Mianserin and trazodone significantly attenuate the intensity of opioid withdrawal symptoms in mice

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Abstract
The aims of this study were to evaluate the effects of trazodone and mianserin on opioid-withdrawal symptoms in morphine-dependent mice. We used a comparative study of the effect of each drug on withdrawal symptoms in one model of acutely high-dose morphine-dependent mice, and two models (high-dose and low-dose) of chronically morphine-dependent mice at the Tel Aviv University Sackler School of Medicine’s laboratory. Trazodone, mianserin or both were given to the morphine-dependent mice together with a high dose of naloxone. Intensity of withdrawal symptoms was evaluated by tail-flick assay latencies and three behavioural measurements (rearing, jumping and grooming) in each group. Trazodone and mianserin, each separately, significantly attenuated withdrawal symptoms in all three models. However, the combined treatment of trazodone together with mianserin was not superior to each drug alone. The combination of trazodone and mianserin has no additive value to each drug alone in the control of withdrawal symptoms in opiate-dependent mice undergoing detoxification. When used in clinical settings, caution is needed in order to prevent the unknown influence of opioid-like drugs in medication-assisted detoxification programmes if complete opiate detoxification is the aim.

Introduction
Good control of opioid withdrawal symptoms is the first step in any withdrawal and rehabilitation programme for opiate-dependent people. Some antidepressant drugs may help to control some of the physical discomfort which is part of withdrawal symptoms, due to their interaction with various neurotransmitter mechanisms, and are therefore usually included in detoxification protocols. In previous studies we evaluated the antinociceptive properties of mianserin and trazodone using the mouse hotplate assay, and found both of them to interact with the opioid system. Mianserin is a tetracyclic antidepressant with potent serotonergic properties, being a strong 5-
HT2 (and to a lesser extent also 5-HT1 and 5-HT3) receptor agonist, with mild to moderate noradrenergic sedating properties due to a pre-synaptic α2 blockade and a strong antihistamine (H1 receptor antagonism) effect. Mianserin and its active metabolites have a half-life of 24–32 hours in the plasma; they lack anticholinergic side effects and are much less cardiotoxic than tricyclics, thus employed in the treatment of depression in elderly and in physically-ill patients. When injected alone i.p., mianserin elicited a biphasic dose-dependent antinociceptive effect abolished by naloxone, indicating a possible involvement of opioid mechanisms. Further evaluation with various opiate agonists and antagonists indicated a significant interaction with μ-, κ1- and κ3-opioid receptor subtypes, but not with the δ-opioid receptor.3

Trazodone is a triazolopyridine derivative with an antidepressant activity and has been in clinical use for more than 20 years. Its overall pharmacological profile differs from each of the other classes of psychotropic drugs, of which it bears some resemblance to their action, i.e. benzodiazepines, antipsychotic phenothiazines and the tricyclic antidepressants. In vitro trazodone is a weak but specific inhibitor of the synaptosomal uptake of serotonin and it binds to α1- and α2-adrenoreceptor sites. Whereas mianserin shows equal affinity for α1 and α2 sites, trazodone reacts with central serotonin receptors in the frontal cortex, an antagonistic activity unlike that of mianserin. Moreover, trazodone has been shown to bind to opioid receptors as well, but only at much higher concentrations than those needed for the interaction with the serotonin- and noradrenergic receptors. When studied in the mouse hotplate assay model, trazodone was found to induce a potent μ1- and μ2-opioid receptor-mediated antinociception.4

Since the interactions of trazodone and mianserin with the opioid system differ regarding the opioid-receptor subtypes involved, we conducted a two-arm study, one animal (basic study), the other—human (clinical study,5 submitted) in order to evaluate the possible use of these two antidepressants to attenuate opioid withdrawal symptoms. The present study reports our findings regarding the effects of treatment with trazodone or mianserin, or both drugs together in three different models of morphine-dependent mice: chronically low-dose morphine-dependent mice,6 chronically high-dose morphine-dependent mice7 and acutely high dose morphine-dependent mice.8,9

