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The Effect of Chlorpromazine, Diclofenac and their Combination on Four Models of Induced Pain in Mice

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الملخص

خلفية الدراسة : ان الادوية المساعدة لمسكنات الالم مثل الادوية المضادة للذهان ، هي أدوية لها استطبابات أخرى غير تسكين الالم، الا انها مسكنة في بعض الحالات المؤلمة، أو يمكنها أن تقلل من الاثار الجانبية للادوية المسكنة للالم. وتعطى عادة مع أحد المسكنات الرئيسة.

الهدف : أ جريت الدراسة الحالية لتقصي تأثيرات الدواء المضاد للذهان: الكلوربرومازين في أربعة نماذج من الالم المحدث، ومقارنة تأثيره مع الدكلوفيناك صوديوم. والدراسة تهدف أيضا» لاختبار فعالية توليف الدوائين مع بعضهما في هذه النماذج من الالم.

طريقة البحث : أجريت التجارب جميعها على الفئران المختبرية، وقيمت استجابة الفئران للمنبهات المؤلمة باستعمال أربعة اختبارات هي: اختبار الومضة الذنبية واختبار الصفيحة الحارة واختبار الفورمالين واختبار التلوّي المحدث بواسطة حمض الخليك. هذه التأثيرات تم قياسها قبل وبعد ساعة من اعطاء الادوية داخل الصفاق، وفي بعض التجارب تمت متابعتها لست ساعات و لاربع وعشرين ساعة.

النتائج : بشكل عام، أظهر الكلوربرومازين لوحده فعالية واضحة في تسكين الالم في نماذج الالم المحدث بواسطة الحرارة (اختبارات الومضة الذنبية والصفيحة الحارة) وذلك بزيادة وقت الكمون بنسبة 34% و80% في الاختبارين على التوالي نسبة الى وقت الاختبارين قبل اعطاء الدواء. وذلك يقارن بزيادة 83% و88% نتجت عن اعطاء الدكلوفيناك صوديوم في اختبار الومضة الذنبية والصفيحة الحارة على التوالي. أما في الطور الاولي للالم الجسدي المحدث كيميائيا» (اختبار الفورمالين) وفي نموذج الالم الحشوي (اختبار التلوّي) فان الكلوربرومازين له تأثير مشابه للدكلوفيناك صوديوم. وعندما أعطي الكلوربرومازين مع الدكلوفيناك صوديوم، لم يعزز الكلوربرومازين تأثير الدكلوفيناك في اختبار التلوّي، الا ان الكلوربرومازين تسبب في تعزيز معتد لتأثير الدكلوفيناك في اثنين من النماذج الثلاثة المتبقية للالم وبمدى تراوح بين 46% و 55 أكثر من تأثير الدكلوفيناك و قد حدث التأثير المسكن الاعلى ساعتين بعد الزرق ليقلّ بعد ذلك.

الاستنتاج : ظهر الكلوربرومازين فعالية مسكنة للالم اختلفت باختلاف نموذج الالم المستعمل. وكانت الفعالية المسكنة للالم مشابهة للدكلوفيناك صوديوم (في اختباري التلوّي والفورمالين) ، وتراوحت بين 41% و91% من تأثير الدكلوفيناك صوديوم في اختباري الومضة الذنبية والصفيحة الحارة. هذا التأثير بلغ أقصاه ساعتين بعد اعطاء الادوية. وقد عزز الكلوربرومازين من التأثير المسكن للدكلوفيناك صوديوم عند استعمالهما معا» في اختبارات الومضة الذنبية والصفيحة الحارة والفورمالين. ولم تكن هناك زيادة للتأثير المسكن للدكلوفيناك صوديوم في اختبار التلوّي. توصي هذه الدراسة باجراء دراسات أخرى حول فعالية هذا التوليف في الممارسة السريرية.

Abstract

Background : Adjuvant or co-analgesic drugs, such as antipsychotics are commonly administered in combination with one of the primary analgesics.

Aim : The present study is carried out to investigate the effects of the antipsychotic drug; chlorpromazine in four animal models of induced pain and to compare its effects with diclofenac sodium and with their combination.

Materials and Methods : All experiments were performed on albino mice (Balb/C) strain. Mice were evaluated for their responsiveness to noxious stimuli using four tests: tail-flick test, hot-plate test, formalin test and acetic acid-induced writhing test.

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These effects were measured before and one hour after intraperitoneal drug administration. In some experiments, they were followed for 6 and 24 hours.

