Original Paper

Evaluating the dose-dependent mechanism of action of trazodone by estimation of occupancies for different brain neurotransmitter targets

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Abstract

Trazodone is a drug that was introduced in the clinic almost 40 years ago. It is licensed to treat depression, but it is also commonly used off-label to treat insomnia. A recent study shows that it could be promising in preventing neurodegeneration in mice, and clinical trials to assess its possible beneficial effects on dementia and Alzheimer's disease are expected to start soon in humans. In this study, we describe the dose-dependent pharmacology of trazodone by carrying out pharmacokinetic simulations aiming to predict the brain concentrations of trazodone for different drug-dosing regimens and calculating occupancy for 28 different targets for which published trazodone-binding data are available. Our study indicates that low doses of trazodone (typically 50 mg daily) should suffice to block specific receptors responsible for the hypnotic effect, and to provide the protective effect against neuroinflammation and neurodegeneration that could be beneficial in dementia. Higher doses are required for an antidepressant effect. The occupancy of specific receptors at therapeutic doses also explains peculiar side effects reported by patients treated with trazodone (e.g. dry-mouth, hypotension and priapism).

Keywords

Trazodone, pharmacokinetic modelling, receptor occupancy, dose-effect

Introduction

Trazodone was introduced in the clinic in the early 1970s (Khouzam, 2017). Currently it is only licensed for treating depression, but it is commonly used off-label for other conditions, mainly to treat insomnia (Wong et al., 2017). Stahl reported that even if 'trazodone was never officially approved as a hypnotic, nor marketed as a hypnotic, it nevertheless accounts for up to half of all prescriptions for hypnotics' (Stahl, 2013: 453). Trazodone is also used off-licence to treat other conditions such as the behavioural and psychological symptoms of dementia in Alzheimer's disease (AD) (Lopez-Pousa et al., 2008) and in fronto-temporal dementia (Lebert et al., 2004). It has previously shown to be of benefit in models of Huntington's disease (Kumar et al., 2011), to protect neuronal-like cells from inflammatory insult (Daniele et al., 2015) and to prevent neurodegeneration (in mice) (Halliday et al., 2017). The latter study, in particular, provided promising results, and clinical trials are expected to start soon in humans to assess trazodone's protective effect in dementia and AD.

Given that trazodone is only licensed for depression, there is a need to know the dose required for treating the variety of other conditions for which it is used off-label, and the pharmacological mechanism of its action. The pharmacological activity of trazodone is dependent on the dose given and Stahl has tried to rationalise the dose-dependent pharmacological effects of trazodone by discussing occupancy in different targets. This was calculated using predicted plasma concentration of trazodone administered orally in different drug-dosing regimens (Stahl, 2009).

In the present study we wanted to improve the understanding of the mechanism of action of trazodone described in Stahl's analysis by (a) calculating receptor occupancy using brain concentration rather than the plasma level of trazodone and (b) considering a larger number of pharmacological targets. We also aimed to: (c) validate the use of a software called Berkeley Madonna (BM) (Krause and Lowe, 2014); (d) reproduce the experimental pharmacokinetic (PK) profile of trazodone, and compare the performance of the model produced by BM with the methodology used by Stahl and co-workers (Lemaire et al., 2009; Stahl, 2009). Finally (e), we aimed to suggest a pharmacodynamic mechanism by which trazodone is able to reduce the levels of activating transcription factor 4 (ATF4) (a key mechanism in slowing neurodegeneration) without affecting the level of phosphorylated eukaryotic initiation factor 2α (eIF2α-P) as reported by Halliday and co-workers (Halliday et al., 2017).

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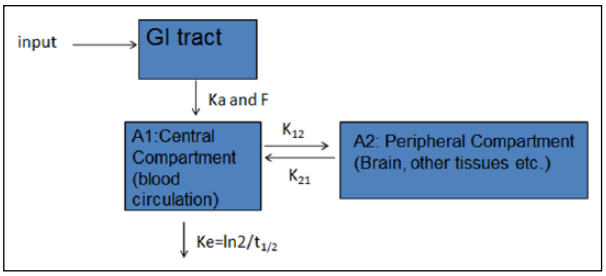

Figure 1. The two-compartment pharmacokinetic (PK) model developed for trazodone using Berkeley Madonna (BM). GI: gastro-intestinal.

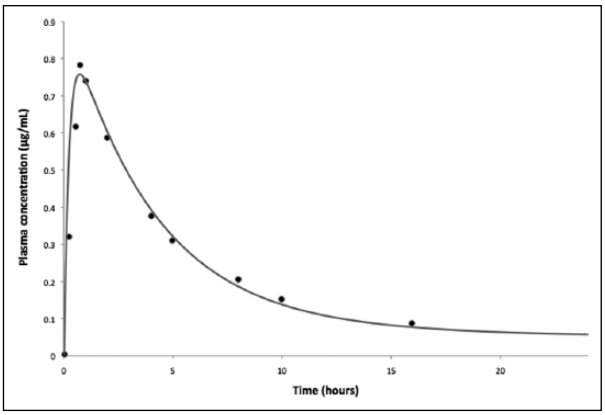

Figure 2. Pharmacokinetic (PK) model fitting in Berkeley Madonna (BM). The data points were extracted from Figure 2 published by Gammans and co-workers (Gammans et al., 1984). The continuous line represents the PK fitted curve obtained using BM.

