaneous administration of test and reference compounds (the standard method as labelled formulations can be distinguished on account of their different molecular weights) should not saturate any processes affecting bioavailability or PK [27, 29]. Furthermore, in order to make the study acceptable to regulatory authorities synthetic processes should be those used in commercial-scale drug production [29] and in-process and finished product specifications for the isotope formulations should conform to those for the commercial products. Additionally, as the stable isotope method represents a novel paradigm for a bioequivalence study intended to gain regulatory approval, the agreement of regulatory authorities should first be sought. A major advantage of the stable isotope method is that it would obviate the need to modulate or correct for endogenous substance levels, or administer supratherapeutic drug doses. Another advantage is that administration of both drug formulations within a single study period avoids biological variability over time, hence increases study power and reduces sample size requirements [27, 29].

Competing interests

None to declare.

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