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Rapid screening for polymorphisms in dextromethorphan and mephenytoin metabolism

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1 The phenotyping parameters for dextromethorphan and mephenytoin were assessed in 48 normal male volunteers following administration of each metabolic probe drug on separate occasions and together according to a randomized 3-way crossover design.

2 Neither the urinary S-/R-mephenytoin ratio nor the dextromethorphan metabolic ratio were altered significantly by coadministration of the probe drugs.

3 Five-hundred and nineteen subjects were screened for expression of mephenytoin 4-hydroxylase and dextromethorphan *O*-demethylase activity following the coadministration of mephenytoin and dextromethorphan. The activity was determined in each case by methods not requiring any quantitative measurements.

4 Nineteen (3.7%) of the subjects were identified as poor metabolizers (PMs) of mephenytoin and 35 subjects (6.7%) as PMs of dextromethorphan.

5 All PMs of dextromethorphan were confirmed by more rigorous evaluation of the metabolic ratio.

Keywords dextromethorphan mephenytoin polymorphic metabolism drug coadministration

Introduction

Interest in polymorphic drug metabolism has grown rapidly over the last 10 years. There is now increasing evidence to link the expression, or lack thereof, of specific cytochrome P-450 enzyme(s) with altered metabolism, response and toxicity to certain drugs and to disease (Ayesh *et al.*, 1984; Dayer *et al.*, 1982; Kaisary *et al.*, 1987; Shah *et al.*, 1982; Siddoway *et al.*, 1987). As a result of these associations, interest has grown in characterizing individuals within the population with respect to the expression of certain P-450 enzymes and further defining the clinical implications associated with their expression (Britto *et al.*, 1989; Fonne-Pfister *et al.*, 1987; Gueguen *et al.*, 1989; Ward *et al.*, 1989). Research directed toward defining the clinical implications of these polymorphisms, however, is hampered somewhat by phenotyping costs and labour-intensive phenotyping procedures. Work on the clinical and toxicological consequences of polymorphic oxidative drug metabolism could be enhanced substantially by further development of rapid, reliable and inexpensive methods capable of characterizing the phenotypes of individuals in the population-at-large.

Several recent trends in pharmacogenetic screening have been aimed at broadening its general utility and availability. These trends include: (1) the coadministration of two or more probe drugs for defining multiple oxidative phenotypes (Breimer *et al.*, 1986; Drohse *et al.*, 1989; Inaba *et al.*, 1988); (2) the utilization of more universally available probe drugs like dextromethorphan (Roy *et al.*, 1984; Schmid *et al.*, 1985); and (3) the development of more cost-efficient screening procedures (Ebner *et al.*, 1989; Guttendorf *et al.*, 1988). In spite of these trends, however, there has been little work

directed specifically toward simplifying phenotyping procedures.

Historically, phenotyping with respect to these polymorphisms has entailed the administration of mephenytoin and debrisoquine or sparteine on separate occasions, followed by the analysis of the collected urine samples (Inaba *et al.*, 1984; Kupfer & Preisig, 1984; Nakamura *et al.*, 1985; Wedlund *et al.*, 1984). Although several investigators have advocated the coadministration of probe drugs (Inaba *et al.*, 1988; Jacqz *et al.*, 1988; Schellens & Breimer, 1987), there has been little experimental justification for such an approach (Baumann & Jonzier-Perey, 1988; Drohse *et al.*, 1989; Sanz *et al.*, 1989; Schellens *et al.*, 1989). The current study was performed to determine whether the coadministration of dextromethorphan and mephenytoin would lead to an interaction of sufficient magnitude to interfere with phenotyping assessments by either of these probes. The results of this study were then applied to a large scale screening of individuals using phenotyping methods devoid of time consuming and labour intensive quantitative measurements.

Methods

Subjects

The subjects were recruited from the general and student populations of Central Kentucky. All subjects were healthy adult male volunteers, 18 to 71 years of age. The studies were approved by the University of Kentucky Institutional Review Board and all subjects provided informed consent and completed a brief medical history. No subjects were admitted to the study who were under a doctor's care, taking medication, had a history of drug abuse (including alcohol), a history of drug sensitivity or a history of renal, hepatic or haematological disease.