With the prediction of occupancies, we also suggest a likely dose of trazodone that could be used in future clinical trials to evaluate its possible protective effect in dementia and AD.

Methods

A two-compartment PK model was developed using BM, beta version 9.0.123 (Krause and Lowe, 2014), in order to predict the pharmacokinetic profile of trazodone administered orally. A simple representation of the model developed in BM is shown in Figure 1 (the description of the compartments is similar to the structure of the model published by Lemaire and co-workers (Lemaire et al., 2009)).

Two differential were used for the modelling in BM: the first describes the absorption from the gastro-intestinal (GI) tract:

$$d/dt\ (\text{GI tract}) = \text{input} - [K_a \times (\text{GI tract})]$$

where 'input' accounts for the amount of trazodone taken orally in a specific time interval (24 h in this study), and K_a is the constant of absorption from the GI tract to the central compartment. The amount of drug considered in the input function is the dose of trazodone multiplied by its bioavailability (F=0.65) (Truven Health Analytics Inc, 2013) in order to account for the fraction of the administered dose that reaches the systemic circulation as unchanged drug. The 'input' administration by the GI tract is modelled by the pulse function in BM (Krause and Lowe, 2014).

The second equation describes the distribution in the central compartment A1 and in the peripheral compartment:

$$d/dt\ (A1) = +[K_a \times (\text{GI tract})] - [K_e \times A1] - [K_{12} \times A1] + [K_{21} \times A2]$$

where K_e is the constant of elimination from the central compartment A1; K_e is also equal to ln2 divided by the half life of elimination of trazodone (7.3 hours in humans (Obach et al., 2008)), thus a K_e of 0.095 h^{-1} assuming linear PK and first order elimination. K_{12} and K_{21} are the constants for the first order distributions of trazodone between the central and peripheral compartment. All the parameters of the model, the central (A1) and peripheral compartment (A2) are shown in Figure 1.

In order to solve the differential equation, the integration method Runge–Kutta 4 in BM was used; the time intervals to be used in the numerical solving of the differential equation system were 0.02 h.

To create the trazodone PK model, the curve fit and sliders functions in BM were used for the estimation of K_a, K_{12} and K_{21} by modelling the experimental plasma concentration curve of a specific dose of trazodone previously published (Gammans et al., 1984).

WebPlotDigitizer (Rohatgi, 2017) was used to extract the experimental data points of the trazodone plasma concentrations against time measured after a single oral administration of 50 mg of trazodone given individually to six patients (having a mean weight of 70 kg) using the plot in Figure 2 published by Gammans and co-workers (Gammans et al., 1984). These points were imported as an external dataset in BM and used as a template to fit the PK model using the 'curve fit' tool in BM by exploring different combination of K_a, K_{12} and K_{21} (K_e was instead set as 0.095 h^{-1} as explained above). BM allows also for the adjustment of each parameter individually to better fit the curve using the sliders function.

The central compartment was approximated to represent the blood circulation compartment where trazodone is distributed as seen in the report published by Lemaire and co-workers (Lemaire et al., 2009). In order to estimate the plasma concentration, the amount of trazodone in the central compartment was divided by the volume of distribution of trazodone in humans (36.4 L for a 70 kg individual (Obach et al., 2008)). The human brain concentration was estimated considering the unbound plasma/brain ratio measured experimentally in mice (the experimental $C_{u,plasma}/C_{u,brain}$ ratio reported in mice is 1.8 (Maurer et al., 2005); in this study we assumed that the unbound plasma/brain ratio in humans is the same as the ratio measured in mice).

Different doses of trazodone were simulated (50 mg, 100 mg, 150 mg administered daily (od) and 100 mg given three times daily (tds)), and the integration interval was set as 24 h.

The E_{max} sigmoid model (Rosenbaum, 2011) was used to calculate the receptor occupancy. The formula is:

$$\left[\text{trazodone}\right]_{brain} / \left(K_i + \left[\text{trazodone}\right]_{brain}\right)$$

where $[\text{trazodone}]_{brain}$ is the brain concentration of trazodone and K_i is the constant of binding experimentally measured for trazodone for a specific receptor.