Results : In general, chlorpromazine, on its own, showed a significant analgesic activity in heat-induced pain models (tail-flick and hot-plate tests) increasing latency by around 34% and 80% in the two tests respectively. This is compared to 83% and 88% increase by diclofenac sodium. In the early phase of chemically-induced somatic type of pain (formalin test) and visceral-type of pain (writhing test), chlorpromazine had similar effect to diclofenac sodium.

When chlorpromazine was given in combination with diclofenac sodium in writhing test, it did not enhance diclofenac effect. In the other three models, chlorpromazine resulted in a significant enhancement of diclofenac effect in at least two of the remaining three pain models to an extent, ranging from 46% to 55% more than that of the diclofenac effect.

Conclusion : Chlorpromazine showed different analgesic activity according to the type of pain model utilized. The analgesic activities were either similar to diclofenac (in writhing and formalin tests), or less than diclofenac effect in tail-flick and hot-plate tests. Chlorpromazine increased the analgesic effect of diclofenac when used in combination in tail-flick, hot-plate and formalin tests.

Key words : Chlorpromazine, diclofenac, pain

Introduction

Improved pain relief can be achieved and adverse effects minimized, by multimodal analgesic combination.[1] An analgesic drug can be combined with a co-analgesic in a specific manner to achieve additive, if not synergistic effect with fewest possible adverse consequences.[2] Co-analgesic drugs, are defined as drugs that have a primary indications other than pain but are analgesic in some painful conditions or are capable of decreasing the side effects of analgesic drugs. These include antidepressants, antipsychotics, anticonvulsants, antiarrhythmics, corticosteroids, and others.

There has been a dramatic increase in the number of these drugs during the past two decades and they now play an important role in the management of chronic pain.[3]

Analgesics are complemented with co-analgesic (adjuvant) drugs, specially in pain of inflammatory and neuropathic type.[4] In case of neuropathic pain, treatment with opioids may be of limited efficacy and combination with co-analgesics is necessary.[5]

Chlorpromazine was found in previous studies to enhance morphine analgesia in rats[6] and its combination with ketorolac was safe and efficacious for treatment of exacerbation of chronic pain.[7]

The present study is, therefore, intended to investigate the analgesic potential of chlorpromazine when used alone or in combination with diclofenac sodium in four models of induced pain in mice; representing different types of pain stimuli.

Materials and Methods

Animals

All experiments were performed on albino-mice (Balb/C strains), 8-12 weeks old, weighing approximately 20 to 25g and housed in special plastic cages at an environmental temperature of 24±2°C. The animals had free access to food and water.

Groups

Four groups, 6 mice each (3 males and 3 Females), were used. The drugs were administered as shown in the table.

Groups	Volume of drug administered intraperitoneally
Group 1	Normal saline (NS) 0.2 ml
Group 2	NS 0.1ml + diclofenac sodium (3mg/kg) 0.1ml
Group 3	NS 0.1ml + chlorpromazine (0.5mg/kg) 0.1ml
Group 4	Diclofenac 0.1ml + chlorpromazine 0.1ml

Pain assessment methods

Mice were evaluated for their responsiveness to noxious stimuli, using four methods of pain assessment: tail-flick test, hot-plate test, formalin test and acetic acid-induced writhing test. The study protocol had been approved by the College Council and the Ethical Committee of Basrah College of Medicine.

Tail-flick test

Each animal was restrained by hands and the tip of its tail (the last 2 cm) was dipped in a water-bath heated to 48±0.2°C. The time to tail curling or flicking was measured as the latency of the test. Each animal was subjected to three trials separated by a minimum interval of 3 minutes. The average of three measurements was considered as the latency of tail-flick test for each mouse.[8]

Hot-plate test

An animal was placed on a metal plate maintained at 52±0.2°C and the latency of nociceptive responses such as licking or flicking of the hind limb or jumping was measured according to the method described by Kanaan et al.[8]

Only mice that showed the nociceptive response within 18 seconds were used for the experiments. The latency of nociceptive responses

in these animals was expressed as the hot-plate latency. A cut-off time of 45 seconds was selected to prevent tissue damage.

The obtained values for each group were compared with the baseline measurements established before administration of the drugs, using each animal as its own control to account for any possible variation in the nociceptive thresholds produced by stress due to frequent manipulation.

