RESEARCH ARTICLE

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The analgesic effect of different antidepressants combined with aspirin on thermally induced pain in Albino mice

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Background: Combination analgesics provide more effective pain relief for a broader spectrum of pain. This research examines the possible potentiation of the analgesic effect of different classes of antidepressants when combined with aspirin in thermal model of pain using Albino mice.

Methods: Different groups of six animals each were injected intraperitoneally by different doses of aspirin (50, 100, or 200 mg/kg), imipramine (2.5, 7.5, 15 or 30 mg/kg), fluoxetine (1.25, 2.5, 5 or 7.5 mg/kg), mirtazapine (1.25, 2.5, or 5 mg/kg) and a combination of a fixed dose of aspirin (100 mg/kg) with the different doses of the three antidepressants. One hour later the analgesic effect of these treatments were evaluated against thermally induced pain. All data were subjected to statistical analysis using unpaired Student's t-test.

Results: Aspirin had no analgesic effect in thermally induced pain. The three selected antidepressants produced dose dependent analgesia. The addition of a fixed dose of aspirin to imipramine significantly increased the reaction time (RT) of the lowest dose (by 23%) and the highest dose (by 20%). The addition of the fixed dose of aspirin to fluoxetine significantly increased RT by 13% of the dose 2.5 mg/kg. Finally, the addition of the fixed dose of aspirin significantly potentiated the antinociceptive effect of the different doses of mirtazapine (RT was increased by 24, 54 and 38% respectively).

Conclusion: Combination of aspirin with an antidepressant might produce better analgesia, increasing the efficacy of pain management and reduces side effects by using smaller doses of each drug.

Keywords: *analgesia*; *antidepressants*; *aspirin*; *combination analgesics*; *hot plate*

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are nonspecific analgesics and can potentially be used for any type of acute or chronic pain. Because they are both analgesic and anti-inflammatory, the NSAIDs are among the most widely used of all therapeutic classes of drugs (1). Most antidepressant medications are analgesics, and can relieve chronic pain even if the patient has no coexisting depression. The specific antidepressant effects are also important – alleviating chronic depression is important in helping patients deal more effectively with pain (2). Nevertheless, no single analgesic agent is perfect and no single analgesic can treat all types of pain. Yet, each agent has distinct advantages and disadvantages compared to the others. Hence, clinical outcomes might be improved under certain conditions with the use of a combination of analgesics, rather than reliance on a single agent (3). By activating multiple pain-inhibitory pathways, combination analgesics can provide more effective pain relief for a broader spectrum of pain, and might also reduce adverse drug reactions (3). Many combinations have been used for the treatment of different types of pain (4, 5). However, no study of the analgesic effect of the combined treatment of aspirin with antidepressant drugs was reported before. Therefore an experimental study using Albino mice was undertaken to examine the analgesic effect of the combined treatment with aspirin and different classes of antidepressant drugs including tricyclic antidepressants (TCA), selective serotonin uptake inhibitor (SSRI) and noradrenergic & specific serotonergic antidepressants (NaSSAs). This was done by studying the antinociceptive effect of these drugs either given alone or in combination with aspirin in thermal pain.

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Methods

Animals used

Albino mice weighting 25–30 g were used. The animals were bred at the Department of Pharmacology, Faculty of Pharmacy, Tripoli University. The animals were kept at room temperature between 20 and 25°C with constant humidity and 12 h light-dark cycle with food and water ad-lib. All experiments were performed at times between 10.00 and13.00 h to minimize diurnal variations.

Drugs and chemicals

Aspirin ampoules containing 500 mg were obtained from Sanofi Aventis-France. Imipramine hydrochloride ampoules containing 10 mg/2 ml were obtained from Polfa–Poland. Fluoxetine hydrochloride and Mirtazapine were supplied by Sigma – Aldrich dealers in Tripoli. Tween 80 and Acetic acid 1% (v/v) were obtained from Riedel-De Haen AG Seelzf-Hannover Germany.

Drugs administration

Aspirin and Fluoxetine hydrochloride were dissolved in 0.9% saline. Imipramine hydrochloride solution was diluted with 0.9% saline. Mirtazapine was suspended in 1% Tween 80 in water. All these drugs were administered by the intraperitoneal (i.p.) route in a volume of 5 ml/kg body weight. Different groups of six animals each were injected intraperitoneally by different doses of aspirin (50, 100, or 200 mg/kg), imipramine (2.5, 7.5, 15 or 30 mg/kg), fluoxetine (1.25, 2.5, 5 or 7.5 mg/kg), mirtazapine (1.25, 2.5, or 5 mg/kg) and a combination of a fixed dose of aspirin (100 mg/kg) with the different doses of the three antidepressant drugs. One hour later the analgesic effects of these treatments were evaluated against thermally (hotplate) induced pain.