Material and methods
Animals
Male naive (ICR) mice (25–35 g, 5–6 weeks old) were purchased from the Levinstein Colony (Yokneam, Israel). The mice were housed in groups of 20 under standard conditions and maintained on a 12-hour light–12-hour dark cycle, with Purina rodent chow and water ad libitum. The animals were divided randomly into groups of five and transferred to the laboratory at least 1 hour before starting the treatments. Each animal was used once. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Sackler Faculty of Medicine at Tel Aviv University (# 11-01-014) and complies with the guidelines for animal experimentation of the National Institute of Health, Bethesda, USA [DHEW Publication (NIH) 85-23, revised, 1995].

Drugs
Several agents were kindly donated, as follows: morphine sulphate was a generous gift from TEVA (Jerusalem, Israel), mianserin HCl was a generous gift from Rafa (Jerusalem, Israel), trazodone from Unipharm (Ramat-Gan, Israel), naloxone HCl was obtained from the Research Technology Branch of NIDA. All other compounds were purchased from commercial sources. All the drugs were dissolved in saline and injected subcutaneously (s.c.).

Morphine treatment
Three different treatment groups were used. Two of the groups received a chronic morphine treatment, while the third group was injected acutely. Two groups of mice received 8 days of morphine injections. In the first group (high morphine) animals were injected with increasing doses of morphine (10–80 mg/kg) twice a day.7,10 In the second group (low morphine) the animals were injected 10 mg/kg twice a day for 4 days and 20 mg/kg twice a day for the following 4 days.6 The third group (acute morphine) received a single high dose of morphine (100 mg/kg), which produced an acute tolerance to mor-
All the animals in the three groups were divided randomly into the following subgroups: 1, morphine + naloxone; 2, morphine + naloxone + mianserin; 3, morphine + naloxone + trazodone; and 4, morphine + naloxone + mianserin and trazodone. Mianserin was injected 40 minutes before the observation followed by naloxone and trazodone injections 30 minutes later. Naloxone (1 mg/kg), mianserin (25 mg/kg) and trazodone (50 mg/kg) were given at the same dose to all their indicated subgroups. The dosages of naloxone, mianserin and trazodone were chosen based on our previous experiments.3,4

Assessment of physical dependence
The intensity of the withdrawal symptoms was determined using the method that was used by Hamdy and colleagues.11 In brief, immediately after the naloxone injection, each animal was placed into a transparent glass cylinder (20 cm in diameter and 30 cm in height) to observe withdrawal manifestations (jumping, rearing and grooming) for 10 minutes. The withdrawal manifestations were counted manually, by co-workers blind to the treatment protocol.

Antinociception assessment
Antinociception was determined by utilizing the radiant heat tail-flick technique.12 The latency to withdraw the tail from a focused light stimulus was measured electronically, using a photocell. In the chronic treatment groups, baseline latencies (as the means of two trials) were taken twice, one before the beginning of the morphine treatment (2.0–3.0 seconds) and the second time on the day 9 before the naloxone injection (6–10 seconds). In the acute group baseline latencies (2.0–3.0 seconds) were taken before starting the experimental treatments and before the naloxone injection (4 hours after the morphine injection). Post-treatment latencies were determined as indicated for each experiment and a maximal latency of 10 seconds was used to minimize tissue damage. The latencies were taken at the end of the withdrawal observation. Antinociceptive effects were defined by calculating the differences between the baseline latencies and the experimental latencies for each mouse.

Statistical analysis
The basic model which was applied on the data was a 3 × 4 ANOVA and correction by the Bonferroni post-hoc test. Results are expressed as mean ± SD or as mean ± SEM. The main factors were morphine treatment (acute, low-chronic, high-chronic) and drug treatment (naloxone, naloxone + trazodone, naloxone + mianserin, naloxone + trazodone + mianserin). The dependent variables were tail-flick (reaction time) before and after drug injection, and observations of three behaviours: rearing, jumping and grooming.

