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Review

Olanzapine-induced weight gain: Chronic infusion using osmotic minipumps does not result in stable plasma levels due to degradation of olanzapine in solution ☆

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Abstract

The mechanisms underlying olanzapine-induced weight gain have not yet been fully elucidated. To examine the effects of long-term treatment with olanzapine on different aspects of energy balance, we administered olanzapine to male rats. Osmotic minipumps were chosen as preferred mode of administration because the half-life of olanzapine is only 2½ h in rats compared to 30 h in humans. We discovered that, within one week, degradation of olanzapine occurred in the solution used to fill the minipump reservoir. This resulted in a decrease in delivered olanzapine and declining plasma levels over the course of the experiment. Therefore, we caution other researchers for the limitations of using osmotic minipumps to administer olanzapine for longer periods of time.

Keywords: Animal model; Drug administration; Osmotic minipumps; Olanzapine; Body weight; Food intake

Contents

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1. Introduction

Olanzapine is an effective and commonly prescribed antipsychotic drug, used in the treatment of schizophrenia and bipolar disorder (Meltzer et al., 1999; Scherk et al., 2007). It causes less extrapyramidal side effects than most typical antipsychotics and has a positive effect on cognitive deficits (Kapur and Remington, 2001; Leucht et al., 2003; Meltzer et al., 1999). Unfortunately significant weight gain is a common side effect (Allison et al., 1999; Baptista, 1999; Wirshing et al., 1999). This weight gain can compromise patient compliance and is often accompanied by dyslipidemia and hyperglycemia, which increases the risk for diabetes and cardiovascular disease (Fontaine et al., 2001; Mir and Taylor, 2001; Weiden et al., 2004; Wirshing et al., 2002).

Olanzapine has affinity for multiple receptors, including dopamine D2, 5-hydroxytryptamine (5-HT) 2A and 2C, histamine H1, alpha-adrenergic and muscarinic receptors (Bymaster et al., 1996). Many of these receptors are known to play a role in the regulation of feeding behavior or energy balance (Berridge, 1996; Masaki and Yoshimatsu, 2006; Reynolds et al., 2002; Tecott et al., 1995; Yamada et al., 2001), however, so far it is not completely understood which of these receptors are involved in olanzapine-induced weight gain.

We recently set-up an animal model for olanzapine-induced weight gain, using male Wistar rats, to study in detail the effects of this drug on eating behavior, food preference and locomotor activity. In the following report, we describe the technical problems that we encountered using osmotic minipumps to administer olanzapine to rats. Furthermore, we caution other researchers for the limitations of using osmotic minipumps to administer olanzapine for longer periods of time.

2. Experiments using osmotic minipumps to study olanzapine-induced weight gain

2.1. Experiment 1: 4 weeks

2.1.1. Methods

Because the half-life of olanzapine in male rats is only 2½ h (Aravagiri et al., 1999), compared to approximately 30 h in humans (Callaghan et al., 1999; Kassahun et al., 1997) we decided to use osmotic minipumps (Alzet®, model 2ML4, Durect Corp., Cupertino, California, USA) to continuously administer an olanzapine-solution for 4 weeks. By using osmotic minipumps, that deliver a fixed volume of drug-solution continuously over several weeks, we aimed to achieve more constant plasma levels than those achieved by administering olanzapine only once- or twice-daily (via oral gavage or injection) and, therefore, more comparable to the clinical situation in humans.

Twenty-four male Wistar rats were purchased from Charles River Laboratories (Crl-Wu, Germany). They were individually housed in a temperature and humidity controlled room (21±2 °C) under a 12 h/12 h light/dark cycle (lights on at 0700 h). All experimental procedures were approved by the Committee for Animal Experimentation of Utrecht University.

One week after arrival, transmitters (TA10TA-F40, Data Science International, St Paul, Minnesota, USA) were placed in the abdominal cavity, to continuously monitor body core temperature and locomotor activity. Surgery was performed under fentanyl/fluanisone (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium, 0.1 ml/100 g i.m.) and midazolam (Dormicum®, Roche, Woerden, The Netherlands, 0.05 ml/100 g i.p.) anesthesia and took place 4 weeks before the start of drug administration. Carprofen (Rimadyl®, Pfizer Animal Health, Capelle a/d Ijssel, The Netherlands, 0.01 ml/100 g s.c.) was administered as pain medication for 2 days.

