Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

**Research Article**

J Exp Clin Med
2022; 39(1): 156-159
**doi:** 10.52142/omujecm.39.1.31

# Effects of favipiravir on hematologic parameters and bone marrow in the rats

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**Received:** 15.07.2021 • **Accepted/Published Online:** 02.08.2021 • **Final Version:** 01.01.2022

**Abstract**

Favipiravir, a selective RNA polymerase inhibitor agent, is an antiviral drug currently used effectively in treating pandemic diseases such as Covid-19. The present study aims to determine the effects of favipiravir use on bone marrow and blood cells. Twelve male Wistar rats were divided into two groups, namely control and favipiravir groups. Physiological saline at a dose of 1 ml/kg was administered to the rats in the control group by oral gavage for 10 days. Rats in the favipiravir group were administered favipiravir by oral gavage at a dose of 200 mg/kg for 10 days. At the end of the study, the blood tissue was collected from the heart, and bone marrow samples were collected from the femur bone of the rats sacrificed under anesthesia. The hematologic parameters in the blood samples obtained were measured using an auto-analyzer device with the help of rat compatible kits. Bone marrow cell counts were performed by examining structural changes and myeloid and erythroid cell series in the smear samples. The results obtained in the study revealed that favipiravir use caused a decrease in the counts of some hematologic parameters containing erythrocytes, lymphocytes, and monocytes. In addition, it was determined that the ratio of myeloid and erythroid cells in bone marrow smears changed significantly with the use of favipiravir. It was concluded that treatment with favipiravir caused suppression of erythrocyte and some leukocyte series. The suppressor effects were also determined in bone marrow cell series in the rats.

**Keywords:** bone marrow, favipiravir, rats, RBC, WBC

## 1. Introduction

Favipiravir, also known as T-705 (6-fluoro-3-hydroxy-2-pyrazinecarboxamide), is an antiviral agent used against RNA viruses developed by the Japanese Toyama Chemical Co. Ltd. Nowadays; drugs with the active ingredient of favipiravir are particularly used in the treatment of Covid-19 disease caused by the SARS-Cov-2 virus. In addition, favipiravir, an RNA-dependent RNA polymerase enzyme selective inhibitor used against influenza and some viruses, has been determined to be effective against arenavirus, bunyavirus and flaviviruses in rodent and in vitro studies, while it has also been reported to have a potential activity against alphavirus, paramyxovirus and noroviruses (3).

RNA polymerase (RNAP) is a family of enzymes that copy genetic information in the DNA or RNA molecule as an RNA molecule. The process of copying the information contained in a gene into a RNA molecule is called transcription. RNAP in cells allows genes to be read as RNA strands. RNAP enzymes are found in many living organisms as well as in viruses. RNAPs are a nucleotidyl transferase enzyme and allow the polymerization of ribonucleotides at the 3' end of an RNA 20 molecule. Molecular studies have revealed that viral proteases play a critical role in the life cycle of many viruses by affecting the cleavage of high molecular weight viral polyprotein precursors to obtain functional products or by catalyzing the process of structural proteins required for the aggregation and morphogenesis of virus particles. Currently, many studies are in progress on protease inhibitors in the treatment of a large number of RNA and DNA viruses, such as HIV, HCV, picornaviruses, RSV, herpes viruses, rotaviruses, and SARS (4, 8, 11).

Traditionally, cytological examinations of peripheral blood and/or bone marrow smears have been based on the screening of these cell types and their morphology, one after the other and one by one. The classification of bone marrow cell populations, on the other hand, has been carried out as manual determination of myeloid / erythroid (M / E) ratio. These analyses are important in the determination of morphological structures and series of cells (14). It has been determined that drug use may cause certain disorders in the hematological systems, as well as affect the leukocyte and platelet series and the entire coagulation system through various mechanisms (10). Some studies conducted on humans and animals have indicated that hematological values of individuals changed with favipiravir use (15, 16). However, there are no studies in the literature showing the effects of favipiravir use on bone marrow cell lines.

Favipiravir is a broad spectrum antiviral agent used in the treatment of many diseases, including covid-19 pandemic

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disease. The aim of the present study is to examine the numerical and morphological changes of blood cells, and bone marrow cell structures due to favipiravir use.

## 2. Materials and methods

### 2.1. Animals

Twelve male outbred Wistar albino rats to be used in the study were randomly divided into two groups as control (n = 6) and favipiravir (n = 6) groups. The study is in agreement with the principles of scientific research and publication ethics according to ethical norms approved by the Local Animal Care Committee of Bingöl University Veterinary Faculty (15668). Oral favipiravir (Favicor, Atabay, Turkey) was administered at a dose of 200 mg/kg for 10 days to the rats in the favipiravir group. For this purpose, the tablets containing 200 mg favipiravir were ground in a mortar and suspended in saline solution and then administered to the rats by oral gavage for 10 days. The animals were sacrificed after blood samples were taken from their hearts under xylazine and ketamine anesthesia 24 hours after the last favipiravir administration.