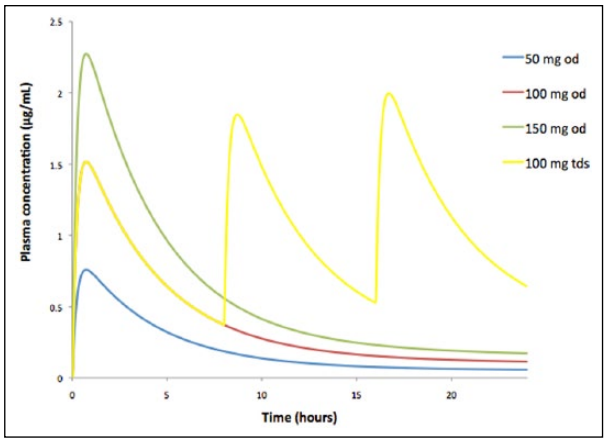

Figure 3. Plasma concentration curves for trazodone administered as 50 mg, 100 mg, 150 mg daily (od) and 100 mg three times daily (tds) predicted with Berkeley Madonna (BM) using the two-compartment model created as described in the Methods section.

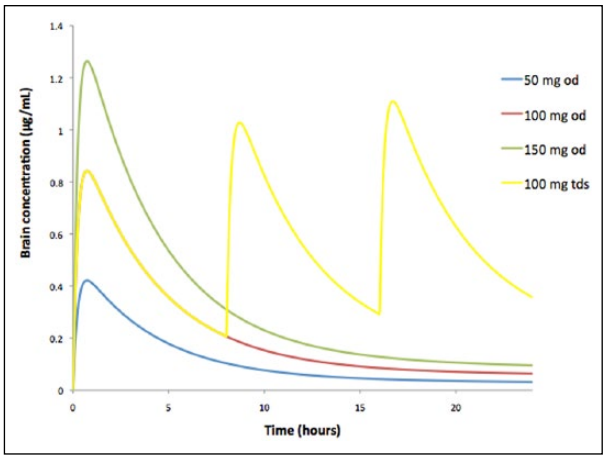

Figure 4. Brain concentration curves for trazodone administered as 50 mg, 100 mg, 150 mg daily (od) and 100 mg three times daily (tds) predicted with Berkeley Madonna (BM) using the two-compartment model created as described in the Methods section.

Results

The experimental plasma concentration curve for trazodone was best modelled with a two-compartment PK model: the parameters for this PK model were the following: K_{12}=0.134 h^{-1}; K_{21}=0.0313 h^{-1}; K_a=4.319 h^{-1}; K_e=0.095 h^{-1}. The curve fit and sliders tools in BM were used to produce the PK model by fitting the data published by Gammans and co-workers (Gammans et al., 1984). The result is shown in Figure 2.

This modelling methodology seems suitable in the case of trazodone as it can be seen in the good fit between the simulated curve produced with our model in BM and the experimental plasma values measured by Gammans and co-workers (Gammans et al., 1984). This is also in agreement with other studies showing that trazodone follows a two-compartment pharmacokinetic model (Lee and Desai, 2007; Lemaire et al., 2009).

The fact that our model shows that the K_{12} is 4.3-fold higher than K_{21} suggests that trazodone distributes substantially to the peripheral compartment (Figure 1). This was expected since trazodone is a basic lipophilic compound, thus this drug is predicted to bind to fat tissues and membranes (Schmitt, 2008).

The plasma concentration and the brain concentration curves for trazodone administered as 50 mg, 100 mg, 150 mg od and 100 mg tds predicted by BM are shown in Figures 3 and 4 respectively.

The therapeutic (antidepressant) window of trazodone is said to be a plasma level of between 0.5 and 1.6 µg/mL, whereas toxic effects are expected to be seen when the concentration of trazodone is above 4 µg/mL (Schulz and Schmoldt, 2003). The drug dosage regimen that best fits this window of plasma concentrations is seen when trazodone is given as 100 mg od or tds (Figure 3). When 50 mg od is given, the plasma level is, for the most part, below the therapeutic threshold. When 150 mg is given, the plasma concentrations are within the therapeutic window but approach the upper limit of the therapeutic window (Figure 3).

Given that the free protein unbound plasma/brain ratio is expected to be 1.8 (assuming that the brain permeability of trazodone in mice (Maurer et al., 2005) is similar to the brain permeability in humans), the brain concentration is expected to be almost half that of the plasma concentration (as can be seen by comparing Figures 3 and 4).

Table 1. C_{max} trazodone (in µg/mL or mg/L) concentrations predicted in the plasma and in the brain after the Berkeley Madonna (BM) pharmacokinetic (PK) simulation of trazodone given as 50 mg daily (od), 100 mg od and 150 mg od.

Dose	Plasma C_{max}[a]	Brain C_{max}[b]
50 mg od	0.76	0.42
100 mg od	1.5	0.83
150 mg od	2.27	1.26

a: Value taken from the curves in Figure 3; b: value taken from the curves in Figure 4.

We calculated the occupancy with the brain concentration using the E_{max} model described in the Methods section. In order to calculate the occupancy we needed (a) the concentrations of trazodone in the brain for each drug dosage regimen and (b) the binding affinity for the receptor or transporter (previously published). These values are reported in Table 1 and Table 2.