Thermally induced pain

Hot-plate Analgesia Meter (model – DS 37 manufactured by Soael Milan-Italy) was used as a mean of inducing thermal pain. The plate temperature was held at a set point by electronic thermostat set at (55 ± 0.2) °C (6, 7). Mice were brought to the testing room and allowed to acclimatize for 10 min before the test begins. The mouse was placed on the hot plate and the latency to respond with either a hind paw lick, hind paw flick, or jump (which ever comes first) was measured in seconds. The mouse was immediately removed from the hot plate and returned to its home cage. If a mouse did not respond within 30 sec, the test was terminated and the mouse was removed from the hot plate (8). Baseline measurement for each mouse was taken just prior to drug administration (pretreatment values or self control) and again 60 min after drug administration. Experiments were performed according to a protocol approved by our animal care committee.

Data presentation and statistical analysis

The degree of antinociception for each mouse was expressed as the percentage increase in reaction time (RT) in seconds calculated according to the formula (% increase in RT = [T*100/C]), where C is the RT before treatment and T is the RT after treatment.

Data generated from the above studies were statistically analyzed with Excel software. Results for each group were expressed as Mean ± SE. One way analysis of variance (ANOVA) was used to see the differences in the effects among the different doses, and unpaired Student's *t*-test (two samples assuming equal variances) to determine which population means were different. A P *value of* <0.05 was considered statistically significant.

Results

Antinociceptive effect of aspirin

Different doses of aspirin (50–200 mg/kg) produced no significant change in the reaction time in the hot plate test (Table 1).

The antinociceptive effect of imipramine alone and in combination with aspirin

Different doses of imipramine produced a significance dose dependent antinociceptive effect against thermal pain starting at the dose 7.5 mg/kg (Table 2). One way ANOVA test showed that the addition of aspirin (100 mg/kg) significantly potentiated the antinociceptive effect of imipramine ($P < 0.01$). This was shown (comparing between the group that was given imipramine only with the group that was given imipramine plus aspirin) to take place with the lowest (2.5 mg/kg) and highest (30 mg/kg) doses of imipramine (Table 2).

The antinociceptive effect of fluoxetine alone and in combination with aspirin

Fluoxetine produced significant dose dependent antinociceptive effect against thermal pain (Table 3). The addition of aspirin (100 mg/kg) significantly potentiated the antinociceptive effect of different doses of fluoxetine ($P < 0.01$, one-way ANOVA). This was shown to take place with 2.5 mg/kg of fluoxetine (Table 3).

Table 1. The antinociceptive effect of different doses of aspirin against thermal induced pain

Treatments	The percentage increase in reaction time
Normal saline	101.4 ± 3.1
Aspirin (50 mg kg)	102.1 ± 2.6
Aspirin (100 mg/kg)	106.5 ± 5.4
Aspirin (200 mg/kg)	108.8 ± 8.6

Values are mean ± SE of six animals.

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Table 2. The antinociceptive effect of different doses of imipramine alone and in combination with 100 mg/kg aspirin against thermal induced pain

Treatment	Imipramine dose (mg/kg)	Percentage increase in reaction time	Percentage change from control group
Normal saline	–	101.4±3.1	0
Aspirin (100mg/kg)	–	106.5±5.4	5.1
Imipramine	2.5	108.8±7.8	7.3
Imipramine+aspirin	2.5	131.2±7.9*#	29.4
Imipramine	7.5	126.2±5.8*	24.5
Imipramine+aspirin	7.5	142.8±11.5*	40.8
Imipramine	15	136.3±7.68*	34.4
Imipramine+aspirin	15	152.5±14*	50.4
Imipramine	30	175.2±7.5*	72.8
Imipramine+aspirin	30	195.7±4.38*#	93

Values are mean±SE of six animals.
*Significantly differs from control normal saline group.
#Significantly differs from the corresponding group that received imipramine only.

The antinociceptive effect of mirtazapine alone and in combination with aspirin

Mirtazapine produced dose dependent antinociceptive effect against thermal induced pain (Table 4). The addition of aspirin (100 mg/kg) significantly potentiated the antinociceptive effect of different doses of mirtazapine ($P<0.001$, one-way ANOVA). This potentiation was statistically significant with all the doses of mirtazapine (Table 4).

Discussion

To the best of our knowledge the analgesic interactions between aspirin and antidepressants that increase the availability of serotonin and/or noradrenaline have not been evaluated previously. Hot plate nociception is commonly used to test for acute CNS analgesia; this test is selective in detecting analgesic action of morphine like drugs. All antidepressants used in this study produced a dose-depended anti-nociception in the hotplate test which was significantly potentiated by a middle dose of aspirin. Aspirin by itself lacks any analgesic effect in this test. These antidepressants are known to increase the synaptic availability of monoamines by different mechanisms, where imipramine inhibits the uptake mechanism for both 5-HT and NA, fluoxetine blocks selectively the uptake of 5-HT, while mirtazapine is believed to increase both noradrenergic and serotonergic neurotransmission via blockade of central alpha2 adrenergic auto- and heteroreceptors increasing the availability of both amines at the synapse (9). Noradrenergic and serotonergic descending inhibitory and excitatory pathways are believed to play an important role in endogenous analgesic

Table 3. The antinociceptive effect of different doses of fluoxetine alone and in combination with 100 mg/kg aspirin against thermal induced pain

