PERSPECTIVE

A Case to Support the Continued Use of Rifampin in Clinical Drug–Drug Interaction Studies

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Reports of 1-methyl-4-nitrosopiperazine (MNP) in rifampin products hampered the conduct of clinical DDI studies with rifampin. Since DDI studies with rifampin are typically conducted for 6 to 14 days, the assessment of acceptable intake (AI) limit of MNP using CPCA and less-than-lifetime adjustment yielded higher than the current AI limits by FDA. Therefore, in this perspective, we make a case for continued use of rifampin in clinical DDI studies based on the totality of data.

THE ROLE OF RIFAMPIN IN CLINICAL DRUG–DRUG INTERACTION STUDIES

Drug–drug interactions (DDIs) can have a profound effect on clinical outcomes by increasing the likelihood of treatment-driven adverse events or reducing efficacy. Generally, DDIs are caused by perpetrator drugs that inhibit and/or induce metabolizing enzymes and transporters that are responsible for the disposition of the victim drug. Hence, the conduct of clinical DDI studies is critical in drug development to assess the magnitude of DDIs.

The antibiotic rifampin (**Figure 1a**) is approved for the treatment of tuberculosis (TB), leprosy, and Legionnaires' disease. Rifampin is also used as a prototypical strong inducer of drug-metabolizing enzymes/transporters, and as an inhibitor of OATP1B1/1B3 transporters. As per ICHM12 DDI draft guidance, rifampin is classified as a strong inducer of CYP3A and CYP2C19 and a moderate inducer of CYP1A2, CYP2B6, CYP2C8, and CYP2C9 enzymes. In addition, rifampin is also listed as a clinical inducer of P-gp, UGTs (UGT1A1, UGT1A4, UGT1A9, UGT2B7, and UGT2B15) and as an inhibitor of OATP1B1/1B3 (single dose).[1] It is established that rifampin exhibits its induction effects primarily by activating the nuclear receptor, pregnane X receptor (PXR), which regulates genes coding for multiple drug-metabolizing enzymes (e.g., CYPs and UGTs) and transporters (e.g., P-gp).[2] For many years, rifampin has been used in clinical DDI studies, resulting in a wealth of data that is available for indexing to other moderate and weak inducers.

NITROSAMINE IMPURITY AND RIFAMPIN

Due to recent reports of nitrosamines observed in pharmaceutical products, the FDA published a notice indicating they became aware of 1-methyl-4 nitrosopiperazine (MNP; **Figure 1b**) in rifampin products. The FDA set a limit of 0.16 ppm for MNP in rifampin products. The FDA did not object to temporarily higher levels (up to 5 ppm) of exposure to ensure patients continue to have access to this life-saving medication for the treatment of tuberculosis (FDA Updates and Press Announcements on Nitrosamines in Rifampin and Rifapentine|FDA). In January 2021, the FDA published analysis results of MNP levels in 11 different manufactured lots of rifampin products (Laboratory analysis of rifampin/rifapentine products|FDA). The results indicated MNP levels were above 0.16 ppm, but below 5 ppm in all lots tested (**Figure 1c**). Contrary to approving the continued use of rifampin products in patients, health authorities advocated that rifampin products with higher than the acceptable limit (0.16 ppm) of MNP levels should not be used for clinical DDI studies typically conducted in healthy volunteers. As a result, sponsors have been seeking alternatives to rifampin to use in clinical DDI studies.

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Received December 18, 2023; accepted March 6, 2024. doi:10.1002/cpt.3256

