ORIGINAL PAPERS

# The Analgesic Effect of 5- fluorouracil in Mice

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## Abstract

The analgesic effect of 5- fluorouracil (5-FU) was analyzed in two experimental tests in mice. Two analgesia test, writhing test and hot plate test have been performed in swiss albino mice. The tested substance used was 5-Fluorouracil, i.p. administered in doses of 10 (Lethal Dose 50/10) and 20 mg/kg body weight (bw) (Lethal dose 50/5), 1 hour before tests. In the writhing test, 5-FU has significant analgesic effect when it is administered in dose of 20 mg/kg bw, but has no significant analgesic effect when administered in dose of 10 mg/kg bw. In the hot plate test, 5-FU increased jumping latency, that was statistically significant at both administered doses of 10 mg/kg bw and 20 mg/kg bw. In the same conditions (hot plate test), the fore paw licking latency was not statistically significant influenced.

These results suggest that the analgesia produced by 5-FU probably involves a central opioid mechanism. The similarity of 5-FU and opioid analgesic in analgesic effect is also found in gemcitabine, that seemed to act via cGMP. Still, given its chemical structure, 5-FU is more likely to act on cAMP and not cGMP. The fact that both gemcitabine and 5-FU interact with cGMP and cAMP, respectively and have analgesic effect suggest the need of developing new analgesic drugs which use this mechanism, but devoid of important toxicity as with these anticancer drugs.

**Keywords:** analgesia tests, 5-fluorouracil, opioid mechanism, cAMP.

## Rezumat

Efectul analgezic al 5-fluorouracilului (5-FU) a fost analizat în 2 experimente la șoarece. Au fost efectuate 2 teste de analgezie la șoareci albinoși tulpina swiss: testul torsiunilor și testul plăcii încălzite. Substanța testată a fost 5-FU, administrat intraperitoneal în doze de 10 (doza letală 50/10) și 20 mg/kg corp (kgc) (doza letală 50/5), cu o oră înaintea testării. În testul torsiunilor, 5-FU a avut efect analgezic semnificativ atunci când a fost administrat în doză de 20 mg/kgc dar nu și la doza de 10 mg/kgc. În testul plăcii încălzite, 5-FU a crescut semnificativ statistic latența saltului la ambele doze (10 și 20 mg/kgc). În aceleași condiții de testare (testul plăcii încălzite), parametrul numit linsul lăbuțelor din față nu a fost influențat semnificativ statistic.

Rezultatele sugerează că analgezia produsă de 5-FU probabil implică un mecanism opioidergic central. Similaritatea între mecanismul de acțiune al 5-FU și morfinei a fost semnalată și pentru gemcitabină, care pare să acționeze prin intermediul GMPc. Totuși, datorită structurii chimice, 5-FU pare să acționeze prin intermediul AMPc nu prin intermediul GMPc. Faptul că și gemcitabina și 5-FU interacționează cu GMPc respectiv AMPc și au efect analgezic sugerează nevoia dezvoltării de noi analgezice cu acest mecanism de acțiune dar fără toxicitate importantă ca în cazul acestor anticanceroase.

**Cuvinte cheie:** teste de analgezie, 5-Fluorouracil, mecanism opioid, AMPc.

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## INTRODUCTION

Pain is defined by IASP (*International Association for the Study of Pain*) as an unpleasant sensorial and emotional feeling which is produced by an actual or potential tissue injury[1]. Chronic pain management is currently a major public health challenge throughout the world, especially for patients with oncologic disorders. Data show that up to 90 percent of cancer patients suffer from pain during the course of their illness[2] and 50-80 percent receive an inadequate pain management[3,4].

Generally, two main groups of drugs are used for pain relief: opioid analgesics and non-opioid analgesics. Opioid analgesics are natural or synthetic drugs. Their main members are opium and morphine and their analgesic properties are known for thousands of years. These drugs act on certain receptors called opioid receptors which correspond to a series of agonists: enkephalins, endorphins, dynorphins and endomorphins.