Conduct of studies

The first study was designed to test whether the coadministration of mephenytoin and dextromethorphan would influence the parameters used to characterize either of the phenotypes determined by these probe drugs. Forty-eight (48) healthy male volunteers, aged 22 to 40 years, who were judged normal by physical examination, medical history, and laboratory screening (haemoglobin, haematocrit, white blood cell and platelet count and blood chemistries) were admitted to the study. At weekly intervals, each subject received one of three treatments: Treatment A—60 mg dextromethorphan hydrobromide (as four 15 mg Mediquell® cough squares, Warner-Lambert Co.); Treatment B—100 mg of racemic mephenytoin (Mesantoin® tablet, Sandoz Pharmaceuticals); and Treatment C—60 mg dextromethorphan hydrobromide plus 100 mg racemic mephenytoin. All subjects received each treatment according to a randomized three-way crossover design.

On the evening of the study, subjects were instructed to void their urine at home immediately prior to drug ingestion and to collect an aliquot of this blank urine sample in a polypropylene tube. The subjects were then to ingest the probe drug(s) with a glass of water and to collect all urine over the ensuing 8–12 h period. The next day the urine samples were returned to the Drug Product Evaluation Unit at the University of Kentucky College of Pharmacy. The urine volume was measured, recorded and a 30 ml aliquot of the urine sample was transferred to a polypropylene tube and frozen for later analysis.

The second study was designed to characterize the phenotypes of a large group of subjects by employing coadministration of the probe drugs dextromethorphan and mephenytoin using a simplified study protocol. Five-hundred and forty-nine (549) normal healthy male subjects, aged 18 to 71 years, were admitted to this study. Admission to the study was governed by the exclusion criteria used in the first study. However, in view of the limitations imposed on routine screening procedures, no physical examinations or preclinical laboratory tests were performed on subjects participating in the second study. Drug administration and urine collection proceeded as described for the first study.

Laboratory methods

A 1 ml aliquot of the urine sample from Treatment A and C in the first study was analyzed by thin layer chromatography (t.l.c.) to determine the initial dextromethorphan phenotype (Guttendorf *et al.*, 1988). The relative colour intensities of the dextromethorphan and dextrorphan t.l.c. spots from each sample were assessed visually and a phenotype was assigned based on the following criteria: If the intensity of the dextromethorphan spot was equal to or greater than that of the dextrorphan spot, the subject was assigned a poor metabolizer phenotype. If the metabolite (dextrorphan) spot was of greater intensity than the parent drug spot, the subject was deemed to be an extensive metabolizer (EM). For further verification of the t.l.c. phenotyping procedure and for quantitative analysis of the effect of coadministered mephenytoin, samples from

treatments A and C were also analyzed by a sensitive and specific h.p.l.c. procedure (Guttendorf *et al.*, 1988). Dextromethorphan and dextrorphan were measured in the urine samples and a metabolic ratio was calculated (MR = μmol dextromethorphan/μmol of dextrorphan). Phenotypes were assigned using a MR cutoff of 0.3; a MR in excess of 0.3 being characteristic of PMs while EMs manifest MRs less than 0.3 (Henthorn *et al.*, 1989; Larrey *et al.*, 1987; Schmid *et al.*, 1985). In the second study, the dextromethorphan phenotype was determined based upon interpretation of the t.l.c. results. As a further check, however, the urine of all PMs and a group of 130 randomly selected EMs were also retested by h.p.l.c. and a MR-based phenotype was assigned.

Mephenytoin was analyzed in samples from both studies using a chiral capillary GC method (Wedlund *et al.*, 1984). The urinary mephenytoin S-/R-ratio was used to distinguish the poor (S/R $>$ 0.9) from the extensive (S/R $<$ 0.7) mephenytoin metabolizer.

Statistics

In the first study, the phenotype of each subject with respect to dextromethorphan and mephenytoin was determined in the absence and presence of the other probe drug. A paired Student's *t*-test was performed to assess the level of significance between the dextromethorphan MRs from treatments A and C (MR_A and MR_C, respectively) and the level of significance between mephenytoin S/R ratios from treatments B and C (S/R_B and S/R_C, respectively). To provide homogeneous variances across the range of EM and PM MRs, a log transformation was performed on MRs before applying the test statistic. The resulting *P*-values were adjusted for multiple comparisons using a modified Bonferroni's inequality correction (Simes, 1986). Significance was set at $P \leq 0.05$. The relationships in the MR between treatments A and C and in the urinary S/R mephenytoin ratio between treatments B and C were examined using correlation coefficients.