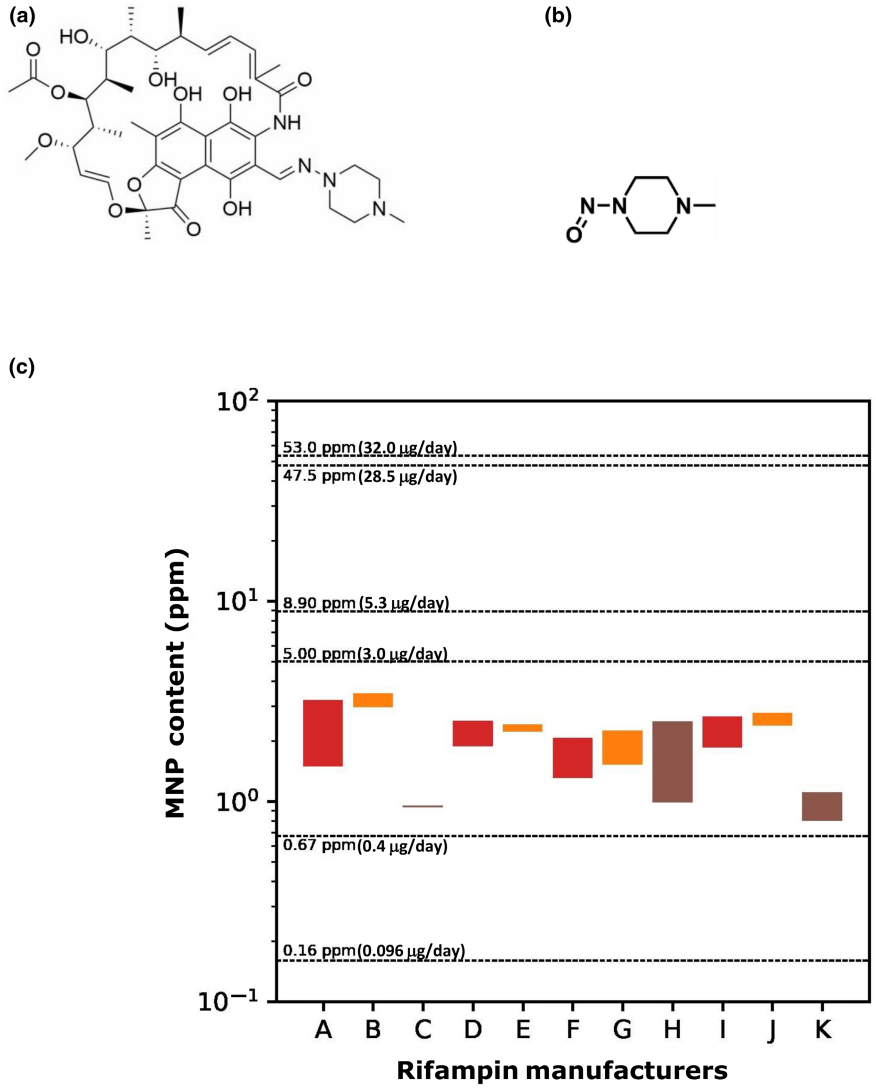

Figure 1 Structures of (**a**) rifampin (**b**) MNP; (**c**) Comparison of MNP level in rifampin lots manufactured by various companies (A & B: Akorn; C: Fresenius Kabi; D&E: Lannett; F&G: Lupin Pharmaceuticals Inc; H: Mylan; I&J: Sandoz/Epic; K: Sanofi Pharmaceuticals), as reported by the US FDA. Color indicates dose strength (orange: 150 mg; red: 300 mg; brown: 600 mg) and vertical bars represent range of MNP levels in different tested lots. The dotted line represents AI limits based on various categories as shown in **Table S1**. AI limits (µg/day) were calculated based on rifampin daily dose of 600 mg.