Table 1 shows the C_{max} concentrations in the plasma and brain for the three different daily drug dosage regimens taken from Figures 3 and 4. The brain C_{max} concentrations were used to calculate the occupancies, since the dose-dependent pharmacological actions of trazodone originate in the extent of binding with the different targets (receptors and transporters) in the brain.

It can be seen in Figures 3 and 4, that the first part of the 100 mg od and 100 mg tds simulation curves overlap. Therefore, as expected, the C_{max} after the first dose is identical in both simulations. However, a significant fluctuation of concentration can be seen in the tds simulation given the relatively short half life of trazodone.

Table 2 reports the binding affinity of trazodone in key receptors and transporters obtained from the Psychoactive Drug Screening Program (PDSP) database (Roth and Driscol, 2011).

We calculated the occupancy for all the neurotransmitter targets (receptors and transporters) in Table 2 using the brain

Table 2. Binding affinities (where data available) of trazodone in different transporters and receptors. Trazodone is an antagonist/blocker for all the targets in the table except for the serotonin (5-HT)$_{1A}$ receptor, where it behaves as a partial agonist/agonist. Values taken from the Psychoactive Drug Screening Program (PDSP) database (Roth and Driscol, 2011).

Protein	Ki (nM)
SERT	367.3
NET	>10,000
DAT	>7000
5-HT$_{1A}$[a]	118
5-HT$_{1B}$	>10,000
5-HT$_{1D}$	106
5-HT$_{1E}$	>10,000
5-HT$_{2A}$	35.8
5-HT$_{2B}$	78.4
5-HT$_{2C}$	223.9
5-HT$_{3}$	>10,000
5-HT$_{5A}$	>10,000
5-HT$_{6}$	>10,000
5-HT$_{7}$	1782
α_{1A}	153
α_{1B}	ND
α_{2A}	728
α_{2B}	ND
α_{2C}	155
β_1	>10,000
β_2	>10,000
D_1	3730
D_2	4142
D_3	ND
D_4	703
D_5	>10,000
H_1	220
H_2	3290
H_4	>10,000
mAChRs	>10,000
nAChRs	>10,000

5-HT: 5-hydroxytryptamine(serotonin) receptors (different subtypes); α: alpha-adrenergic receptors (different subtypes); β: beta-adrenergic receptors (different subtypes); D1–5: dopamine receptors; DAT: dopamine transporter; H: histamine receptors (different subtypes); mAchR muscarinic receptors; nAChRs: nicotinic receptors; ND: not determined; NET: norepinephrine transporter; SERT: serotonin transporter.

[a]Trazodone behaves as agonist/partial agonist for 5-HT$_{1A}$ (Odagaki et al., 2005).

concentrations from the three drug dosage regimens in Table 1 and reported the results in Table 3.

Discussion

First, we will discuss the performance of BM in reproducing PK simulations reported in previous publications. Then, we will look at the effect of the dose on the occupancy of different neurotransmitter targets in treating insomnia, depression and possible neuroprotection. We will also rationalise why trazodone causes some peculiar side effects such as hyposalivation, hypotension and priapism.

BM is able to reproduce PK simulations reported in previous publications

The plasma concentration curves predicted with BM for the trazodone doses of 50 mg od, 100 mg od and 100 tds (Figure 3) are very similar to the plasma concentration curves for the same dosage regimens published by Stahl (Stahl, 2009). This demonstrates the strength and reliability of the methodology used in our study and validates our PK model created with BM since this produced results very similar to published PK simulations (Lemaire et al., 2009; Stahl, 2009).

Trazodone has a half-life of 7.3 h (Obach et al., 2008) and it requires frequent administrations in order to provide a sustained exposure: the ideal time interval for a continuous exposure of a drug is usually an interval as close as possible to the half-life of the drug administered if the oral immediate-release (IR) formulation is given (Rosenbaum, 2011). The C_{max} experimentally measured for a 300 mg od administration of extended-release (ER) formulation of trazodone has been reported in different studies (Herr and Caspi, 2011; Lemaire et al., 2009; Stahl, 2009). According to these publications, ER formulations for 300 mg of trazodone administered od, generated plasma trazodone levels that rose slowly (with a C_{max} obtained almost 8 h after the oral administration) providing an experimentally measured plasma C_{max} at steady state of ~1.5 mg/L of trazodone. This can be rationalised by the fact that an IR 100 mg formulation administered three times od is equivalent to an ER 300 mg formulation given od. Coincidentally, the measured plasma C_{max} at steady state for an ER 300 mg formulation of trazodone administered od is identical to the plasma C_{max} that we obtained in our 100 mg od simulation (1.5 mg/L; Table 1). The predicted brain occupancy resulting from this concentration can therefore be seen in the third column of Table 3. For IR trazodone, the oral dosage regimen that provides a continuous, although fluctuating, exposure to the drug is three times od, as can be seen in Figure 3 and 4. This fact, in combination with the risk of toxic effects seen when the C_{max} is reached (especially when high doses are administered), explains why the manufacturer suggests administering high doses of IR trazodone in divided doses (Summary of Product Characteristics for trazodone, 2017). An alternative to the administration of trazodone in divided doses during the day is the once daily administration of an ER formulation of trazodone which shows less fluctuation of the plasma concentration in comparison with the tds administration of the IR formulation (Fagiolini et al., 2012; Herr and Caspi, 2011; Lemaire et al., 2009; Sheehan et al., 2009; Stahl, 2009).