Formalin test

The nociceptive response in the formalin test was performed using the method described by Mahmoudi et al.[9]

Before the test, the animals were allowed to adapt to the test environment for 30 minutes before drug injection. They were placed in transparent plastic cages. Twenty microliters of 5% formalin was injected subcutaneously into the planter region of one of the hind paws using a micro-syringe. The number of nociceptive responses such as licking and/or biting of the injected paw was recorded over a 30 minute observation period.

Writhing test

All tested drugs were administered 60 minutes before I.P. administration of acetic acid 0.7% (1ml/kg). Animals were individually housed in a glass container and a mirror was arranged at an angle of 45˚ under the container.

Antinociception was recorded by counting the number of writhes immediately after injection of acetic acid and during 30 minutes thereafter. A writhe is indicated by abdominal constriction and stretching of at least one hind limb. [10,11]

Statistical analysis

The results were expressed as mean ± SD; the data were analyzed statistically by one-way analysis of variance (ANOVA), paired and independent t-test, using SPSS (computer package program version 9), P<0.05 was considered to be the lowest limit of significance.

Results

Chlorpromazine (0.5mg/kg) given intraperitoneally resulted in an analgesic effect ranging from around 34% increase in tail-flick latency to 80% increase in hot-plate latency with respect to pre-injection time. This is compared to around 83% and 88% increase for diclofenac sodium (3mg/kg) in the two tests respectively (Table 1 and 2).

The combination of the two drugs resulted in higher analgesic effect compared to each drug given alone (126% increase versus 83% and 34% in tail-flick test and 138% versus 88% and 80% in hotplate test for the combination, diclofenac sodium and chlorpromazine respectively) (Table 1 and 2).

The maximum analgesic effect occurred about two hours after injection and faded away after four hours for each of the two drugs given alone. The analgesic effect continued slightly longer (for 6 hours) if the two drugs were given in combination, particularly in tail-flick test (Figure 1).

In writhing test, chlorpromazine also reduced the number of abdominal writhings in 30 minutes by 72% with respect to the control group, which is near the result produced by diclofenac sodium, 78.3%. In addition, it reduced the number of licking and biting of the affected paw in formalin test by 35.8% with respect to control group compared to 34.9% by diclofenac sodium (table 3). The combination of the two drugs produced a slightly higher effect in both tests compared to each drug used alone (a reduction by 82.7% and 51% in writhing and formalin tests respectively). (table 3).

Table 1: Effect of chlorpromazine, diclofenac sodium, or their combination on tail-flick test in mice

Experimental group Time of injection	Control (normal saline) n=6	Diclofenac sodium (3mg/kg) n=6	Chlorpromazine (0.5mg/kg) n=6	Diclofenac sodium+ chlorpromazine n=6
Before I.P. injection	6.80±3.5	5.95±2.88	7.19±2.53	6.64±3.97
One hour after I.P. injection	6.51±2.82	10.86±3.67	9.63±2.63	15.06±9.24
Percent change with respect to pre-injection time	↓ 4.4%	↑ 82.5%	↑ 33.9%	↑ 125.9%
Statistical significance	not significant	P<0.001	P<0.01	P<0.001

Data are expressed as mean±SD of tail-flick time in seconds

Table 2: Effect of chlorpromazine, diclofenac sodium, or their combination on hot-plate test in mice

Experimental group Time of injection	Control (normal saline) n=6	Diclofenac sodium (3mg/kg) n=6	Chlorpromazine (0.5mg/kg) n=6	Diclofenac sodium+ chlorpromazine n=6
Before I.P. injection	5.08±1.38	5.37±2.56	4.25±1.11	5.27±2.01
One hour after I.P. injection	5.14±1.38	10.1±5.62	7.64±2.32	12.54±4.40
Percent change with respect to pre-injection time	↑ 1.2%	↑ 88.1%	↑ 79.8%	↑ 137.9%
Statistical significance	not significant	P<0.05	P<0.01	P<0.001

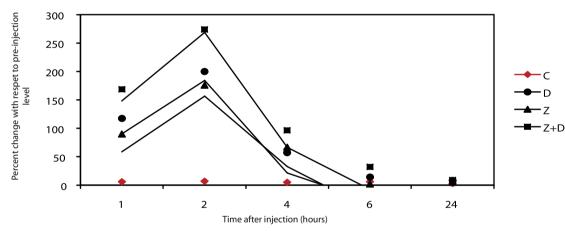Data are expressed as mean±SD of hot-plate time in seconds.

Figure 1: Effect of chlorpromazine, 0.5mg/kg (Z), diclofenac sodium, 3mg/kg (D) or their combination (Z+D) on tail-flick and hot-plate latencies (grouped together) 1,2,4,6 and 24 hours after drug administration in mice in comparison to pre-injection and control (C) latencies.