DERIVATION OF AN ACCEPTABLE INTAKE (AI) FOR MNP

The case of rifampin demonstrates the impact that the risk assessment approach used to derive a *N*-nitrosamine AI has on decisions regarding the availability of medicines. With a rifampin dose of 600 mg/day, a MNP limit of 0.16 ppm translates to an AI of up to 96 ng/day, whereas the temporary AI of 5 ppm for patients would result in potential exposure up to 3 µg/day (**Figure 1c**; **Table S1**). To date, the direct scientific basis for these regulatory limits has not been published; however, it can be inferred that the AI of MNP is derived from the known carcinogenicity data of the N-nitrosodimethylamine (NDMA) with an AI of 96 ng/day or 0.16 ppm. The ICH M7 guidance recommends that compound-specific AI be determined for mutagenic carcinogens following a review of the available carcinogenicity data. When multiple carcinogenicity studies exist, the AI should be derived from the most robust study and from the most sensitive site of tumor induction in that study (https://database.ich.org/sites/default/files/ICH_M7%28R2%29_Guideline_Step4_2023_0216_0.pdf). MNP does have carcinogenicity data, but not sufficient to generate an AI.[3] Several carcinogenicity studies were previously identified, but deficiencies including only dosing a single dose group, lack of control data, limited numbers of animals tested per dose group, exposure less than 50% of a lifetime, and high (100%) tumor incidence led to uncertainty in accurately assessing the AI. Carcinogenicity data for piperazine-containing nitrosamines have been reviewed and demonstrate that this class of compounds are not potent carcinogens, with corresponding AI that would be higher than the ICH M7(R2) AI for mutagenic impurities (1.5 µg/day). An alternative method of AI determination is read across to a closely related surrogate; the closest in this case being *N*-nitrosopiperazine (CAS RN 5632-47-3), for which the corresponding AI would be 28.5 µg/day (**Figure 1c**; **Table S1**).[3]

Recently, health authorities have published a structure-based framework for deriving AI for *N*-nitrosamines, which is referred to as the carcinogenic potency categorization approach (CPCA).[4] This approach considers the number of α-carbon hydrogens (adjacent to the *N*-nitroso group) and the presence of activating and deactivating features to derive a potency score (1–5) that is associated with a specific AI. Using the CPCA framework, MNP is assigned to Category 3 with an AI of 400 ng/day (**Figure 1c**; **Table S1**). The European Medicines Authority (EMA) and Health Canada (HC) have also recently published an AI of 400 ng/day for MNP based on CPCA. To date, the FDA has not published any updates to the recommended limits of MNP in rifampin. Based on the available carcinogenicity data for piperazine nitrosamines (summarized above), an AI of 400 ng/day based on the CPCA framework can be considered conservative.[4]

CONSIDERATION OF DURATION OF TREATMENT

Duration of exposure is another important consideration for the risk assessment of mutagenic carcinogens within the framework of the ICH M7(R2) guidance. The less-than-lifetime framework (also referred to as the "staged-threshold of toxicological

concern (TTC))"[5] was adopted for the control of mutagenic impurities during clinical development and for marketed products not intended for chronic treatment. The chronic treatment TTC (i.e., 1.5 µg/day) is associated with a theoretical excess cancer risk not exceeding 1 in 100,000. Understanding that cancer risk is impacted by both dose and duration,[6] higher TTC values are recommended for shorter administration time. To protect from uncertainties of extrapolating to a very short duration of treatment, the higher TTC values were set conservatively. For example, the TTC for ≤ 1 month (120 µg/day) is 80-fold higher than the chronic (i.e., 70 year) TTC (1.5 µg/day), whereas the difference in duration of treatment is 840-fold. According to ICH M7, an impurity with a compound-specific AI can use the less-than-lifetime (LTL) concept in the same proportion as the TTC. In this case, a clinical trial ≤ 1 month would result in an LTL AI 80 times higher than the chronic AI.

For induction DDI studies, 600 mg/day of rifampin for a minimum of 5 days is required to achieve the maximum induction effect and the total duration of rifampin administration depends on the half-life of the victim drug, although most rifampin DDI studies conducted are between 6 and 14 days.[7] Given the short duration of clinical use, the LTL AI would be 32 µg/day (400 ng/day × 80; **Figure 1c**; **Table S1**).

This stepwise evaluation for acceptable limits of MNP in rifampin intended for use in DDI studies illustrates the impact of a data-driven and transparent risk assessment approach. The current FDA limits have resulted in halting the use of rifampin in DDI studies. This was carried out without considering its value as a well-established inducer or the availability of appropriate alternatives that can replace rifampin as an inducer and their associated safety risks. In contrast, following the application of the CPCA framework and LTL adjustment, the resultant AI has no consequence on the clinical use of rifampin as MNP would be considered reliably controlled.

