https://doi.org/10.31925/farmacia.2024.5.24 **ORIGINAL ARTICLE**

ANTI-INFLAMMATORY EFFECTS OF LOCALLY *VERSUS* SYSTEMIC INJECTED MORPHINE ON CHEMICALLY INDUCED INFLAMMATION: AN EXPERIMENTAL ANIMAL STUDY

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Abstract

The current role of opioids in medical practice focuses on their well-known analgesic effect, but recently different studies were aimed to analyse their potential anti-inflammatory effect at both local and systemic levels. Our experimental animal study was aimed to determine the effects of different concentrations of morphine injected either locally or intraperitoneally on chemically inflamed paw volumes. Male Wistar rats were randomly assigned to six groups and injected with a single intraplantar injection of carrageenan 1% and subsequently another injection with either normal saline or morphine according to their group. Measurements with a plethysmometer were taken at 0, 6, 24, 48 and 72 hours after the first injection. Our experiment shows a statistically significant decrease in paw volume of rats injected with 5mg/kg of intraplantar morphine sulphate, thus demonstrating a local anti-inflammatory effect of high-dose morphine. These data provide evidence that opioids do have a role in inflammation and suggest that they could play a therapeutic role in anti-inflammatory treatment.

Rezumat

Rolul actual al opioidelor în practica medicală se concentrează pe bine cunoscutul lor efect analgezic, dar studii recente au avut ca scop analiza potențialului lor efect antiinflamator atât la nivel local, cât și la nivel sistemic. Studiul nostru experimental pe animale de laborator a avut ca scop determinarea efectelor diferitelor doze de morfină injectate local - intraplantar, respectiv intraperitoneal asupra volumului lăbuțelor cu edem inflamator produs prin injectarea locală de caragenină. Șobolanii masculi Wistar au fost repartizați aleatoriu în șase grupuri. Tuturor le-a fost administrat intraplantar în lăbuța posterioară dreaptă 0,15 ml soluție de caragenină 1%, pentru inducerea inflamației și ulterior o a doua injectare cu soluție salină intraplantară sau intraperitoneală (grupul martor) sau cu morfină intraplantară sau intraperitoneală (grupurile testate). Măsurătorile au fost efectuate cu un pletismometru Ugo Basile 7140 la 0, 6, 24, 48 și 72 de ore după prima administrare. Experimentul arată o scădere semnificativă statistic a volumului lăbuței șobolanilor injectați cu 5 mg/kg de sulfat de morfină intraplantar, demonstrând astfel un efect antiinflamator local al morfinei în doze mari.

Keywords: Morphine, carrageenan, anti-inflammatory effect, intraplantar administration

Introduction

Opioids and anti-inflammatory drugs represent the most commonly used treatment to manage both post-operative pain, as well as pain associated with a significant number of chronic or degenerative musculo-skeletal disorders [20] Currently, most non-steroidal anti-inflammatory drugs are readily available as over-the-counter medications, however, they do come with renal, gastric, cardiovascular, haematological and hepatic adverse effects [3, 7, 15]. On the other hand, opioids, with their extensive pharmacological profile, are commonly used as extremely efficient pain relievers, but they also have been associated with significant well-known side effects such as constipation, respiratory depression and sedation, as well as the potential for abuse [2, 12].

Various studies have debated whether opioids might also have an anti-inflammatory mechanism of action [23, 27, 32] which may play a synergistic role in achieving their analgesic effect [14]. In colitis-induced mice, the administration of μ-opioid receptors agonists both preventively and therapeutically showed reduced mortality and reduced inflammation compared to the untreated mice [17]. Another experimental study found that morphine had an anti-inflammatory effect by reducing oedema and neutrophil buildup in carrageenan-induced peripheral inflammation [6]. These different experiments and the underlying mechanisms lead to the hypothesis that opioids not only possess an anti-inflammatory effect but that it can be triggered *via* different methods of drug administration [1, 29].

Based on these findings, we designed and conducted an experimental study to investigate the potential

effect of morphine sulphate on experimentally induced inflammation.