Non-opioid analgesics – aspirin-like drugs or nonsteroidal antiinflammatory drugs are substances which block the activity of the cyclooxygenase, thereby reducing the production of prostaglandins.

However, researches are now studying the potential use of new types of analgesics, different from the opioid and nonsteroidal antiinflammatory drugs. Some examples are the substances which interfere with the endogen cannabinoid system or with the NO-dependent system[5]. Additionally, it has been recently demonstrated that nucleoside metabolism system is also involved in pain modulation[6,7].

Nucleoside metabolic inhibitors are known for almost fifty years as efficacious antineoplastic and antiviral pharmaceutical agents[8,9].

We hypothesize that, because pain is one of the parameters used for evaluating the clinical efficiency of some anticancer drugs, it may be possible that these oncologic drugs have an analgesic effect independent from the analgesic effect resulted from the tumor shrinkage.

For example, gemcitabine, a pyrimidine analog used as antineoplastic agent, has been shown to produce analgesic effect in mice. Another interesting fact is that gemcitabine potentiated the antinociceptive effect of morphine[10]. Additionally, the analgesic effect of gemcitabine was blocked by aminophylline, a known adenosine receptor antagonist and an inhibitor of phosphodiesterase. The chemical structure of gemcitabine is very similar to that of cytosine, thus making gemcitabine to bind guanine. Consequently, we consider that gemcitabine acts through cGMP pathway and not through cAMP.

We chose to study an antineoplastic agent, Fluorouracil (5-FU). 5-FU has a chemical structure that makes him specifically bind adenine and thus interfere with cAMP levels.

This paper investigates the antinociceptive effect of 5-Fluorouracil (5-FU). 5-FU is, as gemcitabine, a pyrimidine analog used as an antineoplastic agent. WHO (*World Health Organization*) recommends 5-Fluorouracil in the treatment of early stages of breast, colon, rectal and nasopharyngeal cancer, as well as in metastatic colon cancer[11]. *In vivo,* fluorouracil is metabolized to 5-fluorouridine monophosphate (F-UMP). F-UMP inhibits cell growth by replacing uracil and integrating itself into RNA, thereby inhibiting RNA processing[12].

5-5-fluoro-2'-deoxyuridine-5'-O-monophosphate (F-dUMP), another active metabolite of 5-FluoroUracil inhibits the enzyme thymidylate synthase. This causes a depletion of thymidine triphosphate (TTP), which is used in the synthesis of DNA. Other metabolites of 5-FU incorporate into both RNA and DNA, resulting in dysfunctions of RNA processing and functioning[13].

Two analgesia experiments involving 5-FU were conducted. Two types of analgesia tests were used: the writhing test and hot plate test in mice. Experiment 1 assessed the relationship between dose and the analgesic effect of 5-FU in the writhing test in mice. Experiment 2 assessed the relationship between dose and the analgesic effect of 5-FU in the hot plate test in mice.

## MATERIALS AND METHODS

11 mice and 8 mice, respectively, have been used for each group to perform test 1, respectively test 2. Swiss albino mice, weighing 20-25 g, kept under normal conditions of temperature, and day/night alternation, with food and water supplied ad libitum have been used. The mice have been brought in the laboratory one week before the beginning of each experiment. All experiments took place between 9 am and 5 pm. The experiments were conducted according to the agreement of the Institutional Ethic Committee, previously obtained, respecting directive 86/609/EU[14]. The animals were obtained from the biobase of the „Carol Davila" University of Medicine and Pharmacy in Bucharest, and were brought to the laboratory more than 48 hours before the experiments. 24 hours after the experiments, the animals were euthanized. The mice were not used in two consecutive experiments.

The substance used was 5-fluorouracil – 5-Fluorouracil EBEWE 50 mg/ml, concentrat pentru soluție injectabilă/perfuzabilă, EBEWE PHARMA G.m.b.H.