Ad libitum standard lab chow and tap water was available throughout the experiment. In addition, rats had voluntary access to a running wheel in their home cage from 2 weeks before implantation of the minipump; in this time, stable baseline running wheel activity is usually achieved. Running wheel activity was continuously recorded using Cage Registration Program (Department Biomedical Engineering, UMC Utrecht, The Netherlands). Body weight, food and water intake were monitored daily.

The day before the minipumps were implanted, olanzapine (Chempacific Corp, Baltimore, USA) was solubilized in a minimum quantity of glacial acetic acid and diluted with saline to the required concentrations, after which pH was set at ~5.5 with 1 M NaOH. The osmotic minipumps were filled with this solution or a control solution and then immersed in saline at 37 °C overnight, as advised by Alzet®.

Under brief isoflurane anesthesia, minipumps were inserted subcutaneously, through a small incision on the back of the rat, after which the incision was closed using surgical staples. Three different dose groups (1, 2.75 and 7.5. mg/kg/day) and a control group were used (n=6). At the time of minipump surgery mean body weight±S.E.M. was 337±3.8 g.

On the final day of the experiment, animals were sacrificed by decapitation. Trunk blood was collected in lithium-heparin containing tubes (Sarstedt, Nümbrecht, Germany), with 83 μmol of EDTA and 1 mg aprotinine added, and immediately placed on ice. Plasma was stored at −20° for future analysis. In addition, wet weights of mesenteric, perirenal, epididymal and subcutaneous white adipose tissue were determined. One animal did not recover from surgery and was excluded from analysis.

2.1.2. Results experiment 1

In the first week after minipump placement, we observed a small dose-dependent increase in food intake in the olanzapine-

treated groups, which did not reach significance (Fig. 1A). This effect completely disappeared in the second week. Conversely, in the last 2 weeks of the experiment, food intake in the olanzapine-treated groups showed a dose-dependent decrease, although not significant. We observed a significant reduction in running wheel activity in the two highest dose groups during the first week (Fig. 1B). However this effect diminished over the course of the experiment (particularly in the highest dose group) and was no longer significant in the last week. No difference in body weight or adiposity was observed between any dose group and controls.

The decrease in effects of olanzapine over the course of this experiment at first led us to hypothesize that tolerance had developed. However, at the end of the study, we noticed a strong discoloration of the solution remaining in the minipumps of the olanzapine-treated rats (from bright orange to dark green), as well as a dark precipitate in the pumps interior, which was most notable in the highest dose group (see supplemental data).

When we made a test-solution (identical to the one used in the minipumps of the highest dose group) and placed it in an incubator at 37°, we observed a similar discoloration occur within 4–5 days. It occurred regardless of whether acetic acid, citric acid or hydrochloric acid was used as a solvent, although the last solvent seemed to delay discoloration to 6–7 days. Taken together, this led to the hypothesis that a deterioration of the drug-solution was taking place within the minipump reservoir during the course of the experiment and that this could provide an alternative explanation for the decrease in effects we observed in our experiment.

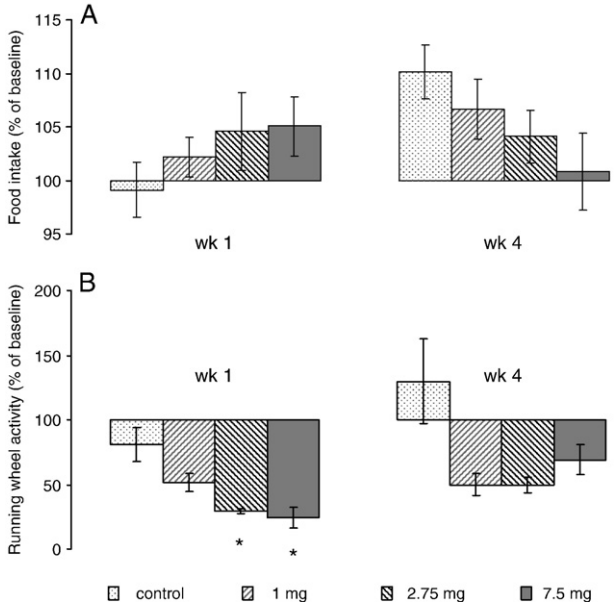

Fig. 1. A Food intake (percentage of baseline) after olanzapine administration in experiment 1. Means±S.E.M. are shown for the first and last week of drug administration. B Running wheel activity (percentage of baseline) after olanzapine administration in experiment 1. Means±S.E.M. are shown for the first and last week of drug administration (ANOVA: $F(3, 22)=8.99$, $P=0.001$. Post-hoc Tukey HSD: $^*P<0.01$ vs control).