However, there are cases where a daily dose of IR trazodone is preferred to a multiple dosing regimen or ER formulations, such as when IR trazodone is used off-license to treat insomnia when the daily dose is given before bed-time.

Dosing trazodone for insomnia

Trazodone is used widely off-label in a low-dose as a hypnotic for the treatment of sleep disorders (Stahl and Stahl, 2011). Trazodone's short half-life is advantageous in treating insomnia because its daytime sedation is minimal when trazodone is given only at night and at low doses, in agreement with the rapid decrease of the plasma and brain concentration curves for single-dose administration (Figures 3 and 4).

Table 3. Occupancy calculated with the E_{max} sigmoid model based on the brain concentrations C_{max} reported in Table 2.

Protein	Occupancy for dose of 50 mg daily	Occupancy for dose of 100 mg daily	Occupancy for dose of 150 mg daily
SERT	0.75	0.86	0.90
NET	<0.10	<0.18	<0.25
DAT	<0.14	<0.24	<0.33
5-HT_{1A}[a]	0.91	0.95	0.97
5-HT_{1B}	<0.10	<0.18	<0.25
5-HT_{1D}	0.91	0.95	0.97
5-HT_{1E}	<0.10	<0.18	<0.25
5-HT_{2A}	0.97	0.98	0.99
5-HT_{2B}	0.94	0.97	0.98
5-HT_{2C}	0.83	0.91	0.94
5-HT_{3}	<0.10	<0.18	<0.25
5-HT_{5A}	<0.10	<0.18	<0.25
5-HT_{6}	<0.10	<0.18	<0.25
5-HT_{7}	0.39	0.56	0.66
A_{1A}	0.88	0.94	0.96
A_{1B}	ND	ND	ND
A_{2A}	0.61	0.75	0.82
A_{2B}	ND	ND	ND
A_{2C}	0.88	0.94	0.96
B_1	<0.10	<0.18	<0.25
B_2	<0.10	<0.18	<0.25
D_1	0.23	0.37	0.48
D_2	0.21	0.35	0.45
D_3	ND	ND	ND
D_4	0.62	0.76	0.83
D_5	<0.10	<0.18	<0.25
H_1	0.84	0.91	0.94
H_2	0.26	0.40	0.51
H_4	<0.10	<0.18	<0.25
mAChRs	<0.10	<0.18	<0.25
nAChRs	<0.10	<0.18	<0.25

5-HT: 5-hydroxytryptamine(serotonin) receptors (different subtypes); α: alpha-adrenergic receptors (different subtypes); β: beta-adrenergic receptors (different subtypes); D1–5: dopamine receptors; DAT: dopamine transporter; H: histamine receptors (different subtypes); mAchR muscarinic receptors; nAChRs: nicotinic receptors; ND: not determined; NET: norepinephrine transporter; SERT: serotonin transporter.
[a]Trazodone behaves as agonist/partial agonist for 5-HT_{1A} (Odagaki et al., 2005).

Different studies have been performed to determine the optimal dose of trazodone to treat insomnia. The first study was carried out by Muratorio and co-workers showing that low doses of trazodone (50 mg od) were not effective as a hypnotic in four non-depressed patients, whereas higher doses (>250 mg od) given to depressed patients helped the patients to sleep (Muratorio et al., 1974).

In contrast, Karniol and co-workers studied the effect of trazodone on sleep in 10 healthy volunteers and concluded that patients were drowsier when taking a dose of 0.33 mg/kg (~25 mg daily taking into account an average weight of 70 kg for the patients) rather than a dose of 0.57 mg/kg (~50 mg daily) (Karniol et al., 1976).

Both of these previous studies should be interpreted carefully given the small number of patients involved. Another study involving 75 patients analysed the effect of the dose of trazodone on the treatment of insomnia associated with depression and concluded that a daily dosage of 50–100 mg (at night) improved sleep disorders, particularly when given at a dose of 100 mg (Mashiko et al., 1999). This finding was confirmed by another study in depressed insomniacs (Saletu-Zyhlarz et al., 2002). Recently Savarese and co-workers performed a retrospective cross-sectional study on 33 patients treated with trazodone given at different doses to treat insomnia for three months (Savarese et al., 2015) and reported that when patients were given a daily dose of 25–75 mg (at night) there were 37.93%, 31.03% and 20.68% of responders when the night dose of trazodone was 25 mg, 50 mg and 75 mg respectively. When the dose was 100 mg or 150 mg daily at night, responder rates were only 10.43% and 0% respectively (Savarese et al., 2015). Taken together these studies show that a dose between 25 mg and 100 mg seems to have a significant hypnotic effect that justifies the use of trazodone for insomnia at low doses.

