SUPPLEMENTARY INFORMATION FOR

A SYSTEMATIC REVIEW AND COMBINED ANALYSIS OF THERAPEUTIC DRUG MONITORING STUDIES FOR LONG-ACTING RISPERIDONE

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Supplementary Text with 2 sections subdivided into subsections

11 Supplementary References (These references are in addition to the references included in the text.)

Supplementary Box S1

Supplementary Figure S1

S1. SUPPLEMENTARY METHODOLOGICAL ASPECTS

S1.1. Supplementary Information on the Article Search

The identified risperidone articles came from four sources: 1) 15 articles were obtained on January 25, 2017, in a PubMed search using the terms ("risperidone/blood"[Mesh] AND (long-acting OR Consta; 2) 15 articles are from the authors' files; 3) 14 articles were obtained on January 26, 2017, in an Embase search using the terms ("risperidone AND 'long acting' AND 'drug concentration'" and activating the limit for risperidone), which led to only 3 new articles; and 4) 1 article is from the list of references in another article. After duplications were eliminated (Supplementary Figure S1), 34 articles were reviewed by the first and last authors after developing a list of 5 exclusion criteria which were successively applied (1 = not risperidone-related, 2 = reviews or comments, 3 = mathematical modelling, 4 = animal studies and 5 = posters) (Supplementary Excel File). The successive review of each abstract and then each article led respectively to the exclusion of 4 and 9 articles, meaning that there were 21 articles left for our systematic review of therapeutic drug monitoring (TDM) for risperidone long-acting (LAI) formulations [40, 41, 46-64].

The first and last authors then realized that they were dealing with two formulations: microspheres (20 articles) and RBP-7000 (1 article). Therefore, they decided to include the 20 studies on the microsphere formulation in Table 1. As they had only found one article using the RBP-7000 formulation, they conducted another PubMed search on February 15, 2017, using the search term "RBP-7000" in the title, which led to 5 additional articles. All 6 of these articles were considered in the writing of a separate Results section titled RBP-7000 Formulation [41, 65-69]. On February 3, 2017, the first author found in a Google search an article about another risperidone LAI formulation called in Situ Microparticle (ISM). A further search yielded two conference abstracts referring to this risperidone LAI formulation; this material is presented in a distinct Results section entitled Risperidone ISM Formulation [43-45].

S1.2. Supplementary Information on the Pharmacokinetic Quality of Studies of the Microsphere Formulation

S1.2.1. Steady State

Most of the studies embraced the same definition of steady state: after ≥ 6 weeks from the first injection or at the time of the fourth injection, assuming they are given every 2 weeks and taking into account that oral risperidone supplementation is needed in the first 3-4 weeks [40, 46-50, 53, 57, 61]. As Table 1 indicates, only 3 of 20 studies [54, 56, 58] did not consider the need for steady state before collecting TDM samples, while 1 does not provide information on that [63].

S1.2.2. Confounders

Based on our review of the literature on oral risperidone, we decided that there were 4 major confounders. In order of importance they are: 1) intake of oral risperidone at the time of TDM, 2) CYP2D6 PM status, 3) intake of CYP inhibitors/inducers, and 4) renal or hepatic impairment (Table 1). Regarding oral intake, 12 of 20 studies were not contaminated by oral risperidone (Table 1). Regarding CYP2D6 PMs, 4 of 20 provided CYP2D6 genotyping. Regarding CYP inhibitors/inducers, 5 studies excluded them, 6 allowed them (details provided in the footnotes of Table 4), and 9 provided no description. A patient taking potent CYP2D6 inhibitors may be "phenoconverted" to CYP2D6 PM status [S1]. Regarding renal or hepatic impairment, 2 studies excluded them from included patients and 18 provided no description (See Table 1).

S1.3. Supplementary Information on the Quality of the Analytical Laboratory Used in Microsphere Studies

There are two quality issues regarding the laboratory: method and method validation, as described in Table 1 and the Supplementary Information.

S1.3.1. Laboratory Methods

Table 1 describes: 1) 5 studies that did not provide information on the method used to quantify risperidone and 9-hydroxyrisperidone (9-OH-R), 2) 3 studies that used radioimmunoassay methods (RIA), and 3) 12 studies that used various chromatography methods.

S1.3.2. Validation of Laboratory Methods

Table 1 describes that, of 12 studies with described methods: 1) 10 studies included information on deviation and accuracy rates, and 2) 2 studies referred to external validation in additional published articles.