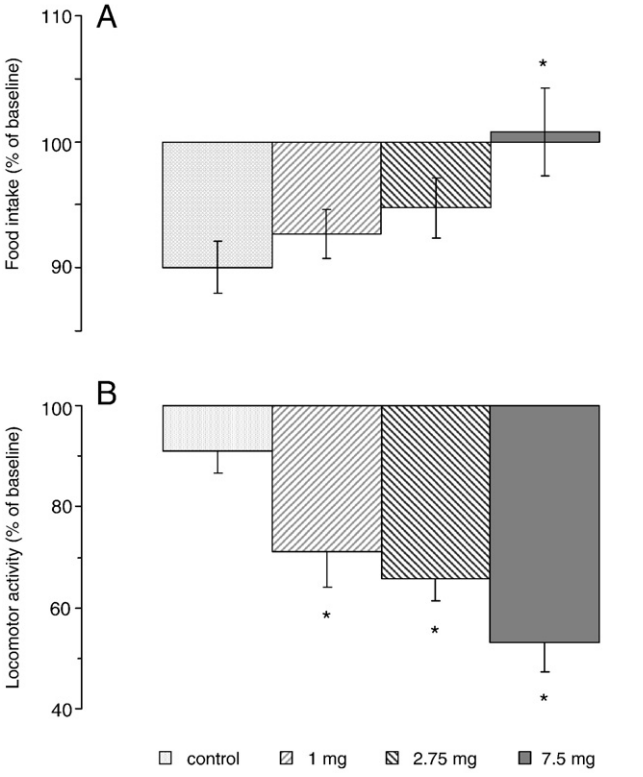

Fig. 2. A Food intake (percentage of baseline) during 10 days of olanzapine treatment in experiment 2. Means±S.E.M. are shown (ANOVA: $F(3, 21)=3.3$, $P=0.044$. Post-hoc Tukey HSD: $^*P<0.05$ vs controls). B Locomotor activity (percentage of baseline) during 10 days of olanzapine treatment in experiment 2. Means±S.E.M. are shown (ANOVA: $F(3, 21)=13.5$, $P<0.001$. Post-hoc Tukey HSD: $^*P<0.05$).

2.2. Experiment 2: 10 days

2.2.1. Methods

Because of the presumed deterioration of the olanzapine-solution in the minipumps, we decided to end our second experiment after only 10 days of drug administration. Methods were identical to the previous experiment, with three exceptions. Firstly, rats did not have access to a running wheel in their home cage, in stead locomotor activity was measured using the intra-abdominal transmitter. Furthermore, we decided to use hydrochloric acid and distilled water to dissolve the olanzapine, because this delayed discoloration of our test-solution for a few days compared to using acetic acid. Additionally, in order to monitor plasma levels of olanzapine over time, we took a blood sample, by tail nick, 4 days after placement of the olanzapine-containing minipump. Blood was collected in tubes containing heparin (Leo Pharma BV, Breda, the Netherlands, 500 IU) and immediately put on ice. Plasma was stored at −20° and at the end of the experiment, trunk blood was collected as described above in experiment 1. Mean body weight±S.E.M. at the time of minipump surgery in this experiment was 362.8±6.0 g. Due to complications, 2 rats did not complete this experiment and were excluded from analysis.

2.2.2. Results experiment 2

The effects we observed on food intake and locomotor activity in this experiment were similar to those observed in the

first week of experiment 1: a small, but significant, increase in food intake in the highest dose group and a clear reduction in locomotor activity in all dose groups (Fig. 2A and B). We did not find a significant effect on body weight or adiposity.