We postulated that intraplantar, compared with intraperitoneal, injections of morphine sulphate could reduce carrageenan-induced paw inflammation and oedema in mice. Also, we aimed to assess the comparative effect of two different dosages to determine whether a greater dose would have a greater anti-inflammatory effect.

Materials and Methods

The current study protocol was approved by the Ethics Committee of "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania (7590/ 22065) for studies involving animals, in conformity with the legislation regarding animal protection used in scientific purposes.

Animals

Forty-two male Wistar rats, age 6 weeks and weighing approximately 300 grams each were provided by the bio-base of "Carol Davila" University of Medicine and Pharmacy in Bucharest. The number of animals *per* group was calculated based on the expected variance, so that the test power would be at least 80%. Animals were given one week to adjust before commencing the experiment. The study was carried out in the Department of Pharmacology. The experiment took place over 3 days and after the experiment, the animals were euthanised under general anaesthesia.

Experimental Procedures

Animals were randomly assigned into six groups and kept in a separate housing with unlimited access to food and water for the duration of the study. The environment remained unchanged (light, temperature, humidity). All animals were marked at the level of the tibio-tarsal joint of both hind paws with a water-resistant marker. Baseline measurements with a plethysmometer (Ugo Basile 7140, Gemonio, Italy) of both hind paws were initially performed and this was designated as time zero (T0). After that, all groups received a single intraplantar injection of 0.15 mL carrageenan 1% in the right paw, and another injection according to their group: Group 1: treated with an intraplantar injection of 0.9% saline (Negative control); Group 2: treated with an intraperitoneal injection of 0.9% saline (Negative control); Group 3: treated with an intraplantar injection of morphine sulphate 1 mg/kg (test 1); Group 4: treated with an intraplantar injection of morphine sulphate 5 mg/kg (test 2); Group 5: treated with an intraperitoneal injection of morphine sulphate 1 mg/kg (test 3); Group 6: treated with an intraperitoneal injection of morphine sulphate 5 mg/kg (test 4). All animals received the same volume of injectate (either morphine or 0.9% saline). Volume measurements of the right and left paws were performed using the same plethysmometer for all groups at 6 hours (T1), 24 hours (T2), 48 hours (T3) and 72 hours (T4). Volume was expressed as mL of dislodged fluid. The two negative control groups injected with 0.9% saline solution were used to evaluate inflammation. All measurements were also compared with the untouched left hind paw.

Statistical analysis

Statistical analyses were performed using SPSS 28.0 (SPSS Inc®, Chicago, IL, USA). Data are presented as mean ± standard deviation of the mean. Data distribution was examined to insure the proper statistical examination. Quantitative data were analysed with independent samples t-test. Mann-Whitney test was used when the analysed data did not follow a normal distribution. All P values are two-tailed and a P value of less than 0.05 was considered statistically significant.

For the interpretation of the statistical data below, we analysed the results of the Shapiro-Wilk test, which is used to assess whether data sets follow a normal distribution. In the Shapiro-Wilk test, the significance value (Sig.) is crucial in deciding whether to reject the null hypothesis, which states that the data are normally distributed.

For the control groups, most tests show that the data are not normally distributed at several time points. In the case of morphine administration at 1 mg/kg, most groups show a normal distribution. In the case of morphine administration at 5 mg/kg, there are some time points where the data are not normally distributed (T2 intraperitoneal and T1/T3 intraplantar). This suggests that statistical tests should be chosen carefully based on the normality of the data distribution. If the data are not normally distributed, non-parametric tests should be used instead of parametric ones. Given the above (that most of the data are not normally distributed), we opted to use the non-parametric Mann-Whitney test instead of parametric tests.

Results and Discussion

Based on the control group we observed a significant increase in paw volume from T0 to T1 ($p = 0.01$), and a non-significant decrease thereafter throughout the duration of the experiment, reaching a non-significant difference at T4 compared to T0 ($p = 0.15$) (Figure 1).

Firstly, we compared the anti-inflammatory effect of 1 mg/kg body weight of intraperitoneal morphine with the intraperitoneal control at each time point of the plethysmometric measurements. Following statistical analysis, we can state that, regardless of the chosen time point, the effect of administering 1 mg/kg intraperitoneal morphine is similar to the progression of inflammation in the control group throughout the 72-hour period, with the Mann-Whitney U test confirming that there are no statistically significant differences (Table I).