S1.4. Supplementary Information on the Quality of Genotyping in Microsphere Studies

Four genes were genotyped in at least one study: cytochrome P450 2D6 (CYP2D6), cytochrome P450 3A4 (CYP3A4), ATP-binding cassette sub-family B member 1 (ABCB1), and the nuclear receptor 1 (NR1).

S1.4.1. CYP2D6 Genotyping

CYP2D6 is well established and used in clinical practice with many commercial laboratories using different genotyping methods in Europe and the USA [S2]. Besides the already described CYP2D6 UMs and PMs, genotyping laboratories also classify patients as extensive metabolizers (EMs), who are the normal metabolizers, and as intermediate metabolizers (IMs), who have lower than normal CYP2D6 activity. There is not complete agreement among the various genotyping labs about the distinction between EMs and IMs, but all agree on the presence of PMs, who are characterized by having 2 null alleles [S2].

There were 4 studies using CYP2D6 genotyping: 1) 2 studies with no description of their methodology [50, 63], and 2) 2 studies [55, 58] using polymerase chain reaction (PCR) [73]. Footnotes 28 and 29 in Table 1 provide details about the CYP2D6 alleles tested. Both studies appear to do a good job of identifying CYP2D6 PMs and 1 [55] appears to do an excellent job in identifying CYP2D6 UMs by

additionally cross-validating their method by applying copy number analysis [S3], yielding 100% agreement between results of the two methods.

1.4.2. CYP3A Genotyping

There are two CYP3A genes: CYP3A4 and CYP3A5, but their genotyping is not well established and is not ready for clinical practice since there is little agreement concerning the genotype-phenotype relationships of the CYP3A4 and CYP3A5 genes [S4]. The CYP3A alleles tested in one of the studies [60] are described in footnote 29 of Table 1.

The only study on CYP3A polymorphisms found no effect on dose-adjusted plasma levels of R, 9-OH-R, total, and R/9-OH-R ratios [60].

1.4.3. ABCB1 Genotyping

ABCB1 is not well established and is not ready for clinical practice, since many genotyping studies tested single-nucleotide polymorphisms (SNPs) which are not associated with changes in P-gp function; many of the significant findings were not replicated by later studies [S4]. The ABCB1 SNPs tested in one study [60] are described in footnote 29 of Table 1.

The only study on the polymorphisms of the ABCB1 gene found no effect on dose-adjusted plasma levels of risperidone, 9-OH-R, total, and risperidone/9-hydroxyrisperidone (R/9-OH-R) ratios [60].

1.4.4. NR1 Genotyping

Inducers increase the expression of P-gp and the CYPs that can be induced, such as CYP3A4, which is induced by activating nuclear receptors. Carbamazepine appears to have inductive effects by activating two major nuclear receptors: the constitutive androstane receptor (CAR) and the pregnane X receptor [26]. Studies exploring the effects of SNPs at these nuclear receptors on drug metabolism are in very early stages and not ready for clinical use [26]. The NR1 SNPs tested in one study [60] are described in footnote 29 of Table 1. A study described, regarding the NR1 allele (rs7643645A>G), that the carriers

of the GG genotype showed 2.5-fold lower total C/D ratios than carriers of the AA genotype [60]. This finding was replicated in a second cohort of this study consisting of 16 patients.

S2. SUPPLEMENTARY INFORMATION ON LIMITATIONS

Before considering the conclusions, the reader needs to be aware of the limitations on: 1) methodology used for the TDM review, 2) the TDM data, 3) the pharmacokinetic assumptions used to review the TDM data, and 4) the units used to calculate concentrations.

S2.1. Limitations on the Methodology Used for the TDM Review

This section on methodology used for the TDM review has 3 subsections on: 1) terminology, 2) blindness, and 3) peculiarities of the systematic reviews of TDM studies.

S2.1.1. Terminology

This review tries to use a systematic approach to calculate mean values for some pharmacokinetic ratios, which is close to the concept of meta-analysis. The word “meta-analysis” is not used since the authors think it is better reserved for reviews providing a mean outcome of randomized controlled trials (RCTs) which tend to control for patient heterogeneity. Shapiro [74] proposed the term “combined analysis” to describe systematic reviews of naturalistic studies providing a mean outcome, which may be much more reflective of heterogeneous samples. The mean R/9-OH-R and C/D ratios from TDM studies provided in this review fit better with the concept of combined analysis than with meta-analysis.

S2.1.2. Blinding

Epidemiologists have developed guidelines for assessing different types of systematic reviews and meta-analyses [S6]. Unfortunately, this type of approach designed by epidemiologists is not suitable for addressing the quality of TDM studies. One of the major concerns of epidemiologists is that researcher bias may influence the outcome of the data; therefore, blinding is given high priority as a sign of quality in RCT meta-analysis [S7]. The TDM ratios are not likely to be influenced by biases from the original

investigators who published the results due to: 1) blinding in the TDM analyses, and 2) blinding toward future use of the TDM data in our calculations of TDM ratios.