2.3. Olanzapine concentrations in plasma from in vivo experiments 1 and 2

Using high performance liquid chromatography (HPLC) followed by ultraviolet (UV)-detection, we measured olanzapine concentrations in plasma that was collected at the end of both experiments, as well as during the second experiment.

A calibration curve (0–200 ng/ml) was prepared using a stock solution – made by dissolving olanzapine (Eli Lily and Co, USA) in methanol (Riedel de Haen, Seelze, Germany) – added to veal plasma. A control solution of olanzapine (40 ng/ml, SKML, the Netherlands) was used as quality control. To all samples 500 ng promazine HCl (Sigma, Australia) was added as internal standard, as well as 0.1 ml ascorbic acid 2.5% to prevent oxidation. After liquid–liquid extraction, plasma-samples were analyzed by an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) controlled by TotalChrom software (Perkin-Elmer, Waltham, Massachusetts, USA). During HPLC, samples were eluted with a solution containing 95% dichloromethane and 5% methanol on a straight phase column (chromspher 5 Si 100× 3 mm, Varian, Palo Alto, CA, USA). Peak detection was performed at 272 nm. Retention times were 4.4 and 6.4 min for promazine and olanzapine respectively. Peak heights correlated linearly for concentrations of olanzapine in the range of 0–200 ng/ml ($r^2 > .99$). Lower detection limit was 10 ng/ml olanzapine.

We observed that, in the two highest dose groups, plasma olanzapine concentrations at the end of the 28-day experiment were only one fifth of the concentrations measured at the end of the 10-day experiment (see Table 1). Moreover, in the second experiment, plasma concentrations in the blood samples that were taken on the fourth day of olanzapine administration were approximately twice as high as those measured at the end of the same experiment (Table 1). Thus, there was a clear decline in plasma olanzapine concentrations over time.

2.4. Olanzapine concentrations in in vitro experiments

It is well known from liquid chromatography studies that olanzapine easily oxidizes in plasma-samples from patients (Catlow et al., 1995; Gervasini et al., 2003; Olesen and Linnet, 1998). We, therefore, investigated whether this process was also detectable in the drug-solution that we used to fill our minipumps.

A single batch of test-solution was prepared (42 mg/ml olanzapine, identical to the one used for the highest dose group in experiment 2) and divided over 5 test-tubes. One tube was immediately stored at −20 °C and the remainder placed in an incubator at 37 °C. On days 1, 4, 6 and 8 we stored an additional test-tube at −20 °C for future analysis by both HPLC-UV and mass spectrometry (MS).

Both studies indicated a reduction of olanzapine concentrations of 20–30% occurring over the course of 8 days (data not shown, personal communication Dr. E.R.Verheij, TNO, Zeist, the Netherlands). These results confirmed that the concentration of olanzapine in the minipump solution declined significantly in only one week.

Table 1
Olanzapine concentrations in plasma of rats that received olanzapine via osmotic minipump

Olanzapine dose	Experiment 2		Experiment 1
	4 days	10 days	4 weeks
7.5 mg/k/day	100.5±20.0 (322±64)	50.1±7.7 (160±25)	8.8±2.2 (28±7)
2.75 mg/k/day	41.8±7.9 (134±25)	22.3±7.9 (71±25)	4.0±1.4 (13±5)

Concentrations are expressed in ng/ml (nM). Means±S.D. are shown. Two consecutive plasma-samples were taken in experiment 2 (on day 4 and 10); only one sample was taken at the end of experiment 1 (Plasma levels of the lowest dose group are not shown because these levels were often lower than 10 ng/ml.).

3. Discussion

3.1. Experiments

In the experiments described, we were unable to induce weight gain or adiposity by olanzapine administration via osmotic minipump. However, olanzapine concentrations measured in plasma indicated that a decrease in plasma levels occurred over the course of these experiments. Furthermore, *in vitro* studies indicated that, at 37 °C, significant degradation occurred of the olanzapine present in the minipump solution over the course of 8 days (20–30%). This degradation of olanzapine within the minipump reservoir most probably caused the decline in plasma levels over time in our study. Furthermore, we believe it is an important reason why we were unable to induce weight gain or adiposity in our model.