Subsequently, we performed the same measurements on the group that received 5 mg/kg intraperitoneal

morphine, comparing it with the control group at each time point of the determinations (Table II).
In this case as well, the administration of morphine does not seem to produce statistically significant effects in reducing paw inflammation in rats.
The same statistical analyses were performed for the intraplantar control group and the 1 mg/kg intraplantar morphine group, which resulted in the absence of a statistically significant anti-inflammatory effect, with a p-value > 0.05. The Mann-Whitney U test also indicated a lack of statistical significance at any analysed time point (Table III).
Then, we conducted a statistical analysis comparing the intraplantar control group with the 5 mg/kg intraplantar morphine group (Table IV).

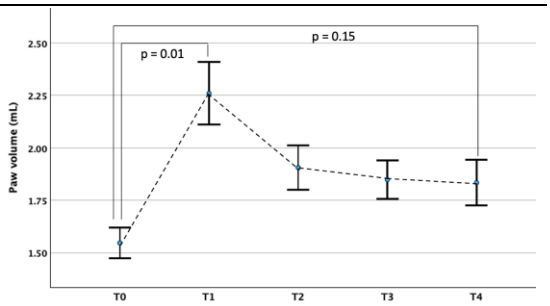

Figure 1.
Inflammatory changes in paw volume from carrageenan 1% injection (T0) to 72-hours after the injection (T4) in the control group
Bars represent 95% confidence intervals

Table I
The anti-inflammatory effect of 1 mg/kg body weight of intraperitoneal morphine compared to the intraperitoneal control

	Lot	N	Mean	Standard deviation	Mann-Whitney U	P
T0	Intraperitoneal control	7	1.5471	0.14032	17.000	0.334
	Intraperitoneal morphine 1 mg/kg	7	1.4443	0.10830		
T1 – 6 h	Intraperitoneal control	7	2.2786	0.31893	21.000	0.655
	Intraperitoneal morphine 1 mg/kg	7	2.2057	0.30165		
T2 – 24 h	Intraperitoneal control	7	1.9329	0.20064	23.500	0.898
	Intraperitoneal morphine 1 mg/kg	7	1.9557	0.28959		
T3 – 48 h	Intraperitoneal control	7	1.7829	0.15756	23.500	0.898
	Intraperitoneal morphine 1 mg/kg	7	1.8057	0.19372		
T4 – 72 h	Intraperitoneal control	7	1.7929	0.20846	24500	1.000
	Intraperitoneal morphine 1 mg/kg	7	1.7900	0.22113		

Table II
The anti-inflammatory effect of 5 mg/kg body weight of intraperitoneal morphine compared to the intraperitoneal control

	Lot	N	Mean	Standard deviation	Mann-Whitney U	P
T0	Intraperitoneal control	7	1.5471	0.14032	12.000	0.109
	Intraperitoneal morphine 5 mg/kg	7	1.4400	0.05538		
T1 – 6 h	Intraperitoneal control	7	2.2786	0.31893	14.000	0.180
	Intraperitoneal morphine 5 mg/kg	7	2.1329	0.17500		
T2 – 24 h	Intraperitoneal control	7	1.9329	0.20064	17.500	0.370
	Intraperitoneal morphine 5 mg/kg	7	1.8571	0.15294		
T3 – 48 h	Intraperitoneal control	7	1.7829	0.15756	19.000	0.482
	Intraperitoneal morphine 5 mg/kg	7	1.7743	0.20329		
T4 – 72 h	Intraperitoneal control	7	1.7929	0.20846	23.000	0.847
	Intraperitoneal morphine 5 mg/kg	7	1.7729	0.19423		

Table III
The anti-inflammatory effect of 1 mg/kg body weight of intraplantar morphine compared to the intraplantar control