Results
Tail-flick latencies are presented in two complementary ways: one as reaction time in seconds (RT) and the other in percentage of changes of reaction time. Baseline tail-flick measures showed normal distribution, with a mean reaction time of 6.88 (SD = 1.73). A one-way ANOVA comparing the three treatment groups was performed on the baseline RTs and revealed a significant difference between treatments ($F(2,117) = 14.75$, $p < 0.001$), due to higher RTs of the acute group compared to both chronic groups (both $p < 0.05$). Test tail-flick measurements showed normal distribution; the general tendency was to lower the RTs with a mean RT of 6.31 (SD = 2.41). A $3 \times 4$ ANOVA in which the main factors were treatment and drug showed a significant drug effect ($F(3,108) = 22.32$, $p < 0.001$) and a significant treatment × drug interaction ($F(6,108) = 4.62$, $p < 0.001$). The source of the interaction was that the two chronic groups did not differ following any of the drug injections, and the acute group was similar to the chronic groups following naloxone and naloxone + trazodone treatments. However, acute morphine treatment led to higher RTs compared to both chronic treatments following naloxone + mianserin (both $ps < 0.01$), while lower RTs were found following naloxone + trazodone + mianserin (both $ps < 0.05$) (Fig. 1).

The percentage change of RT from baseline was calculated for each subject ((test/baseline) × 100). Thus, a score of 100 reflects no change from baseline, scores higher than 100 indicate an increase while lower than 100 a decrease of test RT from baseline. The general tendency was a decrease of test RTs compared to
baseline, with a mean of 92.83 (SD = 30.47). A 3 x 4 ANOVA with main factors of treatment and drug showed significant treatment and drug effects ($F(2,108) = 13.35, p < 0.001; F(3,108) = 18.37, p < 0.001$, respectively) and a significant treatment x drug interaction ($F(6,108) = 3.17, p < 0.01$). The interaction was due to the lack of differences between the two chronic treated groups under any of the drug conditions. The acute group did not differ from the chronic group after naloxone alone or naloxone combined with mianserin. Following the naloxone + trazadone combination no change from baseline was observed for the chronic groups but a reduction to 79% was present in the acute group. This reduction was significantly different from the chronic groups (both $p < 0.05$). Following, naloxone + trazodone + mianserin, the two chronic groups showed a 10% increase from baseline while the acute group a decrease to 56% of baseline RTs (both $p < 0.001$) (Fig. 2).

The analysis of the behavioural observation data showed that of the entire animals used, 35 subjects showed no grooming, 76 no jumping and 45 no rearing. Therefore the logarithmic transformation of the combination of the three behaviours (sum of rearing, jumping, and grooming) was analysed using a 3 x 4 ANOVA model (Fig. 3).

The analysis of the logarithmic transformation of rearing revealed significant treatment and drug effects ($F(2,108) = 8.93, p < 0.001; F(3,108) = 61.79, p < 0.001$, respectively) and a significant treatment x drug interaction ($F(6,108) = 2.20, p < 0.05$). Chronic groups did not differ under any drug condition. Acute treatment led to generation of more rearing compared to the two chronic groups following naloxone + trazodone (both $p < 0.01$), and naloxone + trazodone + mianserin (low: $p < 0.05$; high: $p < 0.01$), but not following naloxone alone or when combined with mianserin (Table 1). The analysis of the logarithmic transformation of jumping revealed a significant treatment and drug effects ($F(2,108) = 16.53, p < 0.001; F(3,108) = 3.63, p < 0.05$, respectively), and a significant treatment x drug interaction.

