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Assessment of riboflavin as a tracer substance: Comparison of a qualitative to a quantitative method of riboflavin measurement

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ABSTRACT

Background: Noncompliance with medications may have major impacts on outcomes measured in research, potentially distorting the validity of controlled clinical trials. Riboflavin is frequently used in trials as a marker of adherence. It can be combined with study medication and is excreted in urine where it fluoresces under UV light. This study compares qualitative visual inspection of fluorescence to quantitative fluorometric analysis of riboflavin concentration in its ability to detect the presence of riboflavin in urine.
Methods: Twenty-four volunteers received 0 mg, 25 mg, and 50 mg doses of riboflavin under single-blind conditions, with 20 also receiving a 100 mg dose. Five serial urine samples were collected over the following 36 h. Quantitative measurement of riboflavin by fluorometric analysis and qualitative assessment of each sample using visual inspection were performed.
Results: The overall false positive rate for qualitative assessment was 53%. For quantitative assessment, a riboflavin concentration of 900 ng/mL was established to classify positive samples. More than 80% of samples were positive 2–24 h following ingestion of 25 mg and 50 mg, and less than 80% were positive at 36 h. At least 95% of observations for the 100 mg dose were above 900 ng/mL at all timepoints.
Conclusions: Quantitative fluorometric assessment is superior to qualitative visual inspection alone in determining medication adherence. The combination of 25–50 mg of daily riboflavin and a cut-off level of 900 ng/mL allows for the acceptable sensitivity of missing detection of non-compliant participants while preserving a high level of power to detect all cases of medication compliance.

1. Introduction

Non-adherence with medications threatens the validity of controlled clinical trials. Assessment of medication adherence is essential to determining whether the study has provided an adequate evaluation of the study medication (Babiker, 1986; Kranzler et al., 1997). Similarly, poor adherence can compromise treatment and lead to poor clinical outcomes.

A number of variables have been identified as contributing to nonadherence, including medication factors, such as dosing frequency, cost, or side effects; illness factors; and patient factors, such as cognitive impairment and comorbid psychiatric illness (Osterberg and Blaschke, 2005; Swift et al., 2011). Individuals with substance use disorders experience many of these factors, contributing to a particular problem with adherence in this population (Weiss, 2004). Substance use can directly impair judgment, thereby negatively impacting treatment adherence (Magura et al., 2002). Additionally, there can be ambivalence surrounding the use of medications in this population, which can affect adherence (Sowers and Golden, 1999). In a study of 577 individuals receiving disulfiram for alcohol dependence, Fuller et al. (1986) found only 20% were adherent to medication. Teter et al. (2011) studied individuals with bipolar disorder and found that rates of medication adherence

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were lower in individuals with current substance use disorders as compared to those with past history of or no substance disorder.

Indirect methods for assessing adherence, such as patient self-report, doctors' estimates of adherence, pill counts and specialized packaging have been shown to be less reliable than direct methods such as measurement of drug levels (Roth and Caron, 1978; Epstein and Cluss, 1982; Kranzler et al., 1997), and these methods may result in overestimates of compliance (Spilker, 1991). Pill counts may provide a better assessment of adherence than self-reports, but depend on the premise that all missing pills were taken by the patient, when they may have been removed from the packaging and subsequently discarded or shared with another individual. Tests of the validity of pill counts have yielded equivocal results (Roth et al., 1970; Epstein and Cluss, 1982; Pullar et al., 1989; Rudd et al., 1989; Young et al., 1984). Micro-electronic monitoring, such as containers that record the time and frequency of opening, provide continuous assessments of compliance, but can be costly and inaccurate. Patients may remove more than one pill at each opening, or not ingest all pills removed from the container (Cheung et al., 1988; Cramer et al., 1989; Rudd et al., 1981). Measurement of drugs or their metabolites in body fluids allows for a more direct assessment of adherence. This method, however, requires special laboratory facilities and can be expensive. Additionally, the interpretation of results can be confounded by differences in drug metabolism and cumulative effects of long-acting compounds. More importantly, the measure of compliance is applied to the active medication group but not to the placebo group, which limits its usefulness.

Tracer assay, in which a compound is encapsulated with study medication and is later detectable in urine, provides an alternative to directly measuring drug levels. A number of substances have been employed as tracers, including sodium bromide, methylene blue, phenol red, fluorescin, bromide, and phenazopyridine (Roth et al., 1970; Epstein and Masek, 1978; Spriet and Simon, 1985; Kraus et al., 1987), but riboflavin has been the most extensively studied (Hobby and Deuschle, 1959; Deuschle et al., 1960; Veterans Administration Cooperative Study Group, 1967, 1970, 1977a,b; Cluss and Epstein, 1984; Cluss et al., 1984; Dubbert et al., 1985; Del Boca et al., 1996). It is a nontoxic, non-allergenic, water-soluble vitamin (B2), which is inexpensive and readily absorbed through oral administration. Riboflavin is excreted in urine at relatively low concentrations, where it can be detected by fluorescence under UV light using quantitative fluorometric assessment or qualitative visual inspection (Hobby and Deuschle, 1959; Spilker, 1991; Lambert et al., 1985). After administration, riboflavin is rapidly absorbed (t_{max} 1.4–2 h) and is eliminated in urine, with more than 91% of the total excretion of riboflavin taking place during the first 24 h (Zempleni et al., 1996), making it a good candidate for the measurement of compliance using a once per day dosing.