	Lot	N	Mean	Standard deviation	Mann-Whitney U	P
T0	Intraplantar control	7	1.5457	0.12421	13.500	0.159
	Intraplantar morphine 1 mg/kg	7	1.4214	0.15636		
T1 – 6 h	Intraplantar control	7	2.2414	0.20449	19.000	0.482
	Intraplantar morphine 1 mg/kg	7	2.1529	0.27378		
T2 – 24 h	Intraplantar control	7	1.8786	0.17286	19.500	0.522
	Intraplantar morphine 1 mg/kg	7	1.9571	0.24404		
T3 – 48 h	Intraplantar control	7	1.9143	0.13710	19.000	0.482
	Intraplantar morphine 1 mg/kg	7	1.8300	0.27282		
T4 – 72 h	Intraplantar control	6	1.8817	0.14303	19.500	0.830
	Intraplantar morphine 1 mg/kg	7	1.8386	0.30580		

Table IV

The anti-inflammatory effect of 5 mg/kg body weight of intraplantar morphine compared to the intraplantar control

	Lot	N	Mean	Standard deviation	Mann-Whitney U	P
T0	Intraplantar control	7	1.5457	0.12421	11.000	0.084
	Intraplantar morphine 5 mg/kg	7	1.4271	0.09759		
T1 – 6 h	Intraplantar control	7	2.2414	0.20449	22.000	0.749
	Intraplantar morphine 5 mg/kg	7	2.1529	0.30302		
T2 – 24 h	Intraplantar control	7	1.8786	0.17286	24.500	1.000
	Intraplantar morphine 5 mg/kg	7	1.8686	0.26448		
T3 – 48 h	Intraplantar control	7	1.9143	0.13710	6.000	0.018
	Intraplantar morphine 5 mg/kg	7	1.6700	0.16207		
T4 – 72 h	Intraplantar control	6	1.8817	0.14303	8.5000	0.074
	Intraplantar morphine 5 mg/kg	7	1.6557	0.23478		

In this case, it should be noted that there is a notable difference between the two groups at 48 and 72 hours, with the group treated with morphine showing a lower average inflammation value, suggesting a pronounced anti-inflammatory effect of morphine administered at a dose of 5 mg/kg intraplantar. The Mann-Whitney U test indicates that there is a statistically significant difference at 48 hours after administration. The large effect size (r = 0.89) suggests that the group treated with morphine behaves differently from the control group. The increase in effect size (r) is very large in all cases, indicating that the change is both practically and statistically significant. This suggests that both morphine and the changes over time in the intraplantar control group have a significant effect on the measured values, although the exact nature of these values is not specified in these descriptive statistics.

We also compared the anti-inflammatory effects of morphine regardless of site of injection (either intraperitoneally or intraplantar) and of dose. There was no statistical difference between the control group and the experiment group at none of the time-points during the follow-up: T1 (2.26 - 0.25 mL *vs*. 2.16 - 0.25 mL, p = 0.25), T2 (1.90 - 0.18 mL *vs*. 1.90 - 0.23 mL, p = 0.95), T3 (1.84 - 0.15 mL *vs*. 1.77 - 0.20 mL, p = 0.22) or T4 (1.83 - 0.17 mL *vs*. 1.76 - 0.23 mL, p = 0.30). We further proceed in comparing the systemic effects of morphine by comparing the control group (Group 2) with the 1mg/kg intraperitoneal (Group 5) and the 5 mg/kg intraperitoneal (Group 6) groups. We did not observe a statistically significant decrease in paw size compared to the control group in either experimental group throughout the follow up (T1 to T4) – Figure 2.

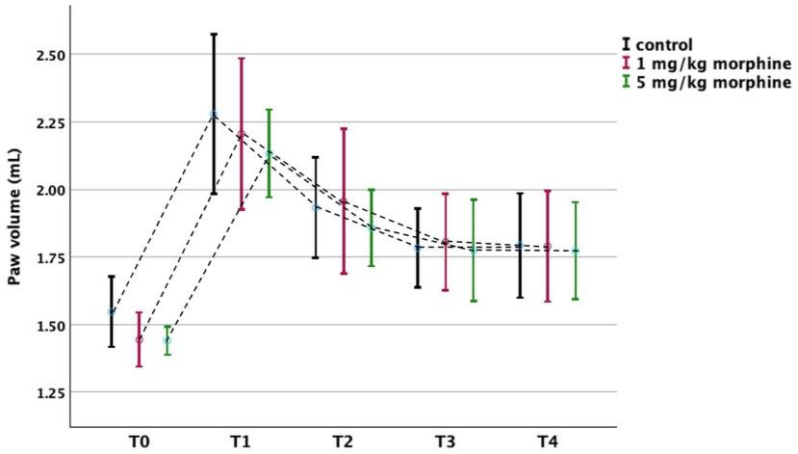

Figure 2.