The first level of blinding in TDM is introduced by the fact that clinicians send blood samples to a laboratory assuming that the patient is taking the drug; then the TDM laboratory provides the results of the drug concentrations based on a complex set of laboratory procedures that are relatively independent of human judgment. The laboratory staff can never be 100% sure that the drug will be identified in the blood sample since absolute non-compliance (not taking the drug at all) is not rare in psychiatric patients [S8-S10]. The second level of blinding refers to the ratios used in Tables 2 and 3 which were calculated by the authors of this systematic review using published data. We calculated R/9-OH-R ratios in 4/10 of the articles (and rechecked the rest) and in 11/12 of the total C/D ratios (and rechecked the other one), since the original articles did not describe these ratios. Therefore, it is very likely that many of the articles' authors did not know that TDM ratios can be calculated with their data, but now they will know the ratios that are used in this systematic review.

S2.1.3. Quality of the Systematic Reviews of TDM Studies

After many years of reviewing TDM studies, we think that in an article like this which tries to provide a systematic review and a combined analysis of TDM ratios, the quality of the laboratory's analytical methods plays a role, but the most important quality issue is control over the relevant pharmacokinetic variables influencing that drug's metabolism.

S2.2. Limitations of the TDM Data

S2.1.2. Other Formulations

There is no published TDM data following repeated dosing in clinical samples using the RBP-7000 or ISM formulations, and these formulations have not been approved for clinical use. We thought that it was important to review the limited data in case either of these two formulations are approved in the next few years.

S2.1.3 Microsphere Formulation

The LAI microsphere formulation was introduced in the US market 14 years ago, but its marketer has published very little of the large TDM database that they probably have from the RCTs used for approval by the drug agencies. In the absence of that data, clinicians wanting to learn how to use TDM to better monitor patients taking LAI risperidone microsphere formulations are left with the studies of independent investigators. As Tables 2 and 3 describe, the number of studies available to calculate mean R/9-OH-R and C/D ratios is relatively small and the studies are not free of confounders. Anyway, with all of their limitations, these are the only TDM studies that are available.

S2.3. Limitations of the Pharmacokinetic Assumptions Used to Review the TDM Data

To analyze these TDM studies, three major pharmacokinetic assumptions were made concerning: 1) steady state for the LAI risperidone microsphere formulation, 2) the time between injection and collection once steady state was reached, and 3) dosing.

S2.3.1. Steady State for LAI R Microsphere Formulation

Most of the studies assumed that steady state is reached at ≥ 6 weeks after the first injection or after the fourth injection if given every 2 weeks, taking into account that oral risperidone supplementation is needed during the first 3-4 weeks. The authors of this systematic review think that this assumption is reasonable but, unfortunately, there is no definitive data in the literature supporting this assumption.

The limited data published [47] by risperidone's marketer is from a study that only included 5 injections, with the fifth injection on day 64; 40 days of follow-up occurred after this fifth injection was given. The data in that article [47] was presented in a figure with median total concentrations from patients switched from oral risperidone to risperidone LAI, including 21 patients switched from 2 mg/day to 25 mg intramuscular every 2 weeks, 31 patients switched from 4 mg/day to 50 mg intramuscular every 2 weeks and 25 patients switched from 2 mg/day to 75 mg intramuscular every 2 weeks. In that figure, the comparison of the total mean concentration after 3 doses reached the peak on the day of the third injection (day 36), decreased on the day of the fourth injection (day 50), and further decreased on the day

of the fifth injection (day 64). Then the authors commented that the mean AUC for 14 days was within the range of 80-125% of bioequivalence, assuming that was good, although the mean AUC for risperidone LAI was 32-42% lower.

It is difficult to reinterpret these values for clinicians since the best figure provides median total concentrations rather than mean values and the median values appear to be slightly higher than mean values on risperidone LAI. It appears to us that steady-state concentrations are reflected on oral risperidone by day 1 and on LAI risperidone by day 64. The respective median concentrations for 2 mg oral and 25 mg intramuscular appear to be around 12 ng/ml and 12 ng/ml, indicating a similar median in these paired doses and a median total C/D ratio around 6. The respective median concentrations for 4 mg oral and 50 mg intramuscular appear to be around 21 ng/ml and 23 ng/ml, indicating a similar median and a median total C/D ratio between 5 and 6. The respective median concentrations for 6 mg oral and 75 mg intramuscular appear to be around 28 ng/ml and 36 ng/ml [47], providing respective median total C/D ratios of 4.7 and 6.7 ng/ml/mg/day.