Only one randomised parallel-group double-blind study has been carried out for trazodone in non-depressed insomniacs. This established the hypnotic efficacy of trazodone in comparison with zolpidem and placebo, and concluded that 50 mg trazodone given daily for two weeks was an effective hypnotic for the

short-term treatment of patients with primary insomnia, being only slightly less efficient than zolpidem (Walsh et al., 1998).

Alongside these studies is the observation that the typical off-licence hypnotic dose of trazodone in non-depressed patients is 50 mg at night. We performed a PK simulation with this dose (50 mg od) to better understand the pharmacological mechanism behind the hypnotic effect of trazodone. This dose afforded, after approximately one hour following oral administration (Figures 3 and 4), a C_{max} in the brain of 0.42 mg/L (Table 1). It is reasonable to assume that the C_{max} is the therapeutic concentration that is responsible for the hypnotic effect. With this concentration, the predicted occupancy for serotonin transporters (SERTs) is 75%, whereas the 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A} and 5-HT_{2B} receptors are expected to be more than 90% occupied. 5-HT_{2C} receptors are expected to show an occupancy of 83% whereas 5-HT_{1B} and 5-HT_{1E} are expected to show an occupancy less than 10% (Table 3).

The calculated occupancy for SERTs is too low to achieve antidepressant activity when a dose of 50 mg is given, since this transporter should be almost fully inhibited in order to exert a pharmacological action (Stahl, 2009). The hypnotic/anxiolytic effect observed at low doses of trazodone can be understood by taking into account the occupancy predicted in our analyses as an antagonist for (a) 5-HT_{2A} receptor (97%); (b) alpha$_{1A}$ receptor (88%) and (c) H_1 receptor (84%). The antagonism for each of these receptors is known to cause a hypnotic effect (Fagiolini et al., 2012; Stahl, 2009). In addition, the activation of the 5-HT_{1A} (91% occupancy as agonist/partial agonist for a daily dose of 50 mg) is likely to contribute to the anxiolytic effect of trazodone at these doses (Odagaki et al., 2005).

Trazodone has been shown to decrease gamma-aminobutyric acid (GABA) release (Garrone et al., 2000; Luparini et al., 2004). Luparini and co-workers showed that this is mediated by trazodone at low doses through 5-HT_{2a} receptors on GABA neurons and also that this decrease in GABA level is accompanied by an increase in 5-HT release (Luparini et al., 2004). Our study confirms this finding since 50 mg od dosing causes 97% occupancy for the 5-HT_{2a} receptors.

Less is known about the 5-HT_{1E} receptor, however, and it has been proposed that it could regulate memory given that it is highly localised in the cortex, hippocampus and olfactory bulb (Bai et al., 2004). The fact that trazodone does not block this receptor suggests that it could be used as a hypnotic without affecting memory. This might suggest that trazodone can be safely prescribed in patients affected by dementia, not least because trazodone does not block muscarinic receptors to any extent (Table 3). A recent randomised double-blind and placebo-controlled study in AD patients confirmed that trazodone (given with a dose of 50 mg at 22:00 for two weeks) was effective as a hypnotic and did not have any effect on cognition using different rating scales (Camargos et al., 2014).

Dosing trazodone for depression

Trazodone has been extensively studied to treat depressed patients (Fagiolini et al., 2012 and references therein). Two clinical studies investigated the optimal dosing of trazodone for treating depression: Mukherjee and Davey compared the treatment of trazodone dosed 25 mg tds against 50 mg tds and reported the superiority of the latter dose regimen in treating this condition (Mukherjee and Davey, 1986). Mihara and co-workers reported a significant linear relationship between the steady-state plasma concentration of trazodone and the percentage of patients improving depression as assessed by the Montgomery–Åsberg Depression Rating Scale (MADRS) (Mihara et al., 2002).

Trazodone probably acts as an antidepressant by different pharmacological mechanisms such as antagonism of the SERT, alpha-2 adrenoreceptor, 5-HT_{2A} and 5-HT_{2C} receptors, as well as stimulation of the 5-HT_{1A} (Fagiolini et al., 2012; Stahl, 2009). In particular, Luparini and co-workers reported that with high doses/concentrations of trazodone, the increase in 5-HT release is mediated by a double mechanism: as a result of a decreased release of GABA (already happening at low doses as explained above), but also by the block of the re-uptake of serotonin that is only observed at high doses of trazodone (Luparini et al., 2004). The 5-HT increase is then accompanied by a rise in GABA release. This complex interaction between the GABAergic and serotoninergic systems may explain the sedation and anxiolytic properties that accompany the antidepressant activity of trazodone (Luparini et al., 2004).

