Comparative Pharmacokinetics of Unlabeled and Deuterium-Labeled Terbutaline: Demonstration of a Small Isotope Effect

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Received November 3, 1987, from the *Pharmacokinetics Laboratory and ‡Medical Statistics and Data Management, AB Draco, P.O. Box 34, S-221 00 Lund, Sweden. Accepted for publication July 22, 1988.

Abstract □ An equimolar mixture of terbutaline and [$^2H_6$]terbutaline was given as an oral solution to six healthy volunteers (three men and three women). Frequent blood samples were collected during a 24-h period and the plasma concentrations of unlabeled and deuterium-labeled terbutaline were measured by GC-MS. The overall geometric mean plasma concentration ratio of terbutaline to [$^2H_6$]terbutaline (isotope ratio) was 1.04 and differed significantly from unity. The difference can be explained by a difference in lipophilicity between the analogues, affecting their absorption. No trend in isotope ratio over the experimental time was observed. For unknown reasons, the isotope ratio was higher for women (1.07) than for men (1.00). Deuterium-labeled terbutaline can be used, intravenously or orally, as an absolute reference in bioavailability studies on terbutaline. If deuterium-labeled terbutaline is given orally in a single-day relative bioavailability study, a correlation should be made for the observed isotope effect.

The bioavailability of a new drug formulation is traditionally determined in a crossover study with another formulation as reference. The underlying assumption for this design is that absorption, distribution, metabolism, and elimination in each subject are stable over the two study periods. As this is not always the case, a large number of subjects must be included to detect an existing difference between the formulations. If both formulations are given at the same time, the influence of the intraindividual variations can be eliminated, which will increase the power of the statistical tests. To differentiate between drug substance derived from the two formulations, a stable isotope-labeled analogue can be used in one of the formulations. Both substances can then be determined simultaneously by mass spectrometry. Absolute or relative bioavailability of a number of drugs has been assessed by this technique, which has been thoroughly reviewed.[1-3] The studies have shown that the number of subjects can be drastically reduced without sacrificing the power of the statistical tests. The stable isotope-labeled drug can also be used as a common reference (pharmacokinetic internal standard) in comparative bioavailability studies.[4]

Incorporation of a heavy isotope, particularly substitution of deuterium for hydrogen, can give rise to an isotope effect[5] that could alter the pharmacokinetics of the drug. This effect is usually insignificant if the label is placed in a metabolically inert position of the molecule. Yet, any isotope effect must be investigated to prevent drawing misleading conclusions from studies with stable isotope-labeled drugs.

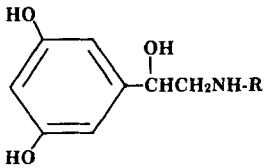
Terbutaline is a β_2-receptor agonist that is widely used for the treatment of chronic obstructive lung diseases, often in the form of slow-release formulations. The drug undergoes extensive first-pass elimination in the gut wall,[6] a fact that makes the stable isotope coadministration technique especially valuable.[7] In the present study we wanted to compare the pharmacokinetics of deuterium-labeled and unlabeled terbutaline with the aim of studying whether it will be possible to use the labeled analogue in future bioavailability studies.

Experimental Section

Subjects—Six Caucasian subjects (three men and three women) participated in the study. Their age was between 23 and 44 years (mean 37 years) and they weighed from 53 to 81 kg (mean 64 kg). They were judged to be healthy by a physician after physical examination and laboratory tests. The study was approved by the local Ethics Committee and registered by the Swedish National Board of Health and Welfare. The performance was in accordance with the Declaration of Helsinki. Informed consent was given in writing.

Drugs—Unlabeled terbutaline [terbutaline sulphate (batch no. 448); powder for oral intake] and deuterium-labeled terbutaline ([$^2H_6$]terbutaline chloride (batch no. OP2); powder for oral intake) were supplied by AB Draco, Lund, Sweden (see structure). Both substances are stable and freely soluble, as defined by USP XXI. The chemical purity was >99% for both substances. Equimolar amounts of the two analogues (9.10 μmol of each), corresponding to a total dose of 5 mg of terbutaline sulphate, were dispensed in six 50-mL glass bottles. The actual weight of terbutaline and [$^2H_6$]terbutaline in each dose was recorded and used in the subsequent calculations. Each dose was dissolved in 50 mL of water at the time of administration and the bottle was rinsed with another 50 mL of water, which was also ingested.