The tested doses of 5-FU were 1/10, respectively 1/5 of LD50 (lethal dose 50). LD50 for 5-FU in mice is 100 mg/kg, when the drug is administered intraperitoneally[15]. In these conditions, the doses used in the experiment were: 5-FU 10 mg/kg (LD50/10), respectively 5-FU 20 mg/kg (LD50/5).

Three groups of mice were used in Experiment 1. Group 1 received saline, group 2 received 5-FU 10 mg/kg, and group 3 received 5-FU 20 mg/kg. The concentration of 5-FU solutions was calculated in such a manner that each mouse received 0.1 mL solution per 10 g body weight (bw). All substances, including the saline, were administered intraperitoneally (i.p.), in a volume of 0.1 mL/10 g bw. One hour after the 5-FU or saline administration, each animal received an i.p-injection 0.1 ml/10 g bw with acid acetic solution of 0.75% v/v.

Five minutes after the acetic acid administration, each animal was put in an exploration cage and it was determined the number of writhes over a 5-minute period. A writhe was considered and counted when the animal touched the floor of the cage with its abdomen and spread the rear legs relative to front legs. Animals were evaluated successively, one by one, in the order control group, 5-FU 10 mg/kg bw, 5-FU 20 mg/kg bw.

Three groups of animals were used in Experiment 2. Group 1 received saline. Group 2 received 5-FU 10 mg/kg, and group 3 received 5-FU 20 mg/kg. The concentration of 5-FU solutions was calculated in such manner that each mouse received 0.1 ml solution per 10 g bw. All substances were administered as i.p. injections. One hour after the i.p. injection each animal was placed upon a plate that was heated to a temperature of 55±0.5˚C. For each animal, the latency (in seconds and hundredths of a second) of fore paw licking (first reaction) and of jumping from the hot plate (second reaction) were recorded. Animals were evaluated one by one successively in this following order: control (saline), 5-FU 10 mg/kg bw, 5-FU 20 mg/kg bw.

Data were presented as the average value obtained for each group, for each variable and experiment. The standard deviation and the standard errors were calculated for each group. The statistical significance of the results was studied with ANOVA and appropriate post hoc test (Tukey test) (if Levene test was significant, $p>0.05$, for homogenous groups). Only the values of $p<0.05$ were considered significant with ANOVA and appropriate post-hoc tests.

# RESULTS

For Experiment 1, the results are presented in Table 1 and Figure 1. For Experiment 2, the results are presented in Table 2, Table 3, Figure 2 and Figure 3.

Table 1. The writhing test in mice using two doses of 5-FU

| | Mean | Standard error | p Value (Tukey test) vs Control group |
|---|---|---|---|
| Control | 15.91 | 1.756 | |
| 5-FU 10 mg/kg bw | 11.91 | 1.756 | 0.074 |
| 5-FU 20 mg/kg bw | 11.55 | 1.756 | 0.048 |

Table 2. The hot plate test in mice using two doses of 5-FU. The first parameter (fore paw licking latency)

| | Mean | Standard error | p Value (Tukey test) vs Control group* |
|---|---|---|---|
| Control | 5.92 | 1.012 | |
| 5-FU 10 mg/kg bw | 6.47 | 1.012 | 0.850 |
| 5-FU 20 mg/kg bw | 6.71 | 1.012 | 0.715 |

*ANOVA: p not significant

Table 3. The hot plate test in mice using two doses of 5-FU. The second parameter (jump latency)

| | Mean | Standard error | p Value (Tukey test) vs Control group |
|---|---|---|---|
| Control | 41.88 | 5.838 | |
| 5-FU 10 mg/kg bw | 58.94 | 5.838 | 0.021 |
| 5-FU 20 mg/kg bw | 57.99 | 5.838 | 0.030 |