Drugs that block the re-uptake of serotonin increase the concentration of this neurotransmitter in the synaptic cleft. This is a well-known mechanism of action for the selective serotonin receptor inhibitors (SSRI): excess of serotonin in the synapse is thought to act on the post-synaptic 5-HT_{1A} receptor providing antidepressant action (Stahl, 2008, 2009). SSRIs do not block post-synaptic 5-HT receptors and are associated with side effects such as insomnia, anxiety and sexual dysfunction (Stahl, 2008). Serotonin agonism on the 5HT_{2A} and 5-HT_{2C} post-synaptic receptors is the mechanism for this (Stahl, 2008, 2009). The potential advantage of trazodone is that, in addition to blocking SERTs, it is an antagonist at 5-HT_{2A} and 5-HT_{2C} receptors. 5-HT_{2C} antagonism is also observed with other drugs such as mirtazapine and agomelatine (Stahl, 2008, 2009). Each of these non-SSRI drugs are known to exert antidepressant activity with a much reduced risk of anxiety or sexual dysfunction.

In addition to this, it has been postulated that simultaneous 5-HT_{2A} and 5-HT_{2C} antagonism combined with SERT inhibition might also potentiate antidepressant effect and improve tolerability (Fagiolini et al., 2012; Stahl, 2009).

As can be seen in Table 3, the occupancy for SERTs is predicted to be 86% when trazodone is given as 100 mg daily and 90% when trazodone is given as 150 mg daily. As expected, at these doses trazodone almost completely blocks the 5-HT_{2A} and 5-HT_{2C} receptors thus, in theory, reducing the risks of sexual dysfunction and anxiety related side-effects (Stahl, 2009). The fact that high doses of trazodone are more effective in treating depression than lower doses (Mihara et al., 2002; Mukherjee and Davey, 1986), suggests that the block of SERT (that is only significantly achieved when high doses are given as explained above), and thus the increase of 5-HT, is more important than the block of alpha-2 adrenoreceptor, 5-HT_{2A} and 5-HT_{2C} receptors or the stimulation of the 5-HT_{1A} receptor for treating depression (the latter receptors in fact show a high occupancy already at lower doses (Table 3)).

In particular, the increase of 5-HT caused by chronic administration of high doses of trazodone most likely causes 5-HT_{1A} receptor downregulation, which may also contribute to the antidepressant properties of trazodone (Subhash et al., 2002).

Different studies report that trazodone usually decreases the amount of rapid eye movement (REM) sleep (Aton et al., 2009;

Brogden et al., 1981; Mendelson, 2005; van Bemmel et al., 1992; Yamatsu et al., 1974). However, to our knowledge, only one study investigated the effect of the dose of trazodone on the amount of REM during sleep following administration of this drug (Yamatsu et al., 1974). According to this investigation, the suppression of REM is proportional to the dose of trazodone administered, with low doses having no effect on the amount of REM during sleep (Yamatsu et al., 1974). We suggest that this could be correlated with the fact that at high doses of trazodone, the increased concentration of serotonin in the synaptic cleft (as a result of SERT-block) activates 5-HT_{1B} receptors. The agonist activation of this receptor has been shown to decrease REM during sleep (Boutrel et al., 1999): the fact that trazodone does not block this receptor (Table 3) could explain the fact that when serotonin concentration increases following SERT block by high doses of trazodone, the effect is a suppression of REM, and this hypothesis would be confirmed by trazodone's dose-dependent suppression of REM. These findings support the fact that low doses of trazodone (typically 50 mg at night) are to be preferred when treating insomnia, as the quality of sleep would be improved as the REM phases would not be majorly affected.

Dosing trazodone for possible neuroprotection in humans

Halliday and co-workers recently proposed a possible beneficial use of trazodone in dementia and AD by showing that this drug is able to reverse unfolded protein response (UPR)/integrated stress response (ISR) activation induced by UPR stressors such as tunicamycin. The authors showed that trazodone was able to reduce the levels of ATF4 without affecting the level of eIF2α-P (Halliday et al., 2017). However, the authors of this study did not explain the mechanism by which trazodone produced this effect. Given that it has been showed that p38 activates the expression of ATF4 in tunicamycin-induced stress-related protein kinase RNA-like endoplasmic reticulum kinase (PERK)/eIF2α/ATF4 pathway (Jiang et al., 2014) and also that 5-HT_{2A} agonism activates the mitogen-activated protein kinase p38 (Kurrasch-Orbaugh et al., 2003), it is evident that trazodone's antagonist activity on the 5-HT_{2A} receptor is expected to decrease the activity of p38 and this should decrease also the formation of ATF-4. Thus, we suggest that 5-HT_{2A} blockade could have a protective role against dementia and AD and explain the effects described in the recent study published by Halliday and co-workers (Halliday et al., 2017).