PERSPECTIVES ON CONTINUED USE OF RIFAMPIN IN CLINICAL DDI STUDIES

Due to the health authority's concern about higher than acceptable limits of MNP in rifampin products, pharmaceutical industry sponsors have sought alternatives for clinical DDI studies. Recently, carbamazepine and phenytoin were identified as fit-for-purpose alternatives to rifampin for conducting CYP3A induction DDI studies.[8,9] However, there are limitations and risks associated with these two alternatives. Rifampin is a widely recognized perpetrator for conducting induction-mediated clinical DDI studies and its impact on the metabolism of CYP and non-CYP substrates and transporters is well-established based on decades of clinical and nonclinical research. The potency of induction, pleiotropic effects, and safety at clinically relevant doses make rifampin a unique inducer for clinical DDI studies. For studying induction-mediated DDI effects of enzymes and transporters that are expressed both in the intestine and liver (e.g., CYP3A, P-gp) and dual substrates of intestinal metabolic enzymes and transporters, it is important to consider an agent that provides maximum induction/DDI effect. Except for rifampin, no other inducer available is capable of characterizing the induction effects broadly. Both proposed alternatives, carbamazepine and phenytoin are CAR activators, the induction effect of these agents on the enzymes (e.g., CYP2C8 and UGTs) and transporters (e.g., P-gp) that are regulated primarily by PXR is considered less than rifampin. Although both carbamazepine and phenytoin are listed as strong CYP3A inducers based on the limited available clinical data, both agents are relatively less effective inducers than rifampin.[8] Therefore, the calibration of induction risk by using comparison to a strong inducer like rifampin needs to be adapted to less effective inducers like carbamazepine and phenytoin. Both carbamazepine and phenytoin are narrow therapeutic index drugs with warnings for severe adverse events including life-threatening dermatological reactions (i.e., Steven-Johnson syndrome or toxic epidermal necrolysis; Tegretol (carbamazepine USP) (fda.gov); DILANTIN (phenytoin sodium) Label (fda.gov)). The treatment of both these drugs are also associated with drug reaction with eosinophilia and systemic symptoms (DRESS) and multiorgan hypersensitivity. In addition, carbamazepine requires dose titration for 3–6 days to manage tolerability and genotyping to exclude participants with HLA*1502 allele. Phenytoin has saturable pharmacokinetics and therefore small changes in dose can result in disproportionately larger changes in exposure. Phenytoin also possesses a highly variable induction response with sensitive CYP3A substrates.[8] Rifampin has a more favorable safety profile as a single agent at 600 mg daily dose level. In addition, physiologically based pharmacokinetic (PBPK) models are more progressed for rifampin compared with alternatives to simulate DDI risk of newer drugs as victim.[10] Based on the totality of the data (**Figure 2**) including the toxicological risk assessment of MNP (based on the CPCA framework and ICH M7 principles), the short duration of clinical DDI studies with rifampin, stronger induction potential and considering shortcomings of alternative inducers including severe adverse events and tolerability, we recommend that that rifampin should remain the drug of choice for clinical DDI studies.

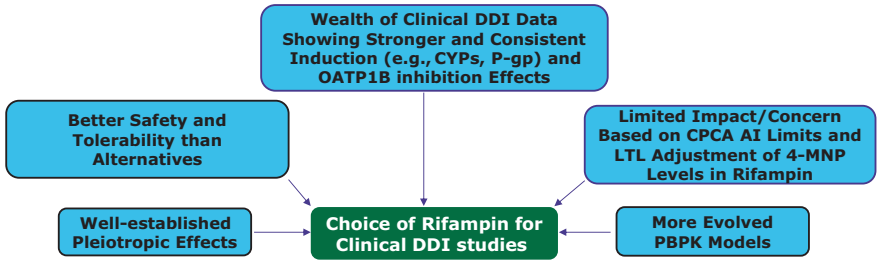

Figure 2 Enablers to support continued use of rifampin in clinical DDI studies with healthy volunteers.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

FUNDING

No funding was received for this work.

CONFLICTS OF INTEREST

JPB is an employee of Gilead Sciences, Inc. SLR and KLD are employees of Pfizer. JB is an employee of EMD Serono. JPB, SLR, KLD, and JB holds stock in their respective companies. DJP is an employee of Lhasa Limited, a nonprofit organization. RT is an employee of the University of Washington and declares no conflict of interest.

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