Comparison between the intraperitoneal control and the 1 mg/kg intraperitoneal and 5 mg/kg intraperitoneal experiment group throughout the 72-hours follow-up

Bars represent 95% confidence intervals

The local anti-inflammatory effects of morphine were analysed by comparing the control group (Group 1) with the 1 mg/kg intraplantar (Group 3) and the 5 mg/kg intraplantar (Group 4) groups. We did not observe a statistically significant decrease in paw size compared to the control group in the 1 mg/kg intraplantar morphine experimental group throughout the follow up (T1 to T4), but we did observe a statistically significant decrease in paw size at T3 and T4 in the 5 mg/kg intraplantar experimental group compared to control – Figure 3.

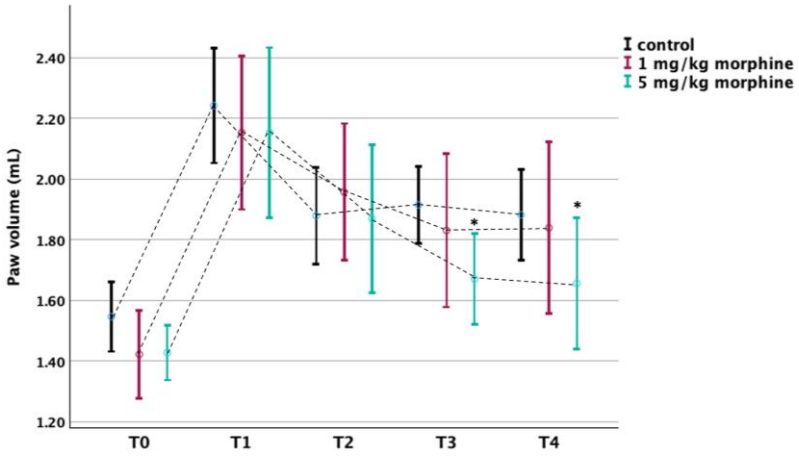

Figure 3.
Comparison between the intraplantar control and the 1 mg/kg intraplantar and 5 mg/kg intraplantar experiment group throughout the 72-hours follow-up
Bars represent 95% confidence intervals; *denotes statistical significance

Inflammation is the body's natural response to harmful stimulus such as infections, injuries, or toxins, aiming to protect and heal the affected tissues. It involves the activation of the immune system and is characterised by redness, swelling, heat, pain and sometimes loss of function [10, 31].

A well-established method for assessing the anti-inflammatory treatments is the carrageenan-induced rat paw oedema model, extensively used to evaluate the anti-oedematous effects of drugs [28].

Our study intended to evaluate the potential anti-inflammatory effects of morphine sulphate administered locally compared to systemically administration, and also the assessment of the dose-dependent increase in the anti-inflammatory effect of morphine sulphate. The scientific data pointed out that naloxone was found to attenuate the anti-inflammatory effect of morphine, thus suggesting the involvement of opioid receptors in the inflammatory pathway [16, 17, 25].

Previous studies have identified peripherally distributed opioid receptors [22, 27], which might explain our findings of superior anti-inflammatory effects achieved through peripherally injected morphine compared to intraperitoneal administration. Inflammation is the vascularised tissue's response to either infections or tissue injury, involving the migration of cells and molecules generated by the host defence system from the systemic circulation to areas where they are activated to eliminate offending agents [4]. Studies conducted over the last years suggest the importance of peripheral opioid receptors in mediating the analgesic and anti-inflammatory effects of morphine, demonstrating that it can act effectively in inflammation without relying on central mechanisms. Additionally, research highlights the advantage of local administration of morphine in managing inflammatory pain compared to systemic administration. These findings underline the potential of morphine as a treatment for inflammatory conditions, indicating that its local use could offer a more favourable therapeutic response [21]

Opioids, especially morphine, appear to have a significant impact on cells involved in the inflammatory immune response [30, 32].