Results

The mephenytoin oxidation phenotype assignments made for each subject were not altered by the coadministration of dextromethorphan. All 48 subjects were classified as extensive metabolizers after both treatments B and C. The mephenytoin S/R ratios obtained in the absence of dextromethorphan ranged from 0.03 to 0.55 and from 0.02 to 0.58 when mephenytoin was administered with dextromethorphan. No statistical difference was observed between the urinary S/R-mephenytoin ratio following treatments B and C (95% confidence limits = −0.0255, 0.0292) and a statistically significant correlation ($r = 0.679$, $P < 0.0001$) was observed between the mephenytoin S/R urinary ratios in both treatment groups.

Dextromethorphan phenotypes were determined in the original 48 subjects by the qualitative t.l.c. procedure and clear distinctions were made between extensive and poor metabolizers. Forty-six subjects were classified as EMs and two as PMs with this technique and the phenotype assignments were unaffected by co-ingestion of mephenytoin. Subsequent to the t.l.c. analysis, dextromethorphan MRs were also determined by h.p.l.c. For each subject, the MR-based phenotype assignment concurred with the qualitative assignment, both in the presence and absence of mephenytoin. Metabolic ratios ranged from 0.001 to 2.350 in the absence of mephenytoin and from 0.001 to 4.030 in its presence. No statistical difference was noted between the two sets of data (95% confidence limits = −0.0718, 0.0968) and a statistically significant correlation was noted ($r = 0.998$, $P < 0.0001$) between the metabolic ratios obtained in both treatment groups (Figure 1).

Of the 549 volunteers who were recruited into the second study, 519 (94.5%) completed the protocol. The remaining subjects were excluded from further analysis owing to poor compliance as judged by the inability to detect one or more of the probe drugs in the urine sample after several attempts. A histogram of the mephenytoin S/R urinary ratio data for all 567 subjects participating in both studies is presented in Figure 2. A

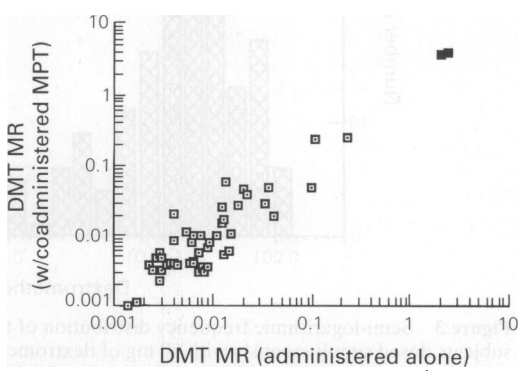

Figure 1 Comparison of the dextromethorphan metabolic ratios (DMT MR) in 48 normal subjects (EMs – □, PMs – ■) phenotyped in the presence and absence of mephenytoin (MPT) (correlation coefficient $r = 0.998$; $P < 0.0001$).

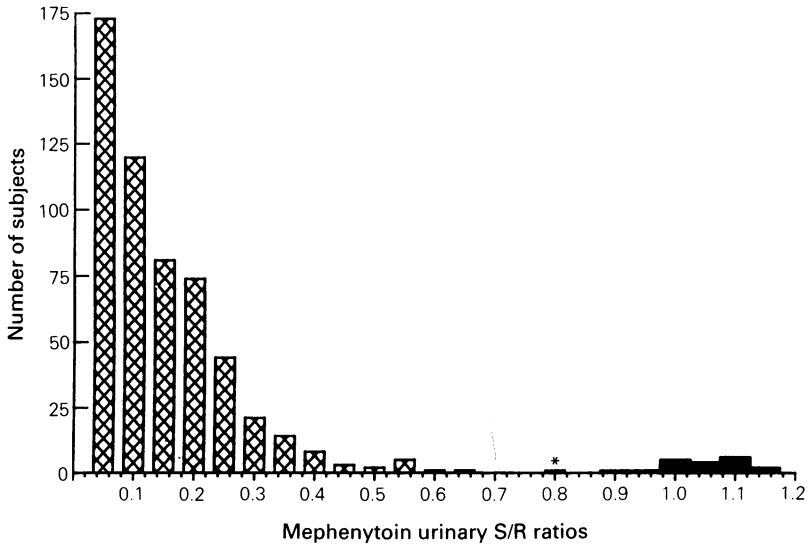

Figure 2 Frequency distribution of the urinary mephenytoin S/R ratios in 567 subjects dosed simultaneously with 100 mg of racemic mephenytoin and 60 mg of dextromethorphan hydrobromide. The data include the ratios of the 48 subjects from study one (S/R_C). ⊠ extensive metabolizers, ■ poor metabolizers, *□ intermediate metabolizer.