Clinical Procedures—The study was open. After fasting for 10 h, the subjects arrived at the clinic in the morning. An indwelling catheter was inserted into an antecubital vein for blood sampling and a blank blood sample was drawn. The terbutaline: [$^2H_6$]terbutaline mixture was administered orally and blood samples were obtained at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h after the dosing. At each sampling time, the first 2 mL of blood from the catheter was discarded and the following 10 or 20 mL collected into a heparinized



Terbutaline R = $C(CH_3)_3$

($^2H_6$)Terbutaline R = $C(C^2H_3)_2CH_3$

($^2H_9$)Terbutaline R = $C(C^2H_3)_3$

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tube (Venoject). After sampling, the catheter was flushed with 2 mL of heparinized saline (10 IU/mL) to keep it patent. The blood was immediately centrifuged at room temperature and the plasma sucked off and stored in polystyrene tubes at −20 °C until assayed. Two hours after the start of the experiment, a standardized breakfast was served.[8]

Terbutaline Assay—Unlabeled and deuterium-labeled terbutaline in plasma (free plus protein bound) were determined by GC-MS, essentially as described previously.[9,10] Briefly, after addition of 20 pmol of [$^2H_9$]terbutaline as internal standard, the plasma sample (2 mL) was extracted on a disposable C_{18} column.[10] The evaporated extract was silylated and the trimethylsilyl derivatives of terbutaline, [$^2H_6$]terbutaline, and [$^2H_9$]terbutaline were analyzed by ammonia chemical ionization GC-MS.[9] Separate calibration curves, in the range 2–80 pmol, were constructed for terbutaline and [$^2H_6$]terbutaline. The lower limit of quantitation was 2 pmol. At this level, the within-day variation (CV), as determined during method development, was 3.9% for terbutaline and 3.6% for [$^2H_6$]terbutaline.

[$^2H_9$]Terbutaline interfered to some extent at the *m/z* value recorded for [$^2H_6$]terbutaline, and vice versa. Corrections were therefore made of the ion intensity ratios *m/z* 442/451 and *m/z* 448/451 according to the equations given below:

$$\text{Corrected ratio } m/z\ 442/451 = \frac{I_{442}}{I_{451} - 0.0441 I_{448}} \quad (1)$$

$$\text{Corrected ratio } m/z\ 448/451 = \frac{I_{448} - 0.0298 I_{451}}{I_{451} - 0.0441 I_{448}} \quad (2)$$

where $I_{442}, I_{448}, I_{451}$ are the measured ion intensities at *m/z* 442, 448, and 451, respectively. The ion intensity ratio *m/z* 448/451 measured for pure [$^2H_9$]terbutaline was 0.0298, and the ion intensity ratio *m/z* 451/448 measured for pure [$^2H_6$]terbutaline was 0.0441. There was no interference from [$^2H_6$]terbutaline or [$^2H_9$]terbutaline at the *m/z* value recorded for terbutaline, or vice versa.

To be able to measure the low plasma concentrations between 10 and 24 h, relatively large volumes of plasma (3.5–8.0 mL) had to be extracted. In those cases, the sample was divided into 2-mL portions and each portion extracted on a C_{18} column. The extracts from each sample were then combined before the GC-MS analysis. This procedure did not influence the experimental values. A sign test for a difference in the isotope ratio from 1.00 resulted in similar p values for measurements performed on 2-mL samples ($p < 0.001$) and larger volumes (3.5–8.0 mL) of plasma ($p = 0.004$).

The plasma samples were analyzed on two separate days. Samples from subject nos. 1, 5, and 6 were analyzed during the first day and samples from subject nos. 2, 3, and 4 during the second day. The geometric mean isotope ratio of the standard samples, used for the calibration curves, was 0.996 and did not differ significantly ($p = 0.576$) from 1.000. The coefficient of variation of the isotope ratio measurement of the standard samples was 3.0%.

Data Analysis—The measured plasma concentrations of terbutaline and [$^2H_6$]terbutaline were normalized, using the actual weight of each dose, to a dose of 9.10 μmol, and the ratio of terbutaline to [$^2H_6$]terbutaline (hereinafter referred to as the isotope ratio) was calculated for each sample. An isotope effect, if any, will be reflected in this ratio. Primary and secondary pharmacokinetic parameters (clearance, distribution volume, and area under the curve) are linear combinations of plasma concentrations and times, and the calculation of these pharmacokinetic parameters would add no extra information.