Figure 1. Time to tail-flick results of the three morphine-dependent models of mice treated with naloxone (Nal), naloxone + trazodone (Nal + Traz), naloxone + mianserin (Nal + Mian) and naloxone + trazodone + mianserin (Nal + Traz + Mian). *$p < 0.05$ from the acute group; **$p < 0.01$ from the acute group; n ≥ 10.
Figure 2. Change of time to tail-flick results of the three morphine-dependent models of mice treated with naloxone (Nal), naloxone + trazodone (Nal + Traz), naloxone + mianserin (Nal + Mian) and naloxone + trazodone + mianserin (Nal + Traz + Mian). *p < 0.05 from the acute group; **p < 0.01 from the acute group; n ≥ 10.

Figure 3. Behaviours (jumping, grooming and rearing) results of the three morphine-dependent models of mice treated with naloxone (Nal), naloxone + trazodone (Nal + Traz), naloxone + mianserin (Nal + Mian) and naloxone + trazodone + mianserin (Nal + Traz + Mian). *p < 0.05 from the acute group; **p < 0.01 from the acute group; n ≥ 10.
(F(6,108) = 2.87, p < 0.05). Similar to rearing, chronic groups did not differ under any drug condition. Acute treatment led to more jumping compared to the two chronic groups following naloxone + trazodone (both ps < 0.05), and naloxone + trazodone + mianserin (both ps < 0.01), but not following naloxone alone or when combined with mianserin (Table 1).

The analysis of the logarithmic transformation of grooming revealed a significant drug effect (F(3,108) = 24.51, p < 0.001) and a significant treatment × drug interaction (F(6,108) = 2.59, p < 0.05). However, unlike the findings with rearing and jumping, the only observed difference for grooming was following naloxone + mianserin due to more rearing of chronic-high subjects compared to chronic-low and acute subjects (both ps < 0.01) (Table 1).

The analysis of the logarithmic transformation of all three behaviours revealed significant treatment and drug effects (F(2,108) = 12.21, p < 0.001; F(3,108) = 28.62, p < 0.05, respectively), and a treatment × drug interaction that almost approached significance (F(6,108) = 2.11, p = 0.058). Acute treatment led to more behavioural activity compared to the two chronic groups following naloxone + trazodone (both ps < 0.05) and naloxone + trazodone + mianserin (both ps < 0.01), but not following naloxone alone or when combined with mianserin, while the chronic groups did not differ (Fig. 3).

**Discussion**

In this study we show, in three different models of addiction, that adding either trazodone or mianserin to naloxone that is given to morphine-dependent mice is effective in attenuating opiate withdrawal intensity in response to a high dose of an antagonist challenge. The possibility to modify the intensity of the opiate withdrawal symptoms using various substances has been extensively studied in animals. In clinical settings the use of various medications in order to reduce withdrawal symptoms is common practice, and the routine protocol of drug treatment during the acute and subacute phases of opiate-dependent people admitted voluntarily to the Jaffa center (Tel Aviv, Israel) includes medications which attenuate physical opiate-withdrawal symptoms, i.e. clonidine (α2-adrenergic agonist), chlordiazepoxide (benzodiazepine), dipyrone (analgesic and antipyretic), papaverine (smooth muscle

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<tr>
<th>Nal</th>
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<tr>
<td>Rearing</td>
<td>Grooming</td>
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<td>27.1 ± 18.1</td>
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<td>Naloxone (Nal), naloxone + trazodone (Nal + Traz), naloxone + mianserin (Nal + Mian) naloxone + trazodone + mianserin (Nal + Traz + Mian). n ≥ 10.</td>
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relaxant), metoclopramide (antiemetic), phenobarbital (sedative barbiturate) and levomepromazine (major tranquilizer) (Herman & Shamir, personal communication). Based on findings in previous studies, which were aimed at assessing the antinociceptive effects and the interaction with opioid mechanisms of antidepressant drugs), we hypothesized the possible benefit of using either trazodone or mianserin or both in order to attenuate withdrawal symptoms in people undergoing opioid detoxification programmes. As a first step, we tested the possible validity of this hypothesis in three different models of morphine dependence in mice, two chronic and one acute. Although bearing the least resemblance to clinical opioid-dependence in humans, we decided to include in the study the model of acute, high-dose morphine dependence in mice in order to encompass different aspects of opioid-addiction.