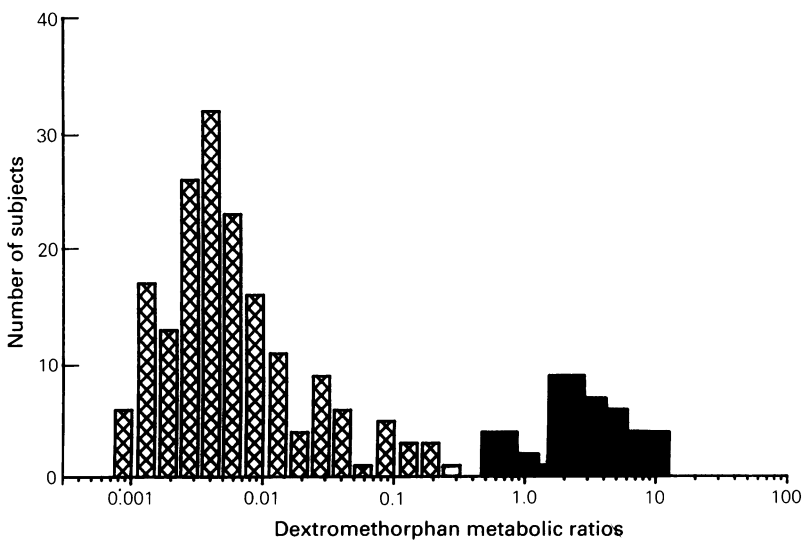

Figure 3 Semi-logarithmic frequency distribution of the dextromethorphan metabolic ratio in 213 subjects dosed simultaneously with 60 mg of dextromethorphan hydrobromide and 100 mg racemic mephenytoin. The data include the ratios for the 48 subjects from study one (MR_C). The subject identified as intermediate was the only individual in the 567 urines analyzed in whom a definite phenotype could not be assigned using the t.l.c. method. ⊠ extensive metabolizers, ■ poor metabolizers, □ intermediate metabolizer.