Treatment	Fluoxetine dose (mg/kg)	Percentage increase in reaction time	Percentage change from control
Normal saline	–	101.4±3.1	–
Aspirin (100 mg/kg)	–	106.5±5.4	5.1
Fluoxetine	1.25	112.6±2.7*	11.1
Fluoxetine+Aspirin	1.25	113.2±6.21*	11.6
Fluoxetine	2.5	116.2±3.3*	14.7
Fluoxetine+Aspirin	2.5	129.31±3.06*#	27.5
Fluoxetine	5	117.57±4.12*	15.9
Fluoxetine+Aspirin	5	121.91±5.6*	20.2
Fluoxetine	7.5	124.67±6.4*	22.9
Fluoxetine+Aspirin	7.5	126.8±5.6*	25.0

Values are mean±SE of six animals.
*Significantly different from control normal saline values.
#Significantly different from the corresponding group that received fluoxetine only.

Table 4. The antinociceptive effect of different doses of mirtazapine alone and in combination with 100 mg/kg aspirin against thermal induced pain

Treatment	Mirtazapine dose (mg/kg)	Percentage increase in reaction time	Percentage change from control
Normal saline	–	101.4±3.1	–
Aspirin (100 mg/kg)	–	106.5±5.4	5.1
Mirtazapine	1.25	135.9±9.85*	34.0
Mirtazapine+Aspirin	1.25	158.3±5.1*#	56.1
Mirtazapine	2.5	135.8±5.76*	33.9
Mirtazapine+Aspirin	2.5	189.8±11.2*#	87.1
Mirtazapine	5	142.3±5.37*	40.3
Mirtazapine+Aspirin	5	180.6±17.3*#	78.1

Values are mean±SE of six animals.
*Significantly different from control normal saline values.
#Significantly different from the corresponding group that received mirtazapine only.

systems (10). Both noradrenaline and serotonin produce membrane hyperpolarization, while decreasing the excitatory transmitter release from primary Aδ and C afferent fibers presynaptically and increasing the inhibitory transmitter release, including both GABA and glycine, from the interneurons (11).

Fluoxetine produced less analgesia when compared with imipramine and mirtazapine (an increases in reaction time for fluoxetine from 11 to 22%, 7.3 to 72.8% for imipramine and 34–40% for mirtazapine). If we believe that both noradrenergic and serotonergic descending pathways play an important role in endogenous analgesic systems (12), then it is logic to suggest that fluoxetine being a selective serotonin uptake inhibitor would produce less analgesic effect than imipramine and mirtazapine both of which are known to increase the levels of both amines at the synapse.

Aspirin have been the traditional treatment for moderate pain and inflammation. Aspirin and its derivatives diminish postoperative hyperalgesia peripherally, primarily through inhibition of cyclooxygenase (COX) enzymes in the spinal cord and periphery (13). The combination of aspirin with the selected antidepressants resulted in a potentiation of analgesia in the following order (mirtazapine [22–53%], imipramine [16–22%] then fluoxetine [0.5–12.8%]. The addition of aspirin resulted in a significant potentiation for all doses of mirtazapine. The same results were obtained with imipramine except that the potentiation was significant only with the smallest (2.5 mg/kg) and the highest (30 mg/kg) doses of imipramine. The insignificant effect of the middle doses of imipramine might be related to the relatively higher standard error seen with these groups. The results obtained with fluoxetine are more difficult to explain, since the addition of aspirin resulted in very small potentiation to the analgesic effect which was significant with only the 2.5 mg/kg dose.

The potentiation of aspirin to the analgesic effect of these antidepressants doesn't seem to be additive, as the magnitude of the final effect of the combination was more than the sum of each response separately. Aspirin, by inhibiting peripheral and central cyclooxygenase and decreasing prostaglandin synthesis (14), likely reduced the activation and sensitization of peripheral primary afferents as well as central spinothalamic tract neurons, while the selected antidepressants likely enhanced descending inhibition of spinothalamic tract neurons (15). When combined, the two pharmacologic mechanisms appear to produce greater-than-additive effects. Moreover, previous studies have shown that analgesia induced by central infusion of NSAIDs was antagonized in monkeys by pretreatment with either the serotonergic receptor antagonist cyproheptadine or the alpha-2 adrenergic receptor antagonist yohimbine (16). In addition, activation of descending serotonergic inhibitory circuits has been proposed as a mechanism to account, at least in part, for the analgesic action of several NSAIDs (17–18). Thus, in addition to the possible pharmacologic interactions between cyclooxygenase inhibition produced by aspirin and the increased availability of the monoamines produced by the antidepressants, aspirin may have enhanced monoaminergic neurotransmission to an additional degree which resulted in the potentiation of the analgesic effect of these antidepressants.

It can be concluded therefore that the differing mechanisms of action of these drugs allows for improved analgesia when they are used in combination, even at reduced doses. Combination analgesic therapy can increase the efficacy of pain management and reduce side effects, minimizing pain and reducing recovery time.

Conflict of interest and funding

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