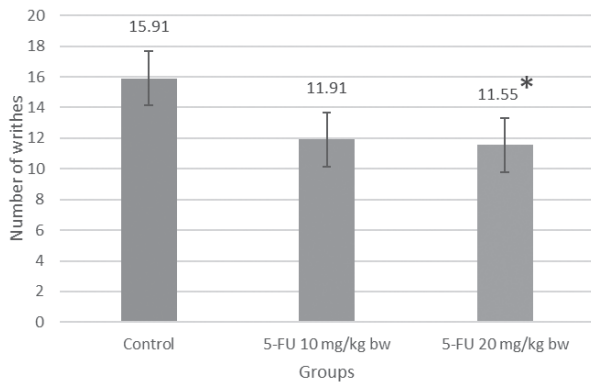


**Figure 1.** The relationship dose-effect (decrease of number of writhes) when two doses of 5-FU were used (10 mg/kg body weight, bw and 20 mg/kg bw, respectively). Control group 15.91±1.76 writhes/5 minutes, 5-FU 10 mg/kg bw 11.91±1.76 writhes/5 minutes, 5-FU 20 mg/kg bw 11.55±1.76 writhes/5 minutes (*p=0.048, Tukey test).

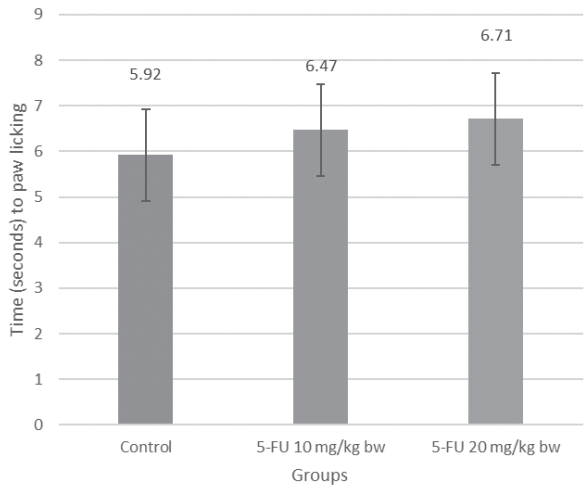


**Figure 2.** The effect (increase of fore paw licking latency) when two doses of 5-FU were used (10 mg/kg body weight, bw and 20 mg/kg bw). Control group 5.92±1.01 seconds, 5-FU 10 mg/kg bw 6.47±1.01 seconds, 5-FU 20 mg/kg bw 6.71±1.01 seconds. There are no significant differences vs. control group (ANOVA).

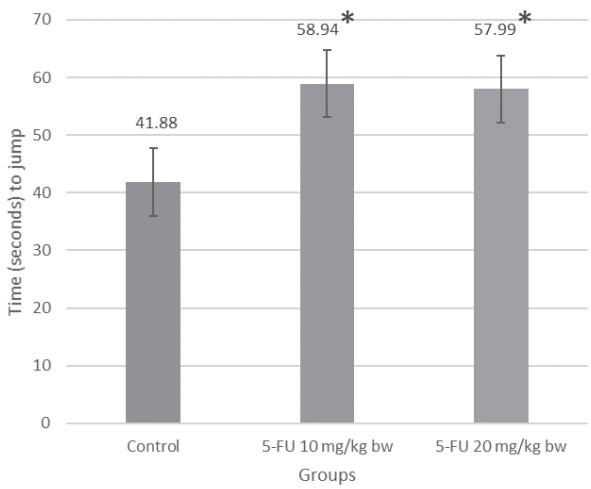


**Figure 3.** The relationship dose-effect (increase of jump latency) when two doses of 5-FU were used (10 mg/kg body weight, bw and 20 mg/kg bw). Control group 41.88±5.84 seconds, 5-FU 10 mg/kg bw 58.94±5.84 seconds (* p =0.021, Tukey test, vs Control group), 5-FU 20 mg/kg bw 57.99±5.84 seconds (* p =0.030, Tukey test, vs Control group).

