A new pharmacological role for thalidomide: Attenuation of morphine-induced tolerance in rats

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ABSTRACT

Objective: Tolerance to the analgesic effect is the main side effect of chronic administration of opioids. Several drugs have been studied to try to find agents to prevent the development of this phenomenon. In the present study we aimed to evaluate the effect of thalidomide on morphine-induced tolerance to the analgesic effect.

Methods: Groups of male rats were randomly rendered and received daily morphine in combination with thalidomide vehicle or thalidomide (2.5 mg/kg, 5 mg/kg, or 10 mg/kg, intraperitoneally). Nociception was measured using the plantar test apparatus. Latency time was recorded when the animal reacted to the light stimulus; licking or raising its hind paw. Treatments and evaluations continued until completion of tolerance to the analgesic effect of morphine.

Results: Our findings indicated that tolerance was achieved following 11 days of morphine administration, while thalidomide postponed the day of tolerance completion for 4 days (2.5 mg/kg and 5 mg/kg thalidomide) or 10 days (10 mg/kg thalidomide). Moreover, thalidomide prevented the morphine-induced shift to the right of the ED50 in the dose response curve.

Conclusion: It was concluded that thalidomide attenuated the morphine-induced tolerance to the analgesic effect.

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There is evidence indicating that thalidomide inhibits the production of human monocyte TNF-α and alveolar macrophages.\textsuperscript{16,17} Thalidomide, a glutamic acid derivative, was approved in 1998 by the US Food and Drug Administration for erythema nodosum leprosum and in 2006 for multiple myeloma.\textsuperscript{18} This drug was a common over-the-counter sedative and antiemetic until 1961, when it was withdrawn because of teratogenicity.\textsuperscript{19} In 1965 an accidental discovery of its immunomodulatory effects was made in erythema nodosum leprosum patients.\textsuperscript{20,21} Today thalidomide and its analogs have shown efficacy against a wide variety of diseases, including inflammation and cancer.\textsuperscript{22} Thalidomide exerts its inhibitory action on TNF-α by enhancing mRNA degradation.\textsuperscript{23} In addition, it was reported to inhibit inflammatory hyperalgesia in rats and the writhing nociceptive response in mice, possibly due to inhibition of TNF-α production.\textsuperscript{7}

The evidence from these studies prompted us to investigate the effect of thalidomide on morphine-induced tolerance to the analgesic effect.

2. Materials and methods

2.1. Drugs

Morphine sulfate was purchased from Temad Company (Tehran, Iran). It was dissolved in normal saline and injected using 1-mL insulin syringes. Thalidomide (Sigma-Aldrich Inc., Chemie GmbH, Germany) was dissolved in vehicle (dimethyl sulfoxide + saline, 4:1) and injected intraperitoneally (i.p.). All the solutions were freshly prepared on the day of the experiment.

2.2. Animals

Male Wistar rats (n = 88) weighing 250–300 g were purchased from the Razi Institute (Tehran, Iran). The animals were kept in temperature-controlled conditions (25 ± 2°C) and standard cages (four rats per cage), on a 12-h light/dark cycle with free access to food and water ad libitum. They were randomly divided into several experimental groups, eight animals per group. In order to minimize the nonspecific stress response, animals were habituated to the testing environment, including transfer to the experimental laboratory, weighing, and handling. All the experiments were in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85–23, revised 1985), and were approved by the research and ethics committee of Kurdistan University of Medical Sciences.

2.3. Experimental details

The experimental groups are described in Table 1.

<table>
<thead>
<tr>
<th>Study sections</th>
<th>Treatment groups (n = 8 per group)</th>
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</thead>
<tbody>
<tr>
<td>Tolerance evaluation groups</td>
<td>Saline (1 mL/kg, i.p.) + thalidomide vehicle\textsuperscript{*} (1 mL/kg, i.p.)</td>
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<td></td>
<td>Morphine (10 mg/kg, i.p.) + thalidomide vehicle (1 mL/kg, i.p.)</td>
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<td>Morphine (10 mg/kg, i.p.) + thalidomide (2.5 mg/kg, i.p.)</td>
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<td>Morphine (10 mg/kg, i.p.) + thalidomide (5 mg/kg, i.p.)</td>
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<td>Morphine (10 mg/kg, i.p.) + thalidomide (10 mg/kg, i.p.)</td>
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<td>Groups for dose–response curves:</td>
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<td>Animals received opposite treatments for 11 days, on Day 12, in separate groups, logarithmic doses of morphine (1/10/100 mg/kg, i.p.) were administered to generate analgesic dose–response curves</td>
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\textsuperscript{*} Thalidomide vehicle = dimethyl sulfoxide + saline, 4:1.

2.3.1. Model of tolerance induction

In order to induce a tolerance to the analgesic effect, morphine (10 mg/kg, i.p.) was injected daily according to our previous study.\textsuperscript{24} It was injected daily 30 minutes after the thalidomide administration.

2.3.2. Assessment of nociception

The nociception was assessed using a plantar test apparatus (IITC Inc. Life Science, Los Angeles, USA) (Hargreaves method).\textsuperscript{25} Rats were placed on a glass plate on the plantar test apparatus and a noxious heat source was held directly under the hind paw. As soon as it was started, the device supplied a continuous beam stimulus to the paw and the withdrawal reflex was produced. The latency time between exposure to the radiant and the paw withdrawal was measured as the analgesia. For each animal the average for three measurements of the baseline paw-withdrawal latency was determined as the baseline latency.