### 2.2. Hematologic analysis

The complete blood count of samples was conducted using a BeneSphera 3 Part Differential Hematology Analyzer (Avantor Performance Materials India Limited, India) with the help of rat compatible kits. The femur bones were collected from the sacrificed rats, and the bone marrow cells were collected.

### 2.3. Bone marrow smear analysis

The femur was cut from the caput femoris region and bone medulla was washed 1 ml DPBS solution containing 1mm-EDTA. In the cytological examination of bone marrow, the preparations were made by spreading the cells onto the slides, drying in the air, and then staining with May-Grunwald-Giemsa (MGG] (2).

**Table 1.** The hemogram analysis results for control and favipiravir administrated groups

| Groups | | WBC (K/uL) | NEU (K/uL) | LYM (K/uL) | MON (K/uL) | EOS (K/uL) | BAS (K/uL) | RBC (K/uL) | HCT (%) | PLT (K/uL) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | Mean | **13.79** | **2.77** | **9.46** | **0.74** | **0.61** | **0.01** | **8.56** | **46.89** | **332.83** |
| | Stand. Dev. | 0.82 | 0.48 | 0.36 | 0.13 | 0.17 | 0.01 | 0.30 | 1.34 | 65.90 |
| Favipiravir | Mean | **12.00** | **2.22** | **8.24** | **0.82** | **0.27** | **0.02** | **7.69** | **51.87** | **631.83** |
| | Stand. Dev. | 0.77 | 0.49 | 0.73 | 0.24 | 0.19 | 0.02 | 0.49 | 2.33 | 115.60 |
| *** P Value** | | **0.04*** | **0.065** | **0.04*** | **0.39** | **0.09** | **0.51** | **0.004*** | **0.002*** | **0.045** * |

* Statistical analysis was conducted with the Mann-Whitney U test. The $p < 0.05$ was accepted as statistically significant. The value is the mean of groups. The Asterix indicate the statistical differences between control and favipiravir administrated groups.

### 2.4. Assessment of bone marrow cells

Two hundred cells were selected from the random regions of each preparation, and myeloid and erythroid cells were identified and counted to determine the ratio of these cells (M:E ratio] in the assessment of bone marrow cells. Myeloid and erythroid cell ratios in these counted cells were determined separately for each animal and averaged. A trinocular light microscope (Leica, DM2500 / DFC295, Germany) was used in the examinations and cell count determination of bone marrow (Bolliger, 2004).

### 2.5. Statistical analyses

The data obtained in the study were statistically analyzed using the SPSS 20.00 (IBM Co. Armonk, NY, USA) software package. Since the Kolmogorov-Smirnov analysis revealed that the data were normally distributed (asymp. Sig.) and the p-value was less than 0.05, the statistical analysis of the data was conducted by applying the Mann-Whitney U test that is used for the comparison of two independent groups. The p-values less than 0.05 were considered statistically significant.

## 3. Results

### 3.1. Hematologic results

The results of hematological analysis indicated that leukocyte (WBC), lymphocyte (LYM), monocytes (MON), erythrocyte (RBC), hematocrit (HCT) and thrombocyte (PLT) counts significantly reduced in the blood samples of rats treated with favipiravir than those in the control group ($p < 0.05$). On the other hand, there was no statistically significant difference in terms of neutrophil (NEU), eosinophil (EOS), and basophil (BAS) counts between the control group and the favipiravir administrated group ($p > 0.05$). Hematological parameters and their comparisons are presented in the Table 1.

### 3.2. Assessment of bone marrow cells

It was determined from the manual counting of the cells obtained from the bone marrow under the light microscope that the percentage ratio of myeloid cells was 42.7% in the control group and 41.6% in the favipiravir group, while there was no statistical difference between the two groups ($p > 0.082$). The percentage ratio of erythrocyte cells in the bone marrow was detected to be 31.4% in the control group and 28.6% in the favipiravir group, and the difference was statistically significant ($p < 0.043$). The M:E ratio was 1.36 in the control group and 1.45 in the favipiravir group and the statistical comparison revealed that the difference was significant ($p < 0.037$). The myeloid and erythroid cell counts and M:E ratios are presented in the Table 2, and bone marrow swab images are shown in the Fig. 1.