In our *in vitro* studies, we observed a clear decrease in olanzapine concentration occur in a test-solution, made with hydrochloric acid and distilled water (as in experiment 2). This most probably explains the decrease in olanzapine plasma levels over the course of experiment 2, as well as the lower plasma levels of experiment 1 compared to experiment 2. We do not have *in vitro* data of a test-solution using acetic acid to dissolve olanzapine (as in experiment 1). However, the fact that discoloration of a test-solution using acetic acid occurred sooner than one using hydrochloric acid, suggests that degradation of olanzapine occurs faster if acetic acid is used. Similarly, we do not have *in vitro* data of a test-solution over more than 8 days, but it is likely that degradation continues. The further decrease of the olanzapine concentration in the minipump solution would thereby lead to a further decline in plasma levels over several weeks.

Other factors could also have played a role in the decline in plasma levels of olanzapine over time, such as an increase in body weight. However, body weight gain did not exceed 17% in experiment 1, or 5% in experiment 2, which is clearly insufficient weight gain to explain the large decrease in plasma levels observed.

Induction of liver enzymes, resulting in a faster drug metabolism, may also have played a role. There are, to our knowledge, no data available on which enzymes are involved in the metabolism of olanzapine in rats, but it has been described that liver enzymes

involved in the metabolism of olanzapine in humans (Cytochrome P450 1A2+2D6, flavin containing monooxygenase and UDP-glucuronosyltransferase enzymes) can be induced by certain xenobiotics (e.g. carbamazepine and indavir), resulting in lower olanzapine levels if co-administered (Kiang et al., 2005; Liston et al., 2001; Wrighton and Ring, 1999). However, to our knowledge, there is no report of olanzapine itself affecting these enzymes and thereby increasing its own metabolism. Furthermore, in a study on olanzapine pharmacokinetics in rats, plasma levels were determined 3 h after oral administration of 6 mg/kg olanzapine in male rats that had received 15 consecutive daily doses of 6 mg/kg previously. These were similar to the levels observed 3 h after just one single dose (Aravagiri et al., 1999), which argues against a major change in drug metabolism taking place in this time course.

The fact that, in experiment 1, a dose-dependent increase in food intake was observed in the first week, but a dose-dependent decrease in the last week at first seemed surprising. However, plasma levels and *in vitro* data indicate that plasma levels declined over the course of this experiment. Therefore it is likely that, only in the first week of the experiment, olanzapine plasma levels were high enough to induce both an increase in energy intake (hyperphagia) as well as a reduction in energy expenditure (running wheel activity), leading to a positive energy balance. Although running wheel activity was decreased in the olanzapine-treated groups throughout the experiment, there was no difference in body weight at the end of the study. Apparently, plasma levels of olanzapine higher than those that reduce running wheel activity are necessary to influence food intake and maintain the positive energy balance necessary for increased weight gain or adiposity.

Over the course of experiment 2, we observed both hyperphagia (increased energy intake) and reduced locomotor activity (reduced energy expenditure), but no significant effect on body weight or adiposity. In this experiment, the short duration and the limited amount of rats used likely prevented a significant effect on body weight and adiposity.

3.2. Review of literature

In several studies, investigating the effects of chronic olanzapine administration in rats, osmotic minipumps have been used. However, we were able to find only four studies that measured plasma levels after minipump administration of olanzapine. Three of these also measured plasma levels after daily s.c. injections of the same dose. A summary of the plasma levels measured in these studies is presented in Table 2.

In a 1-week study aiming to establish in vivo dopamine D2-receptor occupancy levels in male rats that are similar to those seen in humans, Kapur et al. (2003) administered different doses of olanzapine through either daily s.c. injections or osmotic minipump administration. At the end of this 1-week experiment, plasma levels in the dose group receiving 7.5 mg/kg/day olanzapine by osmotic minipump were on average 434 nM (134 ng/ml).

Li et al. (2005) studied the effects of chronic olanzapine administration on maternal behavior in female rats receiving 7.5 mg/kg/day olanzapine via either osmotic minipump or daily s.c. injection. After daily s.c. injections, peak plasma levels in female rats in the 3-week study by Li et al. (2005), were higher than those in male rats receiving the same dose via daily s.c. injection in the 1-week study by Kapur et al. (2003): 5653.33 vs 3124.33 nM respectively.