The intensity of the light was adjusted so that the baseline latencies were 8–10 seconds, with a cut-off time of 20 seconds in order to avoid tissue damage. Two measurements of the latency were averaged for each hind paw in each test session. Maximal possible effect (%MPE) using the following equation was measured for the latency-withdrawal response for each rat:

\[
\text{%MPE} = \frac{\text{[postdrug latency (s) – baseline latency (s)] / [cut-off value (s) – baseline latency (s)]} \times 100}{100}
\]

It is worth noting that the baseline latency was determined once per day for each rat before the daily injection of morphine (10 mg/kg). Later, the drugs or their vehicles were injected. Thirty minutes after drug/vehicle administration, morphine was injected. Finally, 30 minutes after morphine administration, the postdrug latency was measured. Moreover, the baseline and the latency time were registered daily and the %MPE was calculated. The experiments were continued until there was no significant difference in the %MPE between the vehicle- or the drug-treated groups (the tolerant animals) and the vehicle-received group.\textsuperscript{26}

2.3.3. Total analgesic effect assessment

In order to assess the total analgesic effect in different groups, the area under the curve (AUC) for %MPE against the time was calculated. This analysis allows a comparison of the effects from different analgesic tests. The AUC was calculated from the observed values using the trapezoidal rule.

2.3.4. Analgesic dose–response curves

The dose–response curve was plotted for each group for assessment of tolerance induction. Rats received the thalidomide vehicle or the morphine + thalidomide vehicle or morphine + thalidomide once a day for 11 days. On Day 12 (1 day after the morphine-tolerance
completion in the control group), the logarithmic doses of the morphine (1 mg/kg, 10 mg/kg, 100 mg/kg, i.p.) were injected to create the analgesic dose–response curve. In these curves, the morphine antinociceptive 50% effective dose (ED50) values for each group were derived using the linear regression method.27

2.4. Thalidomide analgesic effect assay

In order to find the possible analgesic effect of thalidomide, animals received only the most effective dose of the thalidomide (10 mg/kg, i.p.) or its vehicle. Another test was done 60 minutes later (similar to treatment in assessment of nociception section) to verify whether the observed effect of thalidomide was not simply because of additive mechanism.

2.5. Data analysis

All data from the analgesia tests was presented as the mean of %MPE ± SEM for each group (n = 8). The independent t-test or repeated analysis of variance tests followed by the Bonferroni test were applied to analyze the statistical significance in two or multiple comparisons, respectively. In all analyses, p < 0.05 was considered to be significant.

3. Results

3.1. Tolerance to the analgesic effect of morphine

As shown in Figure 1, on Day 11, there was no significant difference between the control group and the saline + thalidomide vehicle-treated animals; therefore, this day was considered as the date for morphine-tolerance completion. Thus it was found that daily administration of morphine (10 mg/kg, i.p.) for 11 days developed tolerance to the analgesic effect in the control group that received morphine + thalidomide vehicle.

3.2. Effect of thalidomide on the morphine-induced tolerance to the analgesic effect

The results reveal that thalidomide (2.5 mg/kg, 5 mg/kg, 10 mg/kg, i.p.) delayed the tolerance completion for 4, 4, and 10 days, respectively (Figure 1). Furthermore, according to Figure 2, the dose–response curve depicts a significant shift to the right in the animals that received morphine + thalidomide vehicle compared to those which received the thalidomide vehicle or the morphine + thalidomide (10 mg/kg, i.p.) treated group (69.75 mg) compared to the control animals (95.9 mg).

Furthermore, the results showed that the most effective dose of thalidomide (10 mg/kg, i.p.) had no significant analgesic effect (Figure 3). Data analysis for total analgesic effect revealed that the dose of 10 mg/kg thalidomide had the greatest AUC of the %MPE compared to the control group (1627.7), the 2.5 mg/kg thalidomide group (1608.2), or the 5 mg/kg thalidomide group (1724.5) (Figure 4).

4. Discussion

According to the results, chronic administration of morphine for 11 days developed a tolerance to its analgesic effects. Several efforts have been performed to find the possible mechanisms of morphine tolerance. These evidences indicate that chronic morphine administration leads to elevation of proinflammatory mediators and glial activation in the morphine-tolerant animal’s spinal cord;
Moreover, thalidomide has potent immunomodulatory and anti-TNF-α properties. It inhibits the chemotaxis and cytokine-induced nuclear factor kappa B transcription factor gene expression. Therefore, our results also showed that thalidomide prevented the shifting of the dose–response curve and the ED₅₀ to the right, the phenomenon occurring in morphine-tolerant group.

Another possible mechanism for morphine tolerance is an increase of nitric oxide or induction of guanylyl cyclase and agents that inhibit nitric oxide synthase expression, attenuating the development of morphine tolerance and dependence. On the other hand, it was shown that thalidomide has a beneficial effect in neuropathic pain by decreasing cytokines (TNF-α and nitric oxide levels). Therefore, thalidomide may provide a promising novel therapeutic approach for management of morphine-induced tolerance to the analgesic effect.

5. Conclusion

According to the results it was concluded that thalidomide prevented morphine-induced tolerance to analgesic effects. This effect might be related to its properties in the inhibition of TNF-α, prevention of microglial activation, and/or glutamate neurotoxicity.

Acknowledgments

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References


**Figure 4.** AUC of percentage of SMPE was calculated for each group for 21 days. To calculate the AUC, the trapezoidal rule was used. One-way analysis of variance followed by Tukey’s test was used to analyze the differences between the control and treatment groups. In all analyses, p < 0.05 was considered to be significant. * p < 0.001; when compared to the control (Mor + Tal Veh) group. AUC – area under the curve; Mor – morphine; SMPE – maximal possible effect; Sal – saline; Tal – thalidomide; Veh – vehicle.