We found a great similarity between the effects of treatment in the two chronic (high-dose and low-dose) models of morphine-dependence we used (the models with a high resemblance to the clinical picture in humans) and clear differences between the effect of treatment in the chronic vs. the acute, high-dose morphine-dependence models. Nevertheless, as a whole each drug separately (trazodone and mianserin) has attenuated significantly the clinical picture of withdrawal seen in each model, but the combination of the drugs showed little (if at all) benefit.

The reason for the beneficial effect of trazodone and, separately, of mianserin on opioid-withdrawal symptoms is clearly secondary to each drug's profile of interaction with the serotonergic system and with various opioid receptors, as shown in previous studies. However, the lack of a positive effect of the combined trazodone + mianserin treatment is surprising and needs elaboration. One possible explanation to the lack of additive value to the combined treatment with trazodone and mianserin together may derive from mianserin’s “therapeutic window” effect, which could be manifested once trazodone was added. Another possible reason may arise from the differences between the profile of interaction of each drug with the opioid system (different opioid subtypes of receptors) and the difference in the combination of the mixed opioid–serotonergic, opioid–noradrenergic and serotonergic–noradrenergic interactions of each drug. Moreover, while trazodone's serotonergic interaction is at the pre-synaptic reuptake inhibition, mianserin interacts at post-synaptic serotonin receptor sites, inducing a different profile of clinical effects and of side effects. This difference at the pre- vs. postsynaptic serotonergic interaction is evident and at times used sophisticatedly when treating depression, but may be problematic when treating opioid withdrawal symptoms.

The tail-flick assay findings also need some elaboration. On one hand, they replicate the antinociceptive studies, which suggested the possible use of trazodone and mianserin during opiate detoxification. The replication of findings in a different model of acute pain adds to the validity of findings in the original studies. However, each drug's antinociceptive effect is not solely induced through opioid mechanisms, but rather through a complex involvement of serotonergic and noradrenergic mechanisms as well. Thus the findings in pain models alone would not have been enough to indicate possible beneficial effects of adding either trazodone or mianserin or both to opioid-detoxification drug treatment protocols. The clear effect of trazodone and mianserin on the reversal of behavioural changes (as seen in rearing, jumping and grooming) precipitated in morphine-dependent mice by injection of naloxone indicates a major attenuation of withdrawal symptoms when trazodone is added, and a lesser but still significant attenuation when mianserin or both trazodone and mianserin are added.

The findings of the present study suggest a possible beneficial use of either trazodone or mianserin in the routine protocol of treatment of opioid detoxification programmes. However, a problematic question may arise regarding the unknown substitution of morphine with other drugs which possess opioid properties, when substitution is not sought. There already exists different substitute programmes of treatment with either methadone or buprenorphine and the aim of detoxification is not to maintain people on other medications with opioid interactions, even such of low grade. Withdrawal symptoms have already been described upon discontinuation of treatment with some antidepressants. They were usually attributed to the effects of the antidepressants on the serotonergic system, which may result in noradrenergic rebound after discontinuation, or to a rebound excess of cholinergic activity after prolonged anticholinergic effect on cholinergic receptors.
mianserin and trazodone lack cholinergic activity—the presence of withdrawal symptoms upon abrupt discontinuation of treatment with these drugs may derive from their involvement with the opioid system. We therefore conclude this study with a word of caution: trazodone and, separately, mianserin may help attenuate opioid withdrawal symptoms, but they do it through opioid mechanisms.

Acknowledgement
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References