Differences in isotope ratio between subjects, sexes, and sampling times were evaluated by analysis of variance, where the trial was viewed as a "split-plot" design.[11] The Greenhouse-Geisser correction was used in the calculations. The overall deviation of the experimental ratios from unity was also evaluated by analysis of variance. A potential trend in ratio over the sampling time was tested by viewing the within-subject values as repeated measurements and considering the fact that two measurements, close in time, covariate more than two distant measurements. The logarithmic values of the isotope ratios were used in the statistical evaluations; level of significance was set at $p = 0.05$.

Results

The subjects complied well to the study protocol and all blood samples were drawn within 3 min of the scheduled times. Figure 1 shows the mean plasma concentration–time curves of terbutaline and [$^2H_6$]terbutaline. The individual and mean isotope ratios are presented in Table I and plotted in Figure 2 (logarithmic scale on the y-axis). The overall geometric mean isotope ratio of the plasma samples was 1.036 and differed significantly ($p < 0.001$) from 1.000. Thus, the body seems to handle unlabeled and deuterium-labeled terbutaline slightly differently. A further analysis revealed a difference between subjects ($p = 0.005$); the geometric mean ratio was 1.075 for the women and 1.000 for the men. When the ratios for male and female subjects were compared with each other, a significant difference between the sexes was found ($p = 0.042$). In fact, the overall mean isotope effect could be assigned to the observed isotope effect in the women. Within each subject, the ratios seemed to be fairly stable (Figure 2). There was no trend in the ratios at different sampling times ($p = 0.207$) when the measurements within

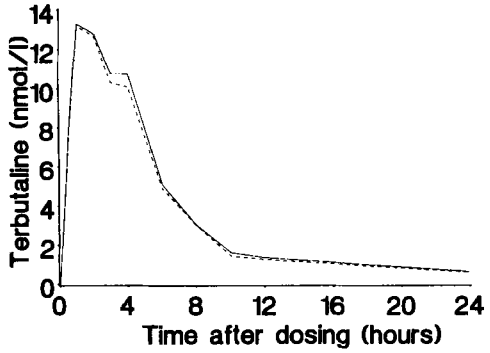

Figure 1—*Mean plasma concentration—time curves of terbutaline (solid line) and [$^2H_6$]terbutaline (broken line). The values were corrected to a nominal dose of 9.10 μmol of each analogue.*

Table I—Plasma Concentration Ratios of Unlabeled versus Deuterated Terbutaline

Subject No.	Sex	Time after Dosing, h										
		0.5	1.0	2.0	3.0	4.0	6.0	8.0	10.0	12.0	24.0	Mean[a]
1	F	1.0746	1.0826	1.0415	1.1269	1.1128	1.1111	1.0729	1.2331	1.2753	1.0134	1.1118
2	F	1.0330	1.0221	1.0349	1.0325	1.0559	1.0138	0.9746	1.0637	1.0618	1.0235	1.0313
3	F	1.0234	0.9698	1.0127	1.1201	1.1510	1.0931	1.1483	1.1016	1.0979	1.1251	1.0827
4	M	1.0039	1.0065	1.0228	1.0459	1.0165	1.0412	0.9040	1.0930	0.9389	1.0533	1.0112
5	M	0.9820	0.9615	0.9679	0.9574	0.9725	0.9673	0.9191	1.0500	0.9937	1.0092	0.9779
6	M	1.0645	1.0145	0.9951	1.0234	1.0276	0.9716	1.0219	0.9707	1.0222	0.9933	1.0101
Mean[a]		1.0297	1.0087	1.0122	1.0494	1.0544	1.0316	1.0032	1.0826	1.0599	1.0354	1.0364

[a] Mean values are geometric means.