In contrast, plasma levels observed after minipump administration of olanzapine in this 3-week study were only 151 nM (47 ng/ml). This is much lower than the 434 nM observed after 1 week of minipump administration of the same dose by Kapur et al. (2003), suggesting that plasma levels decreased during the course of the 3-week study by Li et al. (2005), when olanzapine was administered by minipump, but not when daily s.c. injections were administered.

Due to the length of the experiment, the rats used in the 3-week study by Li et al. (2005) would have gained more weight than in the 1-week study by Kapur et al. (2003), however it does not seem likely that this difference in body weight resulted in the nearly 3-fold lower plasma level observed after minipump administration in the 3-week study by Li et al. (2005). Faster metabolism of olanzapine in female rats could also result in lower plasma levels compared to male rats after minipump administration, however, there are no data on the pharmacokinetics of olanzapine in female rats to support this. Interestingly, the higher peak plasma levels observed in female rats (Li et al., 2005) compared to male rats (Kapur et al., 2003) suggest that metabolism of olanzapine in female rats may be slower. In human studies, women show higher plasma levels and slower clearance of olanzapine compared to men (Callaghan et al., 1999; Weiss et al., 2005). This is, at least in part, due to a lower activity of cytochrome P450 (CYP) 1A2, an enzyme that is important for the metabolism of olanzapine (Aichhorn et al., 2006; Parkinson et al., 2004; Schwartz, 2003). Although this sex-difference is not as well described in rats, one study does describe finding lower baseline activity of CYP1A2 in female rats compared to male rats (Wang et al., 2002), which could,

Table 2
Olanzapine concentrations in plasma of rats that received olanzapine via osmotic minipump or daily injections

Study	Minipump administration			Daily injections (s.c)	
	Duration	7.5 mg/kg/day	10 mg/kg/day	Duration	7.5 mg/kg
Kapur et al. (2003)	1 week	434±291[a]		1 week	3124±823[ab]
Li et al. (2005)	3 weeks	151±35.4		3 weeks	5653±2327[b]
Turrone et al. (2005)	4 weeks[c]	125±10		8 weeks	2677±202[b]
Seager et al. (2005)	1 week[d]		131±8.3[e]		

Concentrations are expressed in nM. Means±S.E.M.are shown ([a]Means±S.D.). [b]Peak plasma level, determined 2 h after final injection. [c]Two minipumps were placed consecutively for 4 weeks each. [d]Three minipumps were placed consecutively for 1 week each. [e]Values calculated from ng/ml.

thereby, result in slower metabolism of olanzapine in female rats.

Taken together, comparison of plasma levels in the studies by Li et al. (2005) and Kapur et al. (2003), suggests that plasma levels of olanzapine decreased during the course of the 3-week study by Li et al. (2005) when osmotic minipump were used.

In an 8-week study by Turrone et al. (2005) on the effects of olanzapine administration on vacuous chewing movements, olanzapine (7.5 mg/kg/day) was administered to male rats for 8 weeks either via daily s.c. injections or via osmotic minipump. After the first 4 weeks of minipump administration, the empty pump was replaced by a new one for the last 4 weeks of the experiment. When determining doses for these minipumps, the authors anticipated on weight gain occurring, thereby preventing a large decline in plasma levels due to weight gain over the course of this experiment.

At the end of this 8-week experiment, rats receiving olanzapine via daily s.c. injection, had peak plasma levels that are comparable to those observed in the 1-week study by Kapur et al. (2003) after daily s.c. injection of the same dose (2677 vs 3124.33 nM); a finding that argues against any major change in drug metabolism taking place over 8 weeks.

Conversely, in the rats receiving olanzapine via minipumps, plasma levels were on average 125 nM (39 ng/ml) at the end of the experiment by Turrone et al. (2005). This is over 3-fold lower than after minipump administration in the 1-week study by Kapur et al. (2003) and also slightly lower than after minipump administration in the 3 week study by Li et al. (2005).

Taken together, comparison of plasma levels in these studies therefore suggests that plasma levels of olanzapine decreased over time during minipump administration in the study by Turrone et al. (2005), similar to the study by Li et al. (2005) and our own.