**Table 2.** Femoral bone marrow myeloid and erythroid cell percentages and M:E ratios in the control and favipiravir administrated groups

| | Control (n = 6) | Favipiravir (n = 6) | p-value |
|---|---|---|---|
| Myeloid (M) cell (%) | 42.7±4.5 | 41.6±5.1 | 0.082 |
| Erythroid (E) cell (%) | 31.4±2.5 | 28.6±3.2 | 0.043 |
| M:E ratio | 1.36±0.3 | 1.45±0.5 | 0.037 |

* Statistical analysis was conducted with the Mann-Whitney U test. The $p < 0.05$ was accepted as statistically significant. The value is the mean of groups

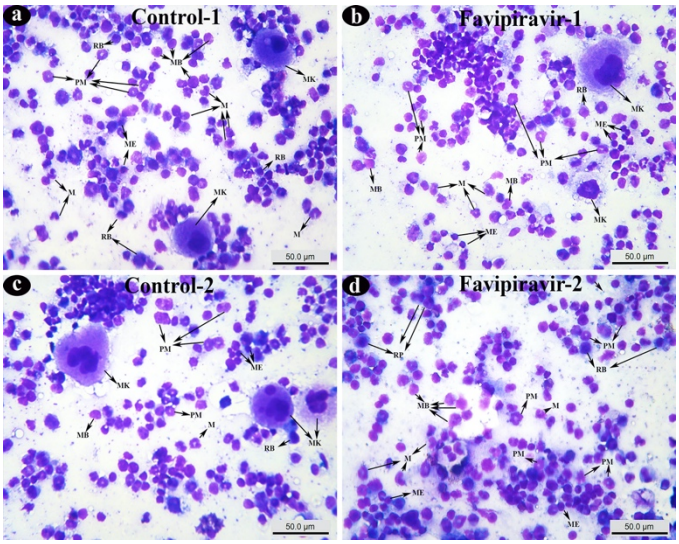


**Fig. 1.** Cytological illustration of femoral bone marrow cells for control and favipiravir administrated groups, MK: megakaryocyte, PM: Premyoblast, MB: Myeloblast, M: Myelocyte, ME: Metarubricides, RB: Rubriblast, May-Grunwald-Giemsa (MGG) staining

## 4. Discussion

Favipiravir, a selective RNA polymerase inhibitor and a broad-spectrum anti-viral agent, has become very widely used with the emergence of Covid-19 pandemic disease. Although favipiravir was originally developed for the treatment of influenza viruses, it is also used in the treatment of other viral diseases as it is effective against RNA viruses with its broad spectrum antiviral activity. In the present study, the effects of favipiravir administration on systemic hematological cell lines were investigated.

It has been reported in the literature that the hematopoietic system was affected with the use of certain antiviral agents (10). McHutchison et al. (2007) found that the antiviral agent ribavirin used for the treatment of hepatitis-C caused anemia. In another study, it was stated that lamivudine used for the treatment of human immunodeficiency virus (HIV) and ribavirin for hepatitis C treatment induced pure red cell aplasia (6). Similarly, a case study indicated that thrombocytopenia developed due to the use of antiviral agents such as daclatasvir and asunaprevir (13, 1). In an animal study, it was reported that the administration of favipiravir in Marburg virus-infected animals caused changes in hemogram parameters, as well as reduced lymphocyte counts, and increased neutrophil counts (16). It was stated in another study that favipiravir use prevented leukopenia and thrombocytopenia in Junin virus-infected guinea pigs (5). However, the results of our study show that favipiravir administration resulted in the development of anemia, in addition to leukopenia and thrombocytopenia in the healthy rats.

In the study, hematopoietic cell lines were evaluated by examining bone marrow cells obtained from all rats. Myeloid (premyeloblast, myeloblast, myelocyte) and erythroid (metarubricytes, rubriblast) serial cells of the bone marrow were counted and their ratios were calculated. It was found that, the ratio of myeloid cells (42.7%) and erythroid cells (31.4%) in the control group was 1.36, while the ratio of myeloid cells (41.6%) and erythroid cells (28.6%) in the favipiravir group was 1.45. A significant difference was determined between the groups in the statistical comparison of the M:E ratio ($p < 0.037$). Schomaker et al. (2002) found that 14-day treatment of Cyclohexanone Oxime (CHO) caused a significant decrease in the M:E ratio (Control: 1.96, CHO: 0.41) based on erythroblastic hyperplasia. It was stated in the same study that there was a decrease in erythrocyte counts as well as hemoglobin and hematocrit levels, and an increase in the counts of reticulocytes and nucleated erythrocytes (12). Several other studies indicated that the use of daunorubicin reduced erythroid and myeloid cell lines (79% reduction in myeloid cell count and 90% reduction in erythroid cell count) and affected erythropoietin responsive cells and erythroid repopulating cells in the rat bone marrow (9, 12). Yaylaci et al. (2020) investigated the effect of favipiravir use on the blood parameters in Covid-19 patients and reported that erythrocyte count significantly decreased.

One of the limitations of the study is that animal models are poor predictors of drug safety in humans. Also, flow cytometric examination of bone marrow cells can enrich our study. Similar studies conducted in the future may focus on these issues.

In conclusion, it was determined in the present study that favipiravir use did not affect the myeloid cell line, suppressed the erythrocyte, thrombocyte, monocytes, and lymphocyte series, and caused a significant decrease in these cells. It is considered that the study would contribute to the relevant literature about the effects of favipiravir use on the hematopoietic and hematological system, on which there are limited studies in the literature.

## Conflict of interest

The authors declare that they have no conflict of interest to the publication of this article.

## Acknowledgments

None to declare.

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