Therapeutic Drug Monitoring
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Consensus Guidelines for Therapeutic Drug Monitoring of Rapamycin: Report of the Consensus Panel

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Federal regulatory agencies, such as the Food and Drug Administration in the United States and the Health Protection Branch in Canada, are placing increasing emphasis on the therapeutic monitoring of drugs with narrow therapeutic windows. It is the intention that early establishment of standardized protocols that encompass analytic, pharmacokinetic, pharmacodynamic, and toxicokinetic aspects of therapeutic drug monitoring will facilitate the approval process of the drug, as well as provide better patient monitoring once the drug is released (1).

Rapamycin (RAPA) (sirolimus), a macrolide antibiotic, has been shown to possess potent immunosuppressive activity both in vitro and in vivo (2–4), while enhancing allograft survival in a number of animal models (3,5–11). Preliminary work with RAPA in animals and clinical trials in humans suggest a relation between trough drug concentrations and immunosuppressive efficacy and toxicity (9,11). This suggests that monitoring of the concentrations of RAPA can be used to optimize the dosing regimen of the drug. It is therefore important that guidelines for monitoring of this drug be established.

It is the hope of the consensus panel that the following recommendations and the rationale for them will provide practical information that will facilitate the establishment of standardized approaches for monitoring of RAPA. As the guidelines are primarily based on animal data, they may not be all applicable to man and may require revision when human data become available.

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RECOMMENDATIONS AND GUIDELINES

1. Until one matrix is shown to be clinically superior to another, analytic reasons should be the basis for selection of sample matrix. Whole blood collected in tubes with EDTA as anticoagulant is the recommended matrix. It is important to insure artifact-free collection of the blood. Therefore, when an i.v. formulation becomes available for patients receiving the drug through an indwelling catheter, venipuncture from the contralateral limb is necessary.
2. The recommended time for collection of the sample in which to measure the drug is within 1 h before the next scheduled dose of RAPA (trough concentrations). If multiple daily dosing is required, collections should be made at the same time during the day to ensure intrapatient consistency.
3. A validated and specific method for measuring RAPA is recommended.
4. Performance criteria for RAPA measurements should meet the criteria for acceptable clinical laboratory practice.
5. No data currently support the routine clinical

monitoring of RAPA metabolites. Should one or more of the metabolites subsequently be shown to be clinically significant, it is recommended that they be measured by a specific method.

6. It is recommended that the time of food intake in relation to RAPA dosing should be consistent.
7. In most clinical situations during the immediate posttransplant period, the maximum frequency of monitoring is once every 24 h.
8. It is recommended that laboratories provide same-day turnaround time for acute transplant patients.
9. Storage of RAPA at room temperature is not recommended. Specimens for RAPA analysis should be shielded from light and stored refrigerated at 2–8°C if analysis will occur within 1 week. For longer storage time, specimens should be stored frozen at −70°C.
10. Therapeutic range(s) for RAPA should be established based on relevant clinical criteria.
11. External quality assurance programs for RAPA should be established to ensure interlaboratory consistency in analysis.

Sample Matrix

In human whole blood, ~95% of RAPA is sequestered within erythrocytes (12). The concentrations of RAPA in whole blood are approximately an order of magnitude greater than those found in the corresponding plasma fractions. The distribution does not exhibit any temperature or concentration dependency up to a concentration of 100 μg/L (12). When immunosuppressive doses of RAPA are administered, the trough whole blood concentrations are within the analytic range of high-performance liquid chromatography (HPLC), whereas those found in plasma generally require a more sensitive HPLC/mass spectroscopy (MS) method (9,11,13). No immunoassays for measurement of the drug have been developed to date. Therefore, to facilitate the implementation of routine monitoring of RAPA using HPLC equipment, the use of whole blood rather than plasma is recommended. The blood samples should be collected in tubes containing EDTA as anticoagulant because this minimizes problems encountered with clotting.

There are no published clinical studies to date in which RAPA concentrations measured in whole blood or plasma from individual patients were compared to determine which of these best correlates with immunosuppression and drug-related side effects. It is only when such studies are performed that a definitive conclusion can be reached on the most appropriate matrix.

Time of Sample Collection

Measurement of the drug at trough concentration, just before administration of the next dose, is the most common practice for the majority of immunosuppressive drugs for which therapeutic monitoring is indicated (14,15). The selection of the trough time is based on its reproducibility; it occurs after absorption and distribution of the drug is complete, which places it in a more reproducible part of the drug concentration versus time curve. Some studies, with other immunosuppressive drugs such as cyclosporine, suggest that area-under-the-curve (AUC) monitoring correlates better with clinical response (16,17). Studies are required to determine whether a similar situation exists for RAPA. However, until such studies are performed, it is recommended that the time for collection of the specimens in which RAPA is to be measured be just before administration of the next dose (trough concentrations). Drug concentrations may also vary with the time of day. Therefore, for multiple daily dosing, collection should be made at the same time during the day to ensure intrapatient consistency.