## DISCUSSION

The results obtained in the writhing test shown that 5-FU has significant analgesic effect when it is administered in dose of 20 mg/kg bw (LD50/5), but has no significant analgesic effect when administered in dose of 10 mg/kg bw (LD50/10).

In the hot plate test, 5-FU increased jumping latency, that was statistically significant at both administered doses of 10 mg/kg bw (LD50/10) and 20 mg/kg bw (LD50/5).

In the same conditions (hot plate test), the fore paw licking latency was not statistically significant influenced.

These results suggest that the analgesia produced by 5-FU probably involves a central opioid mechanism. The similarity of 5-FU and opioid analgesic in analgesic effect is also found in gemcitabine[10]. Still, given its chemical structure, 5-FU is more likely to act on cAMP and not cGMP. It is thus assumed that 5-FU or its metabolites may interfere functionally or metabolically with adenosine, ATP or cAMP.

Adenosine and adenosine triphosphate (ATP) have been shown to modulate pain via P1 and P2 receptors[16]. P1 and P2 receptors are collectively called purinoceptors.

P1 receptors are G protein-coupled receptors with adenosine as endogenous ligand[17]. P1 receptors have four subtypes (A1, A2A, A2B, A3) which are distributed throughout the entire body with involvement in different organic processes[18]. Research shows that activation of A3 receptor in the spinal cord alleviates pain[19].

P2 receptors is separated into two subtypes: P2X and P2Y[20]. P2X receptors are cation liganded channel which open when the receptor binds extracellular ATP. They are involved in modulation of vascular tone, cardiac contractility, platelet aggregation, macrophage activation, apoptosis, neuronal-glial integration and ejaculation and micturition[21].

They also play an important role in nociception with ATPergic mechanisms more prominent in injury and inflammation. Central mechanisms appears to be limited[22].

P2Y receptors are G protein-coupled receptors. P2Y receptors are not stimulated only by ATP, but also by others purine and pyrimidine mono- and dinucleotides[23]. P2Y receptors have eight subtypes[24] and are a therapeutic target for clopidogrel[25], as well as potential drug targets for the treatment of cystic fibrosis and myocardial infarction[26,27].

Recent data has also shown that P2Y receptors are involved in neuropathic and inflammatory pain[28,29]. New therapeutic targets for the treatment of pain and migraines involving P2Y purinergic receptors are now being considered[30].

cAMP is a common pathway of signal transduction for a variety of intracellular substances with signaling function. Usually, decreased cAMP levels have analgesic effect (this is what happens when the opioid receptors are activated)[31-33].

It may therefore be possible that 5-FU binding to the cAMP inactivates it. Thus, 5-FU may provide analgesic effect by depletion of intracellular levels of cAMP.

The fact that both gemcitabine and 5-FU interact with cGMP and cAMP, respectively and have analgesic effect suggest the need of developing new analgesic drugs which use this mechanism. However, because of the toxicity of the cytostatic agents investigated, more research is needed for developing safe analgesic drugs which work through the interaction of these tricyclic nucleotides.

## CONCLUSIONS

1. 5-FU has shown analgesic effects in the experimental conditions described above.
2. In the writhing test, 5-FU decreased the number of writhes for the dose of 20 mg/kg bw, but no effect was registered for the dose of 10 mg/kg bw. The results were statistically significant.
3. In the hot plate test, the analgesic effect of 5-FU was once again proved for the both doses tested.
4. The first parameter, licking, was not influenced by 5-fluorouracil.
5. 5-fluorouracil has increased the jumping latency, with statistically significant results for both doses tested.
6. The fact that both gemcitabine and 5-FU interact with cGMP and cAMP, respectively and have analgesic effect suggest the need of developing new analgesic drugs which use this mechanism.
7. Because of the toxicity of the cytostatic agents investigated, more research is needed for developing safe analgesic drugs which work through the interaction of these tricyclic nucleotides.

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