S2.3.2. Once Steady State Has Been Reached, the Time between Injection and Collection is Not Important

The published TDM articles tend to ignore the time between the injection and the collection of the blood samples; their apparent presumption is that once the patient has reached steady state, total risperidone concentrations do not fluctuate much. From the pharmacokinetic point of view, the ideal point for collecting risperidone TDM would occur before giving an injection of LAI risperidone. Several published studies do not follow that ideal practice. Section 5.5 on Peak and Trough Concentrations provides some idea of the range of variation at different times once steady-state concentration has been reached.

S2.3.3. Limitations in Dosing Calculations

As indicated in the Introduction: 1) a 25-mg intramuscular injection of risperidone microsphere every 2 weeks is considered equivalent to 2 mg/day of oral R by the marketer, versus 1.8 mg/day by Castberg and Spigset [40]; 2) a 50-mg intramuscular injection of risperidone microsphere every 2 weeks is considered equivalent to 4 mg/day of oral risperidone by the marketer versus 3.6 mg/day by Castberg and Spigset [40], and 3) a 75-mg intramuscular injection of risperidone microsphere every 2 weeks is considered equivalent to 6 mg/day of oral risperidone by the marketer versus 5.4 mg/day by Castberg and Spigset [40]. These equivalences were assumed but have never been systematically tested. The impression that we have after reviewing the limited published data by risperidone's marketer [47] is that the equivalence for 25 and 50 mg every 2 weeks may be relatively correct, but the less accurate equivalence may be the one for 75 mg every 2 weeks. Future TDM studies in naturalistic settings will not easily determine whether these dose equivalences are correct since, even when a large group of the same patients are compared using steady-state trough concentrations on 6 mg/day oral risperidone versus steady-state concentrations on 75 mg intramuscular injections every 2 weeks, it is not easy to rule out the possibility that lower concentrations on 6 mg oral risperidone may be due to lower adherence in some patients.

S2.4. Limitations in the Units Used to Calculate Concentrations

The first publication on risperidone TDM from the multicenter study that gained drug approval in the US [10], along with the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP) guideline [1], most of the commercial laboratories in the USA and Europe, and the majority of published TDM studies describe risperidone concentrations in units of ng/ml. Therefore, we used ng/ml as the unit for calculating mean ratios. Ideally, a strict pharmacologist would insist that these two ratios should be calculated after taking into account the different molecular weights of risperidone and 9-OH-R and changing the units from ng/ml to nmol/l. For teaching clinicians, using ng/ml and ignoring this difference in molecular weight appears better to us and only introduces very

minor distortions (the 9-OH-R molecular weight is only 4% higher than risperidone) [8]. In this meta-analysis, AGNP conversion ratios [S11] were used to transform nmol/l to ng/ml.

S2.5. Limitations in the Use of Means to Review Risperidone TDM Data

Means are not good average measures for variables that do not follow normal distributions; unfortunately, the R/9-OH-R ratio does not follow a normal distribution. In an oral risperidone study with 277 patients with risperidone TDM and genotype [13], there were 221 patients who were not contaminated by relevant confounders [36]. The median R/9-OH-R ratio data from 221 non-contaminated patients [36] according to CYP2D6 phenotype, is described in Box 3. The average data per CYP2D6 phenotype is better described by using medians rather than means because any outlier places greater weight on means than on medians. With CYP2D6 there is always the possibility that rare unidentified alleles with low or null function may contaminate the genotyping. The median of the 221 patients was 0.12 while their mean was 0.49. After excluding 14 CYP2D6 PMs, the median of the 204 non-PM patients was 0.10 while the mean was 0.24. This data supports the idea that median R/9-OH-R ratios are less influenced by CYP2D6 PMs and that they definitively should be excluded from mean calculations of R/9-OH-R ratios.