In vitro and animal studies have shown that peripheral opioids can reduce the release of pro-inflammatory cytokines and neuropeptides, decreasing vasodilation, plasma extravasation and tissue damage. Unlike current anti-inflammatory medications, opioids have not shown organ-specific toxicity, making them promising options for pharmaceutical development. However, there is a lack of clinical studies to support these findings [18].

We conducted experiments using two different intraperitoneal dosages of the opioid morphine sulphate and compared them with intraplantar dosages. Subsequently, we analysed the paw volume changes and we observed a statistically significant decrease with 5 mg/kg of intraplantar morphine sulphate, demonstrating a significant anti-inflammatory response already noticeable at 48 hours after an acute injury.

The fact that only the 5 mg/kg dose administered intraplantarly showed a statistically significant anti-inflammatory effect, while the 5 mg/kg dose administered intraperitoneally did not, suggests that morphine exerted its effect through peripheral opioid receptors located in the inflamed paw.

However, other studies have shown that opioid derivatives have a moderate systemic effect in reducing vascular signs of inflammation by inhibiting vasodilation and vascular permeability in rats that received morphine intraperitoneally [8], which we could not demonstrate in the current research.

Certain opioid agents might exhibit some anti-inflammatory action, potentially positioning them as candidates for treating inflammatory diseases alongside their usage as analgesics [19, 24]. In the past 60 years, numerous anti-inflammatory molecules have been

discovered, yet they often exhibit a significant number of side effects and long-term adverse reactions, particularly among chronic users, some of which seem to be dose-dependent [11]. A single study conducted on humans compared the effect of intra-articular injections of morphine sulphate with dexamethasone in patients with osteoarthritis or chronic inflammatory arthritis. In this study, morphine achieved a reduction in the synovial leukocyte count compared to the negative control [23], whereas a high level of synovial leukocytes is associated with inflammation so a reduction in the white blood cell count could be interpreted as an effective reduction in local inflammation.

Enhancing worldwide access to effective and safe anti-inflammatory treatment options is a priority due to unmet needs and common under-treatment of inflammatory diseases. Both novel and traditional opioids offer distinct therapeutic effects based on their bio-chemical profile and receptor affinity [26] Future studies must further demonstrate the potential anti-inflammatory effects of opioid derivatives and their action on peripheral receptors in various animal experimental models and translate experimental animal studies to human medical use [9].

In this study, we have demonstrated the local anti-inflammatory effects of morphine on chemically induced inflammation in rats. However, there are several limitations that should be acknowledged: the study only explored a limited number of morphine concentrations, leaving the effects of lower doses and long-term administration unexamined. The use of carrageenan as an inducer of inflammation may not represent all types of inflammatory responses. The measurement period may not capture long-term effects. The absence of other anti-inflammatory agents limits our ability to compare morphine's efficacy with standard treatments.

Conclusions

In our experimental conditions, the intraperitoneal administration of morphine does not appear to elicit a reduction in paw volume among rats for any of tested doses.

Conversely, intraplantar morphine administration at a dosage of 5 mg/kg body weight demonstrates a statistically significant ($p < 0.05$) reduction in paw oedema after an acute inflammatory insult which demonstrates the potential benefits of opioids as local anti-inflammatory drugs. At 48 hours after the initiation of inflammation, a 12.57% difference was observed between the value of the intraplantar control group and the 5 mg/kg intraplantar morphine group (1.91 ± 0.13 *vs*. 1.67 ± 0.16) and a 22.33% reduction in inflammation compared to the peak of inflammation at 6 hours in the experimental group.

Since the same amount of morphine administered intraperitoneally did not have a statistically significant anti-inflammatory effect, it can be assumed that intra-plantarly administered morphine exerted its effect through certain peripheral opioid receptors located at the site of inflammation, rather than through opioid receptors in the central nervous system.

The current study findings join and support other studies in the literature regarding a possible anti-inflammatory effect of morphine with potential therapeutic use, in addition to the classic effects of morphine.

Conflict of interest

The authors declare no conflict of interest.

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