Table 3: Effect of chlorpromazine, diclofenac sodium, or their combination on acetic acid-induced writhing test and on formalin test in mice

Drug	Dose	N	Writhing test Number of abdominal writhings in 30 minutes	Formalin test Number of lickings or bitings of the affected paw (X±SD)
Control	Normal saline	6	50±8.36	17.66±1.36
Diclofenac sodium	3mg/kg	6	10.83±1.16* (-78.3%)	11.5±1.37* (-34.9%)
Chlorpromazine	0.5mg/kg	6	14±0.89* (-72%)	11.33±1.36* (-35.8%)
Diclofenac + chlorpromazine	3+0.5mg/kg	6	8.66±0.81* (-82.7%)	8.66±0.81* (-51%)

Data are expressed as mean±SD of n=6 with percentage of change from control.
Significant difference with respect to the control group: * P<0.001

When the effect of chlorpromazine was measured as the ratio of percent change caused by chlorpromazine to the percent change caused by diclofenac sodium in the four models of pain, it can be seen that the effect of chlorpromazine was either similar to, or produced less analgesic effect than diclofenac depending on the model used. However, when it is used in combination with diclofenac, it enhanced the analgesic effect of diclofenac by 53% in heat-induced somatic pain (tail-flick and hot-plate tests) and by 46% in chemically induced somatic pain (formalin test), while no enhancement in visceral-type of pain (writhing test) was found.

Discussion

Co-analgesics are defined as drugs that have primary indications other than pain but may be analgesic in selected circumstances.[12] They can also be defined as drugs that have weak or non-existent analgesic action when administered alone, but can enhance analgesic actions when co-administered with known analgesic agents.[13] This combination increases analgesia without increasing the dose of analgesics, and therefore, can reduce the incidence of adverse effects.[14] The combined treatment of the two types of drugs at doses much lower than therapeutic doses may be of great value in pain therapy.[15]

Pain is a common problem, and often associated with inflammatory conditions. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used analgesic agents. NSAIDs can inhibit both forms of cyclooxygenase enzyme (Cox-1 and Cox-2) and are effective in treatment of pain and inflammatory disorders.[16] NSAIDs are clinically effective because they alleviate pain and inflammation and restore patients to more normal daily function.[17,18]

Thus, the NSAID (diclofenac sodium) was selected in the present study as a standard drug to be co-administered with chlorpromazine. Chlorpromazine was found to have analgesic effect when given alone, however, its effects differ according to the pain model used. Its analgesic effect was less than that of diclofenac sodium in heat-induced pain models, particularly tail-flick test. On the other hand, the effects were similar to diclofenac in formalin and writhing tests.

Although it is usual to measure the analgesic effect one hour after drug administration, the analgesic effect found in the present study increases after that time to reach a peak at two hours after administration of both chlorpromazine and diclofenac. This finding is difficult to explain.

Chlorpromazine enhanced the analgesic effect of diclofenac in three pain models (tail-flick, hot-plate and formalin tests) when given together. Chlorpromazine significantly enhanced the effect of diclofenac in formalin test and the effect is just significant in tail-flick test.

Chlorpromazine had been found, in previous studies, to enhance morphine analgesia using hot-water tail-flick test in rats.[6] Similarly, it potentiated the effect of chlordiazepoxide in rats.[19] Chlorpromazine can, also, enhance the effect of other drugs; for example octreotide (a somatostation analogue used in the symptom management in terminal cancer patient).[20] Combination of ketorolac and chlorpromazine was found a safe and efficacious alternative to meperidine plus promethazine for the treatment of exacerbations of chronic pain.[7]

Chlorpromazine, on its own, can inhibit acetic acid induced writhing in mice.[21] Retrobulber chlorpromazine injections was also found to be safe and effective for the management of painful eye in 80% of patients with no permanent complications.[22] Moreover, it appeared to be an excellent option for treatment of migraine[23] and tension-type headache.[24] The analgesic effect of chlorpromazine might result from its ability to alter perception of pain. It might be related to its antiserotonergic activity and its ability to induce an α-blockade.[7]

Our findings, therefore, are in line with the above cited studies, showing that chlorpromazine has an analgesic effect on its own in the four pain models used. The peak of its effect occurs two hours after intraperitoneal administration. In addition, it can increase the analgesic effect of diclofenac when used together in all pain models tested except the writhing test.

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