The detection method used in the first three studies, mentioned above, was identical (using identical HPLC-MS equipment), as well as the method of drug preparation (using acetic acid). Therefore, we feel that direct comparison of the plasma levels in these studies is reliable. Furthermore, at the different time points, measurements after minipump administration differed greatly between these studies, whereas levels after daily s.c. injection were more similar. Together with our own data, this suggests that lower plasma levels of olanzapine occur over time only when osmotic minipumps are used and not with daily s.c. injections.

A different method of drug preparation (using lactic acid) as well as different HPLC-MS equipment was used in the study by Seager et al. (2005). In this 3-week experiment, plasma levels measured after minipump administration were much lower than in the study by Kapur et al. (2003): 131 vs 434 nM respectively. Osmotic minipumps were replaced every week (compensating for weight gain) and the dose administered was higher (10 mg/kg/day) in the study by Seager et al. (2005). Therefore plasma levels would have been expected to be at least as high as in the 1-week study by Kapur et al. (2003).

Similarly, our own measurements after 4 weeks of minipump administration of 7.5 mg/kg/day in experiment 1, are much lower than after 4 weeks of minipump administration of the same dose by Turrone et al. (2005): 28 vs 125 nM respectively. Discrepancies of measured plasma levels are also apparent when studies administering acute injections of olanzapine are compared. Aravagiri et al. (1999) measured plasma levels 3 h after an i.p injection of 6 mg/kg olanzapine using HPLC and electrochemical detection. These levels (38 nM; 12 ng/ml) are approximately 100-fold lower than the peak plasma levels measured 2 h after s.c. injection of 7.5 mg/kg (2677–5653 nM, see Table 2), although the dose differed only slightly. Two other pharmacokinetic studies, using different detection methods, also show very different olanzapine plasma levels: C_{max} 541 vs 1951 nM, measured 1 h after 5 mg/kg and 8 mg/kg p.o. respectively (Gunaratna et al., 2004; Chiu and Franklin, 1996).

Taken together, it seems that differences in methods and materials can have a large effect on the measured plasma level of olanzapine. Nevertheless, similar methods allow us to compare plasma levels of the first three studies described. This comparison confirms that, when osmotic minipumps are used to administer olanzapine, plasma levels decline in experiments lasting several weeks.

4. Closing remarks

To our knowledge, the apparent instability of olanzapine in solution was previously not taken into consideration in chronic experiments administering olanzapine via osmotic minipumps. It is conceivable that experiments using osmotic minipumps to administer olanzapine fail to show effects due to the degradation of olanzapine within the minipump reservoir. Conversely, if a certain dose of olanzapine does reveal an effect after chronic minipump administration, this will be an underestimation of the true effect of this dose, as plasma levels will have declined over the course of the experiment. Therefore, we hereby advise against the use of osmotic minipumps for olanzapine administration in (sub)chronic experiments.

An alternative to minipump administration is repeated injection (i.p. or s.c.) or oral gavage. However, in order to achieve constant plasma levels, several injections/gavages per day would be necessary, over the course of several weeks. This is stressful for the animals and thus could influence experimental outcome. A different, more elegant, solution might be a depot-injection of an olanzapine-salt that gives of olanzapine at a fixed rate for several weeks (e.g. olanzapine-pamoate, currently being developed by Eli Lily). Mixing olanzapine in either food or drinking water is another approach to achieve more constant drug levels, but the change in flavor caused by the addition of olanzapine (it has a bitter taste) could influence experimental outcome. Furthermore, plasma levels will be much lower during the light phase, as animals eat and drink very little in this period.

Continuous infusion, either intragastrically, subcutaneously, intraperitoneally or intravenously, using an external pump filled with an olanzapine-solution would be another option. In this set-up the lower temperature of the solution (21 vs 37 °C) will result in a slower rate of degradation and the solution can easily be refreshed regularly without disturbing the animal.

Finally, when using osmotic minipumps to administer a compound, it is important to take into account the stability in a

warm, humid environment. If degradation occurs, not only will the amount of drug delivered diminish over the course of the experiment, but degradation products that are spontaneously formed within the minipump will also be co-infused with the drug of interest. Only drugs that are (relatively) stable in a solution at body temperature will result in truly constant drug delivery from an osmotic minipump and, therefore, enable accurate and reliable experimental results.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejphar.2007.11.078.

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