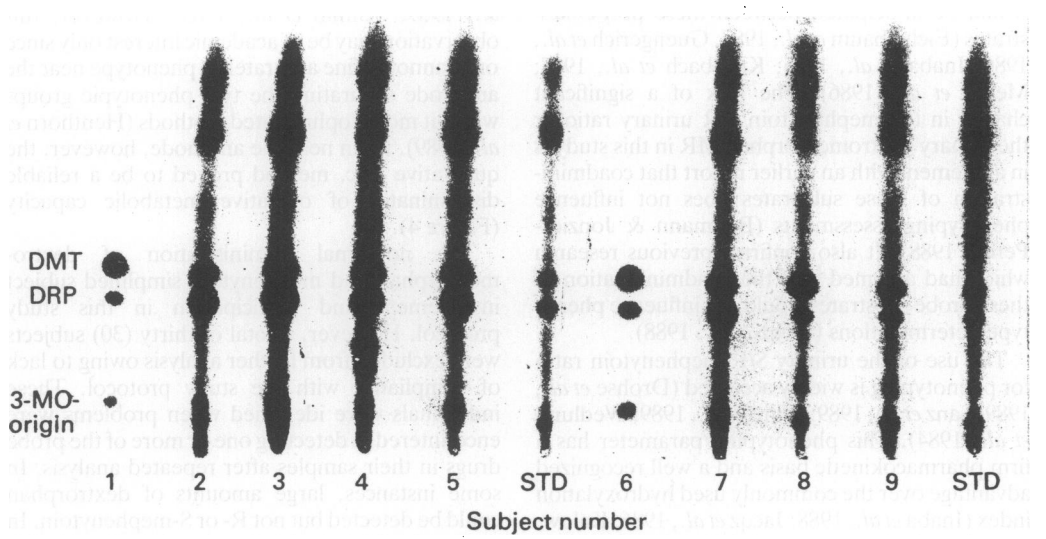

Figure 4 T.l.c. analysis of urine from subjects with dextromethorphan MRs at or near the interphenotypic MR cutoff (0.3). Metabolic ratios increase from left to right. Abbreviations are STD = Standards; DMT = Dextromethorphan; DRP = Dextrorphan; and 3-Mo = (+)-3-morphinanol. Subject 1 MR = 0.128; Subject 2 MR = 0.131; Subject 3 MR = 0.178; Subject 4 MR = 0.179; Subject 5 MR = 0.209; STD = Standards; Subject 6 MR = 0.297; Subject 7 MR = 0.382; Subject 8 MR = 0.431; Subject 9 MR = 0.458.

distinct separation between the subpopulations can be observed. Nineteen (19) of the participants in the second study were identified as poor metabolizers of mephenytoin, representing 3.7% of those who completed the study. One individual (S/R = 0.82) clearly fell between the two phenotypes and was assigned an intermediate metabolizer (IM) status. The remainder were classified as extensive metabolizers.

All of the dextromethorphan phenotyping in the second study was carried out using the t.l.c. technique. Thirty-five (35) of the subjects (6.7%) were classified as poor metabolizers of dextromethorphan, and the remainder as extensive metabolizers. Only one subject out of the 519 tested was classified as indeterminant based upon the t.l.c. results. All poor metabolizers of dextromethorphan and 130 randomly selected extensive metabolizers were also phenotyped by h.p.l.c. to validate further the t.l.c. methodology. In all cases the MR-based phenotype supported the qualitative t.l.c. classification. Because of the subjective nature of the relative colour intensity used for t.l.c. phenotyping, the t.l.c. based method was always conducted prior to the h.p.l.c. analysis. The one subject classified as indeterminant by t.l.c. had a MR of 0.297.

The MRs from all 48 subjects in the first study and 165 of the subjects participating in the second study were combined and are presented graphically in Figure 3. The one individual classified as indeterminant by t.l.c. is labelled intermediate for identification purposes in Figure 3. To demonstrate the capabilities of the t.l.c. screening procedure for phenotype classification near the antimode separating the oxidative polymorphisms, a t.l.c. plate of urine samples from the nine subjects nearest the MR cutoff are shown in Figure 4.

Several subjects participating in this research complained of nervousness, inability to sleep, nausea and headache. Although these side effects were transient and did not represent a major adverse event, they do suggest some concern for safety. These adverse effects along with the potential toxicity of mephenytoin indicate that medical supervision should be available even when carrying out such simple pharmacogenetic screening studies.

Discussion

In theory it should be possible to phenotype subjects accurately and efficiently with respect to the mephenytoin and debrisoquine polymorphisms by coadministration of the probe drugs mephenytoin and dextromethorphan. As the polymorphic oxidation of these drugs is known to be mediated by two distinct cytochrome P-450 enzymes ($P\text{-}450_{MP}$ and $P\text{-}450_{db1}$), no metabolic interaction

would be anticipated between these probe substrates (Eichelbaum *et al.*, 1987; Guengerich *et al.*, 1986; Inaba *et al.*, 1985; Kronbach *et al.*, 1987; Meyer *et al.*, 1986). The lack of a significant change in the mephenytoin S/R urinary ratio or the urinary dextromethorphan MR in this study is in agreement with an earlier report that coadministration of these substrates does not influence phenotyping assessments (Baumann & Jonzier-Perey, 1988). It also confirms previous research which had assumed that the coadministration of these probe substrates would not influence phenotype determinations (Jacqz *et al.*, 1988).

The use of the urinary S/R mephenytoin ratio for phenotyping is widely accepted (Drohse *et al.*, 1989; Sanz *et al.*, 1989; Ward *et al.*, 1989; Wedlund *et al.*, 1984). This phenotyping parameter has a firm pharmacokinetic basis and a well recognized advantage over the commonly used hydroxylation index (Inaba *et al.*, 1988; Jacqz *et al.*, 1986; Kalow, 1986; Wedlund *et al.*, 1984). Furthermore, as the determination of this ratio does not require any quantitative measurements, use of the urinary S/R mephenytoin ratio allows a more rapid evaluation of phenotype. Recently, the 8 h urinary S/R mephenytoin ratio has been criticized as being unable to discriminate oxidative phenotypes as clearly as urine samples collected over later periods of time (Sanz *et al.*, 1989). While the distinction of individual phenotypes is reflected by a more marked difference in the urinary S/R ratio when using later or longer urine collection times, one must weigh this advantage with the practical realities of screening large numbers of subjects in outpatient settings.