Our hypothesis has also been confirmed by the fact that there is a partial reduction of neuro-protective effect in the presence of the 5-HT_{2A} receptor agonist (R)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (R-DOI) (Daniele et al., 2015). The latter ligand displays a high affinity towards 5-HT_{2A} (K_i=3.36 nM) (Knight et al., 2004), approximately 10-fold stronger than the affinity of trazodone for the same receptor. In addition, the decreased activity of p38 as a result of 5-HT_{2A} block is expected to decrease the expression of nuclear factor kappa B (NF-kappa B), which is an important transcription factor in inflammation (Olson et al., 2007). Moreover, trazodone is expected to have beneficial effects on dementia and AD because, as a 5-HT_{1A} agonist, it is expected to lead to ERK1/2 activation which is known to increase the protein expression of brain-derived neurotrophic factor (Daniele et al., 2015; Masson et al., 2012; Polter and Li, 2010). In addition, the block of H_1 and alpha-1 receptors are both expected to decrease the expression of NF-kappa B (Bakker et al., 2001; Gupta et al., 2009; Perez et al., 2009).

5-HT_{1A} downregulation is less likely to occur at lower doses of trazodone because the levels of 5-HT are lower, and 5-HT is a 5-HT_{1A} agonist (Subhash et al., 2002). Thus, the administration of low doses of trazodone (e.g. 50 mg od) would be preferable if the aim is to use this drug as neuroprotective through the activation of the 5-HT_{1A} receptor and the effects on different transcription factors mentioned above.

Given that 5-HT_1, 5-HT_{2A}, H_1 and alpha$_1$ receptors are saturated with a dose of 50 mg daily (Table 3), our study suggests, therefore, that this dose might suffice for observing neuro-protective effects towards dementia/AD and we suggest that it would be reasonable to use this dose for future clinical studies of trazodone in dementia/AD.

Rationale for the presence of unanticipated side effects of trazodone

The fact that trazodone displays minimal central or peripheral occupancy for muscarinic receptors at these doses makes this drug suitable for treating patients with glaucoma, angina, prostatism, constipation and dementia.

The central occupancy of the receptors calculated in our study can also be used to understand the side-effects reported by patients taking trazodone. In particular, we explain how the occupancy of the different receptors for trazodone can explain three peculiar side effects of trazodone (a) hypo-salivation; (b) hypotension; (c) priapism.

It is clear from Table 3 that trazodone does not block the muscarinic receptor, thus hyposalivation is not mediated by antagonism of acetylcholine. Lung and colleagues reported that salivation is also controlled by alpha receptors: in particular hypersalivation can be caused by α_1-agonists and/or α_2-antagonists, whereas hypo-salivation is caused by α_1-antagonists and/or α_2-agonists (Lung, 1994). This is also supported by one of the theories that clozapine-induced hypersalivation is mediated by the block of the α_2-adrenergic receptors (Corrigan et al., 1995; Szabadi, 1996). The binding data reported in Table 2 show that trazodone has a stronger affinity for the α_{1A} receptor than the α_2 receptors (153 nM and 441.5 nM (average value of the affinity for the α_{2C} and α_{2A} receptors) respectively). This is also reflected in the occupancy (Table 4). This binding profile could therefore explain why trazodone can cause hypo-salivation (dry mouth).

A relatively frequent side effect of trazodone is hypotension (Khouzam, 2017). The specific binding profile of trazodone with respect to α-receptors can also help to explain this side effect. It is known that α_1 agonists and α_2 blockers cause hypertension, whereas α_1 antagonists and α_2 agonists cause hypotension (Reid, 1986). Using a similar reasoning as that given for the explanation of the hyposalivation, the fact that the block of α_1 receptors is stronger than α_2 receptors could also explain the presence of hypotension episodes sometimes seen with trazodone use.

There have been several reports of priapism associated with the use of trazodone (Hayes and Kristoff, 1986), mainly with doses of 150 mg/day or less (Khouzam, 2017). Our study agrees with published studies that proposed that the effect is related to the blockade of α-receptors in the absence of sufficient antimuscarinic

activity (Patel et al., 1996). This criteria is fulfilled by the predicted occupancy of trazodone already at low doses (Table 3). Notably, also yohimbine blocks the α_2 receptors and has been studied for potential treatment for erectile dysfunction (Andersson and Stief, 2001). Given the pharmacological profile of trazodone, it is not surprising that it has also been proposed to treat the latter condition (Khouzam, 2017).

Conclusion

In this study we have validated the use of BM for PK simulations as we were able to reproduce PK simulations for trazodone that were previously published. We have shed more light on the mechanism of action of trazodone in the treatment of different conditions (depression, insomnia and in its possible use as protective treatment for dementia/AD). Our study indicates that low doses of trazodone (typically 50 mg daily) should be used for treating insomnia with minimal influence on REM sleep, and possibly should provide pharmacological protection for neurodegenerative conditions such as dementia/AD.

Our findings are corroborated by the prediction of brain concentrations of trazodone using different drug-dosing regimens, and the prediction of occupancy for 28 different targets for which published trazodone binding data are available. The occupancy of specific receptors is also able to explain the rationale for the presence of specific side effects reported for trazodone. The approach presented in this study could be used to investigate further the dose-dependent pharmacology of other psychotropic medications.

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