Method of Measurement

The recommended method for measurement of RAPA should measure the pharmacologically active species of the drug. To date, only HPLC/MS and HPLC methods have been established for measurement of the drug (18–20). The former method has a sensitivity in the ng/L range, while the latter have sensitivities of ~2.0 μg/L (18–20). Receptor assays (21) and immunoassays for the drug are presently under development. When immunosuppressive doses of RAPA are administered to animals as monotherapy, the whole blood trough concentrations are within the μg/L range, which is well within the sensitivity range of HPLC procedures (9,11,13). It is therefore indicated that measurement of RAPA by HPLC will suffice for the majority of clinical indications. Because there is no present evidence to suggest that these methods are subject to interference due to RAPA metabolites, they can therefore be considered specific for parent drug. However,

this cannot be confirmed until all metabolites have been isolated and identified. The absolute analytic recovery of an HPLC method used to measure RAPA should be >60% with the analytic recovery relative to an internal standard in the range of 90–110%. For routine monitoring of RAPA in clinical laboratories, the Committee recommends that immunoassays and receptor assays that are amenable to automation be established.

Performance Characteristics

The between-day coefficient of variation for methods should be ≤10% at a concentration of 5 μg/L and ≤5% at a concentration of 40 μg/L. This is based on estimated trough concentrations that span the therapeutic range of the drug, and variations which may result in an altered therapeutic decision. The dynamic range of the assay should be 1–75 μg/L, which is based on the concentration seen in animals when immunosuppressive doses are administered. Results for new procedures should be compared with a validated one for at least 30 specimens of blood per transplant type or 100 total. Standard statistical evaluation of the linear-regression data should be used, including, but not limited to, the slope and standard deviation of the slope, the *y*-intercept and standard deviation of the intercept, the standard deviation of the estimate ($S_{y/x}$, standard error of the regression, standard error of the residuals). An appropriate statistical test (*t* test) should be performed on the slope and the intercept to determine that they are not statistically different from 1.0 or 0.0, respectively, at the 95% confidence interval. It is recommended that the slope differ by ≤10% from the line of identity (slope 1.0: acceptable 0.9–1.1), the intercept, and the $S_{y/x}$ by no more than 5 μg/L. Validation for accuracy should be based on the use of an appropriate standard. Precautions should be taken when drying and desiccating the reference material before standards are prepared. The relative analytic recovery of RAPA should be 95–100%.

RAPA Metabolites

Studies investigating the metabolism of RAPA have been limited (22,23). Several metabolites, including two that have been structurally characterized, a demethylated and a hydroxylated one, have been isolated to date. Of the metabolites identified, both retain immunosuppressive activity of <10% of the parent drug (22,23). It is presently not known whether the metabolites possess any toxic activities. Further work is required to elucidate the structures of other metabolites, their steady-state concentrations in transplant patients, and their biologic properties prior to a conclusion being reached on their clinical significance. The effect of other drugs on the spectrum and concentration of parent drug and metabolites also needs to be investigated. Such work is also critical to development and assessment of performance characteristics of methods for measurement of the drug. The Committee recommends that the scientific community place a high priority on further investigations in this area.

Effect of Food on RAPA Absorption

Studies investigating the effect of food on RAPA absorption have not been reported. However, based on the hydrophobic nature of the drug, as well as its limited bioavailability as observed in animal studies (18) in the present vehicle, the potential exists for a food effect. However, until studies confirm or reject this hypothesis, it is recommended that in order to reduce intrapatient variability, dosing of RAPA should be consistently timed with respect to food intake.

Monitoring Frequency

RAPA has been shown to have a relatively long half-life (~60 h) in transplant patients (18). Based on the long half-life and the lack of detailed evidence for acute toxicity of the drug, it is recommended that the maximal frequency of monitoring be once every 24 h with no provision for stat turnaround time. However, it is imperative that a clinically relevant turnaround time be provided. For most clinical situations, this will necessitate results being reported within 24 h subsequent to collection. Subsequent to initiation of dosing or a change in dose, it is generally accepted that drug levels should not be monitored until drug concentrations have reached steady state. Early data obtained from transplant patients receiving oral doses of RAPA suggest that drug level monitoring may occur within approximately 5–7 days after initiating or changing the dose.

Sample Stability

RAPA in EDTA-anticoagulated blood has been shown to be stable for up to 24 h at room temper-

ature and up to 7 days at 2–8°C and 3 months at −20°C (19; R Yatscoff, unpublished observations). For prolonged storage of specimens, it is recommended that the specimens be stored at −70°C.

Therapeutic Range

Studies in animals have shown that RAPA concentrations <5–10 μg/L result in an increased incidence of allograft rejection whereas those >60 μg/L result in an increased incidence of drug-related side effects (9,11,24,25). However, prolonged graft survival has been reported at trough concentrations as low as 0.5 μg/L (24). Confirmation of these ranges is required in humans. However, it is recommended that in such studies a standardized criterion for rejection and toxicity be used along with a validated procedure for measurement of the drug. RAPA has also been shown to be synergistic with cyclosporin A (3,4). The type of medication(s) that are coadministered with RAPA must be taken into consideration in the establishment of therapeutic ranges.

Quality Assurance

The experience with other immunosuppressive drugs indicates that to ensure ongoing center-to-center consistency, laboratories must participate in a quality assurance program (26–28). It is recommended that a similar program be instituted for RAPA. Specimens included in such a program should be drug-free whole blood spiked with RAPA, as well as pooled specimens from patients receiving the drug. However, the limited stability of RAPA in whole blood at 2–8°C may pose a problem in the preparation of suitable control materials.

CONCLUSION

The recommendations and their rationale discussed above should provide practical information resulting in consistency in therapeutic drug monitoring of RAPA.

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