The t.l.c. based method for phenotyping patients with respect to dextromethorphan metabolism, while less rigorous than the MR based measurements, is nonetheless just as accurate. In several population studies which have examined the dextromethorphan MR, the average phenotypic difference in the MR between the groups has been 150–350 fold (Henthorn *et al.*, 1989; Larrey *et al.*, 1987; Mortimer *et al.*, 1989; Schmid *et al.*, 1985). Because of this large difference, it may not be necessary to measure actual amounts of dextromethorphan or dextrorphan eliminated in the urine. Rather, a simple visual inspection of the relative amounts of dextromethorphan and dextrorphan eliminated in the urine should suffice for phenotype determinations. All PMs identified by t.l.c. were confirmed by h.p.l.c. The only subject who could not be classified based on the t.l.c. method, out of the 567 urines examined, was found to have an MR of 0.297. This MR is indistinguishable from the antimode of 0.30 reported by others to separate the EM and PM phenotypes of this drug (Henthorn *et al.*, 1989; Schmid *et al.*, 1985). However, this observation may be of academic interest only since one cannot define accurately a phenotype near the antimode separating the two phenotypic groups without more sophisticated methods (Henthorn *et al.*, 1989). Even near the antimode, however, the qualitative t.l.c. method proved to be a reliable discriminator of oxidative metabolic capacity (Figure 4).

The nocturnal administration of dextromethorphan and mephenytoin simplified subject involvement and participation in this study protocol. However, a total of thirty (30) subjects were excluded from further analysis owing to lack of compliance with the study protocol. These individuals were identified when problems were encountered in detecting one or more of the probe drugs in their samples after repeated analysis. In some instances, large amounts of dextrorphan could be detected but not R- or S-mephenytoin. In one instance, a urine sample was returned with a half dissolved pink mephenytoin tablet in it. However, in any large population study where subjects participate on an outpatient basis with minimal preclinical screening, a 100% compliance rate should be considered exceptional. Despite this, with one exception, there is a lack of information about patient compliance with these studies (Inaba *et al.*, 1988).

A total of thirty-five (35) or 6.7% of the subjects in this population were identified as poor metabolizers of dextromethorphan. This is similar to the 5–10% frequency reported for the deficiency in the isozyme responsible for dextromethorphan metabolism reported in other western nations (Brosen *et al.*, 1985; Drohse *et al.*, 1989; Eichelbaum *et al.*, 1979; Evans *et al.*, 1980; Steiner *et al.*, 1988). Most important, all of these individuals were identified initially using the simple t.l.c. based phenotyping method. Most extensive metabolizers of dextromethorphan eliminated so much dextrorphan and so little of the probe drug in the urine that the measurement of an MR for each subject was deemed unnecessary.

A total of nineteen (19) or 3.7% of the subjects in this population were identified as poor metabolizers of mephenytoin. This is also similar to the 2–6% frequency for this phenotype reported by other investigators (Jacqz *et al.*, 1988; Kupfer & Preisig, 1984; Sanz *et al.*, 1989; Wedlund *et al.*, 1984). Furthermore, the 8 h urinary S/R mephenytoin ratio proved adequate for establishing individual phenotypes within this population. Only three subjects in this population expressed a deficiency in the metabolism of both dextromethorphan and mephenytoin. The fact that these individuals represent less

than 1% of the population suggests an independence in the segregation and expression of these polymorphisms.

Beyond simply establishing their frequency in the population, these polymorphisms may have significant clinical implications. Therefore, it is important that rapid, simple, and reliable methods be made available for screening large numbers of subjects and selecting out the individuals of interest in the population. This study demonstrates that it is possible to identify phenotypes for dextromethorphan and mephenytoin metabolism just as effectively with simple qualitative methods as with more expensive and time consuming quantitative procedures.

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