The presence of riboflavin in many foods and vitamin products is a potential disadvantage to its use as a tracer, as dietary riboflavin results in low, background levels of UV fluorescence. However, urine riboflavin levels are 10–20 times higher following the ingestion of supplementary riboflavin at the 20–60 mg dose (Malcolm et al., 1992; Zempleni et al., 1996). With large doses of riboflavin or multiple daily doses, a "spillover" effect has been observed, in that riboflavin can be detected in the urine for longer than 24 h (Babiker et al., 1989; Cluss and Epstein, 1984). This is a significant limitation of the utility of riboflavin in cases of multiple-daily dosing. Riboflavin has been reported to be light-sensitive (Chen et al., 2005), and a number of studies have kept specimens refrigerated and protected from sunlight. Babiker et al. (1989), however, demonstrated no difference in fluorescence among specimens that were refrigerated, those kept at room temperature but protected from light, and specimens kept at room temperature and exposed to light.

Studies regarding the reliability of riboflavin detection have yielded equivocal results. Anton (1996) assessed the accuracy of visual inspection using results from fluorometric analysis as a standard and found an overall error rate of 28%, comprised of a 20% false positive rate and an 8% false negative rate, suggesting that qualitative assessment is less precise than quantitative fluorometry. The VA Cooperative Group (Goldman et al., 1982) found detection of riboflavin fluorescence to be unreliable, dropping this measure from their analysis of medication compliance because of lack of uniform interpretations of fluorescence using visual inspection. Young et al. (1984) also report that riboflavin testing was discontinued in previous VA Cooperative Group studies because of difficulty in detection by the clinician.

Other studies, however, have found that riboflavin fluorescence could be accurately detected even by individuals with minimal training (Cluss and Epstein, 1984; Cluss et al., 1984). Dubbert et al. (1985) used visual inspection to differentiate fluorescence of normal dietary riboflavin from that produced by riboflavin supplementation. The authors found that the accuracy of discrimination increased with riboflavin dose, and reached 100% with measurement 2–8 h after a 50 mg dose, although there was an average 21.2% false positive rate. Babiker et al. (1989) also demonstrated that a single 50 mg dose could be detected via visual inspection within 4–6 h with 95% accuracy in a small study of five participants.

Del Boca et al. (1996) performed six trials in which they assessed the reliability and validity of visual inspection, and determined that raters were most reliable in correctly identifying negative samples and those which contained high concentrations of riboflavin. They used additional measures for validity, employing diaries of pills taken in two of the trials, a capsule count in one trial, and fluorometric analysis in another, and demonstrated accuracy of up to 94%. This study was limited significantly by small sample size, however. Five of the six trials had only two participants, including the one using fluorometric validation of results.

Many research groups use a qualitative assessment of fluorescence, but we were not able to locate studies that have verified the results of qualitative assessment using objective measurements to provide a decisive assessment of the validity of visual inspection. To address this, we compared results of qualitative visual inspection to quantitative fluorometric measurement of riboflavin concentration. We sought to determine whether the qualitative method has acceptable performance to be used as a method of monitoring medication compliance and to determine the dose of riboflavin that is optimal for monitoring medication compliance using the quantitative method of measurement.

2. Methods

2.1. Participants

The sample consisted of 24 healthy individuals recruited through word-of-mouth and posted flyers. Eligible participants were between the ages of 18 and 50, willing to participate and comply with study procedures, and classified as medically healthy volunteers after a general medical history interview. The study was approved by the New York State Psychiatric Institute Institutional Review Board. All study participants provided written informed consent and were compensated for taking part in the study. Individuals who reported current renal or urinary tract dysfunction, pregnancy or lactation, or a history of allergic reaction to riboflavin were excluded from participation.

2.2. Procedures

Riboflavin capsules (0 mg, 25 mg, 50 mg and 100 mg) were administered in single doses at least one week apart. Twenty-four participants received the 0 mg, 25 mg, and 50 mg doses, and 20 of those participants also received the 100 mg dose. In each dosing phase, the riboflavin capsule was administered under single-blind counterbalanced conditions (0 h) and five serial urine samples were collected over the following 36 h (2, 6, 8, 24 and 36 h). At the time of riboflavin administration, participants received 5 tubes for specimen collection, as well as instructions with specific