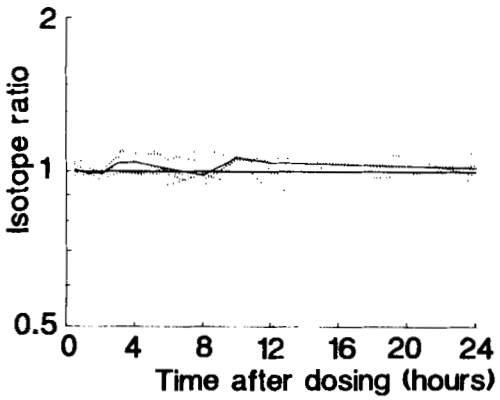

Figure 2—*Individual (dotted lines) and mean (solid line) isotope ratios after simultaneous administration of unlabeled and deuterium-labeled terbutaline. The line corresponding to an isotope ratio of 1.00 is also shown for comparison.*

each subject were regarded as repeated measurements and the covariation of the experimental values at adjacent sampling times was considered.

Discussion

Stable isotope labeling of a drug can alter its physicochemical properties such as pK_a and lipid solubility.[5] These changes may influence the fate of the drug at different steps along its passage through the body. Absorption, distribution, metabolism, or excretion can be changed. Absorption and distribution are processes that depend primarily on the molecular size and the lipophilicity of the substance. Terbutaline is a hydrophilic substance with incomplete absorption (~50%) after oral intake.[12] It has been shown that substitution of deuterium for hydrogen makes a molecule less lipophilic.[13] A lower lipophilicity of [$^2H_6$]terbutaline could reduce its absorption compared with terbutaline. Such an effect should give an isotope ratio above unity, constant over time after the initial absorption phase. As no time-dependent trend in the isotope ratio was observed, a change in absorption is a plausible explanation of the observed isotope effect. The reason for the observed sex difference in isotope ratio is unknown. A difference in distribution of the two terbutaline analogues should result in a smaller distribution volume, and a faster rate of elimination, of the less lipophilic deuterated compound. This would show up as an isotope ratio below unity and as a trend in the isotope ratio over time. These characteristics were not found.

Drug metabolism can give rise to large isotope effects if the breaking of a chemical bond to a deuterium atom is the rate-limiting step in the process.[14] [$^2H_6$]Terbutaline was labeled in the *t*-butyl group, a position in the terbutaline molecule at which no metabolic reactions are known to occur.[6] Renal excretion depends on the physicochemical properties of the molecule, but also on the molecular structure in case an active transport process exists. Metabolism and excretion are both processes that, in contrast to absorption, are effective during the whole study period. If excretion, or metabolism, differed for unlabeled and labeled drug, this would appear as a trend in the isotope ratio over time. No such trend was revealed when the early (0–4 h) ratios were contrasted with the late (6–24 h) ratios. A power analysis showed that the chance to detect a difference in ratios of ~ 0.06 between the first and last values was 80%. It is therefore unlikely that the observed isotope effect is due to differences in excretion or metabolism of the terbutaline analogues.

The observed difference of the isotope ratio from unity was not due to contamination of the deuterium-labeled terbutaline with unlabeled drug, since the same batch of deuterated terbutaline was used to prepare standard samples for the calibration curves. The imprecision of the analytical method (3.0%, CV) was of the same magnitude as the difference of the mean isotope ratio from the theoretical value (4%). Detection of this small difference by statistical methods was possible because as many as six subjects were included in the study. Similar investigations on other drugs reported in the literature have only comprised one to three subjects, and, with such small panels, only relatively large isotope effects can be detected. A study of the pharmacokinetic equivalence of metaproterenol, another β_2-receptor agonist similar in structure and lipophilicity to terbutaline, and a deuterated analogue in two volunteers was recently published.[15] Although it was concluded that no isotope effect was at hand, the data indicated a reduced absorption of the deuterium-labeled compound.

Conclusion

A small in vivo isotope effect of deuterium-labeled terbutaline was observed. The effect can be explained by reduced absorption of deuterium-labeled terbutaline from the gut, caused by a somewhat lower lipophilicity of this analogue.

The results show that the deuterated analogue can be used in bioavailability studies on terbutaline when given intravenously. The labeled analogue can also be used, intravenously or orally, as a pharmacokinetic internal standard in studies on the relative bioavailability of different terbutaline formulations, comprising two or more study periods. In these cases, no corrections of the experimentally obtained data with the presently observed isotope ratios for men and women need to be performed. If unlabeled and deuterium-labeled terbutaline are given orally in a single-day bioavailability study, the observed isotope ratios for men and women should be taken into consideration.

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