SUPPLEMENTARY RERENCES

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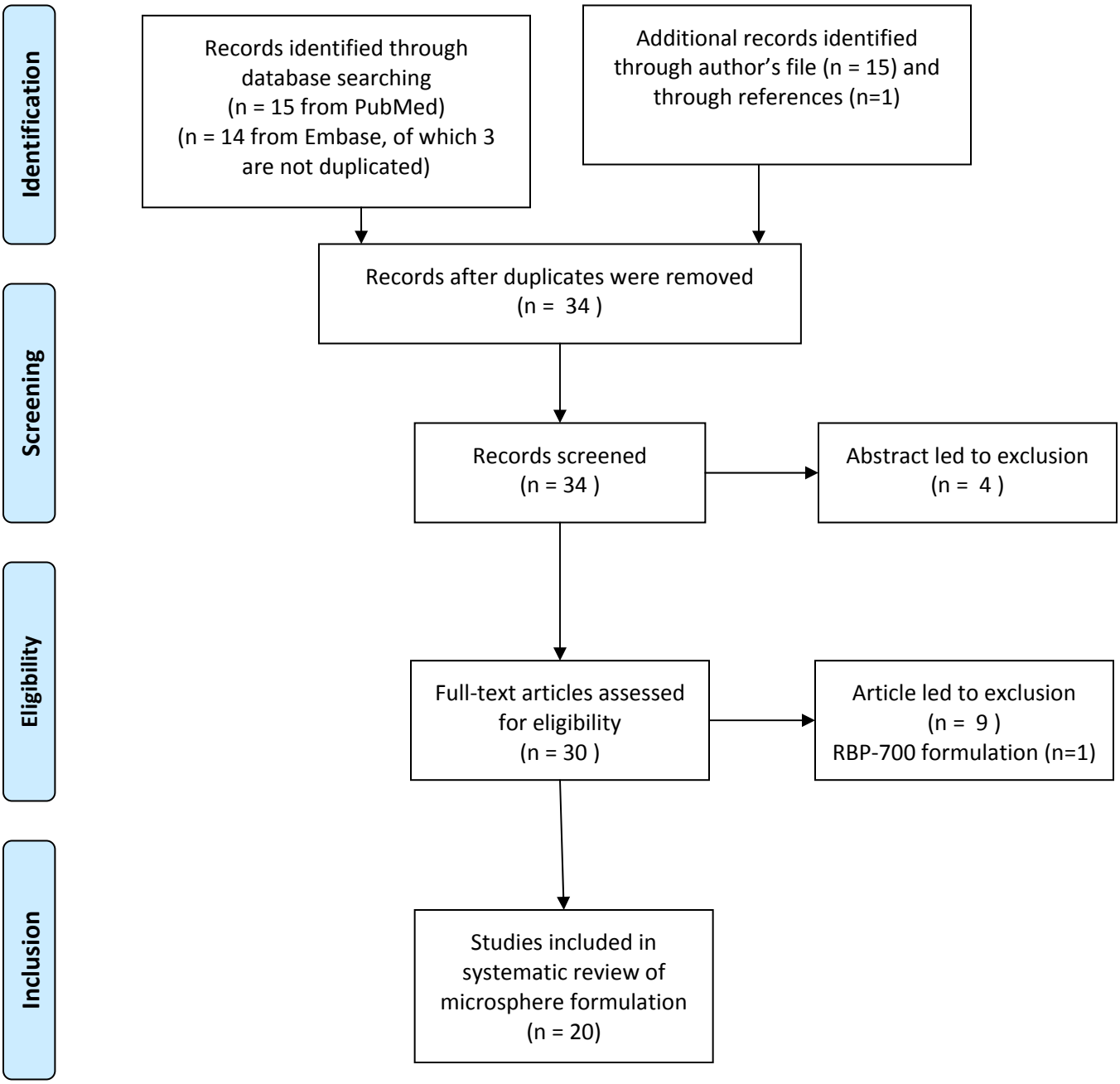
Supplementary Box S1. TDM indications according to AGNP consensus guidelines [1]

1) Dose optimization after initial prescription or after dosage change
2) Drugs for which TDM is mandatory for safety reasons (e.g., clozapine)
3) Suspected complete or partial non-adherence (non-compliance) to medication
4) Lack of clinical improvement under recommended doses
5) Adverse drug reactions under recommended doses
6) Combination treatment with a drug known for its DDI potential or suspected DDI
7) TDM in pharmacovigilance programs
8) Relapse prevention under maintenance treatment
9) Recurrence under adequate doses
10) Presence of a genetic particularity concerning drug metabolism
11) Pregnant or breast-feeding patient
12) Child and adolescent patients
13) Elderly patient (>65 years)
14) Individuals with intellectual disabilities
15) Patients with pharmacokinetically relevant comorbidities (hepatic or renal insufficiency, cardiovascular disease)
16) Forensic patients
17) Switching from an original preparation to a generic form (and vice versa)

AGNP: Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie; DDI: drug-drug interaction; TDM: therapeutic drug monitoring.

Supplementary Figure S1. Flow leading to 20 articles for systematic review on risperidone microsphere formulation.



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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