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Investigation of the Mutagenic Potential of Immunomodulator Arglabin Native in Tablets in the Ames Test

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Abstract: The mandatory preclinical safety evaluation is an essential prerequisite to obtain the qualitative and effective medicines. Due to the fact that drugs may reveal genotoxic properties, the investigation of their mutagenic activity is an obligatory part of the preclinical drug safety program. The aim of the research is to study mutagenic properties of a new original immunomodulator Arglabin native in tablets in the induced test of gene mutations (the Ames test) on *Salmonella typhimurium* strains. Materials and methods: Four strains of *S. typhimurium* TA98, TA100, TA1535, and TA1537 were used to assess the mutagenicity in the Ames test. Results and conclusions: No statistically reliable dose-dependent increase in the number of revertant colonies of *Salmonella typhimurium* has been observed in the presence of the given drug within the investigated dose ranges from 5.0 to 100.0 μg/mL for strains TA100 and TA1535, and from 5.0 to 250 μg/mL for strains TA98 and TA1537 against the baseline of spantaneous mutations. Arglabin native in tablets does not reveal a mutagenic activity within the studied dose ranges on *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537.

Key words: Arglabin native, the Ames test, mutagenic properties.

1. Introduction

Immunomodulating agents are widely used in the preventive and clinical medicine. The immune system is affected practically during any somatic or infectious disease. By now, numerous data have been collected confirming that the immune system disorders can stand as one of the main pathogenetic links of atherosclerosis, coronary heart disease, or myocardial infarction. According to the autoimmune theory of atherosclerosis pathogenesis, the formation of autoimmune complexes results in the disruption of a lipoprotein exchange and the arterial wall damage [1, 2]. Therefore, it is pertinent to use immunocorrecting agents in the complex therapy.

The use of natural biologically active compounds received from plants is viewed as a prospective approach in the immunocorrective therapy. In this regard, Arglabin native in coated tablets, based on the eponymous sesquiterpene γ-lactone isolated from an endemic Kazakhstan plant *Artemisia glabella* Kar. et Kir., deserves special attention. A number of previously conducted researches confirmed that Arglabin native has the pronounced anti-inflammatory and immunomodulatory effects [3, 4].

The combination of immunotropic activity and anti-inflammatory effect of Arglabin was established during treatment of endometritis in the experimental animals. In addition, its pronounced impact on the central link of immunogenesis is highlighted which is characterized by the decrease in thymocytes' content in cortical and medullary substances of the thymus segments, the stimulation of T-lymphocytes efflux from T-dependent periarterial zone and the phagocytosis processes, as well as the increase in the inflow of macrophages and the volume of lymphocyte-macrophage infiltrations in the red pulp. The therapeutic effect of Arglabin at a dose of 10 mg/kg was comparable to that of Timalin. Moreover, in

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both cases similar processes in regards to speed and volume were observed when the inflammation symptoms subsided and the endometrium regenerated [3, 4].

The possibility of mutagenic effects development is not quite excluded when actively using Arglabin native; thus, the risks have to be evaluated which became the subject of the given research.

To assess the mutagenicity in the Ames test, strains of the bacterium *Salmonella typhimurium* are usually used in which histidine operon point mutations are introduced resulting in the histidine biosynthesis disruption, and bacteria inability to grow on a histidine-free medium. Induction, as a result of mutagenic effect of the reverse mutations according to the base pair substitution mutation or frameshift types in His gene of these tested strains, leads to a return to a "prototrophic" state of bacteria in these amino acids and ability of cells to grow on a histidine-free medium. Tested strains are exposed to various concentrations of the investigated compound and grow on a histidine-free medium. The mutagenic potential is estimated based on the revertants' induction from auxotrophic to prototrophic state on histidine, which can survive and grow on a histidine-free medium. Strains TA100 and TA1535 of *Salmonella* register the induction of base pair substitution mutations, whereas strains TA98 and TA1537 of *Salmonella* indicate frameshift mutations [5-7].

The aim of the given research is to investigate the mutagenic properties of a new original immunomodulator Arglabin native in tablets in the induced test of gene mutations (the Ames test on *Salmonella typhimurium*).

To pursue this aim we researched the mutagenic properties of Arglabin native in tablets in the induced test of gene mutations, the Ames test on *Salmonella typhimurium* in a microplate format (MPF), Ames MPF™ Penta I kit, Xenometrix, Switzerland [8-10].

2. Materials and Methods

The object of research is a solid dosage form of the drug Arglabin native in 50 mg tablets. Tablet mass was compressed on Erweka EP1 tablet press using a biconvex form with a diameter of 9.0 mm without a pill groove. Core tablets were covered with the enteric coating on the Tablet Coating System SFC 30 FSH using 20% aqueous suspension of Acryl-EZE White 93O18359 filming agent. After coating the surface of tablets was even and smooth without visible defects (chips, cracks, or adhesion). The produced tablets of Arglabin native underwent a disintegration testing. According to the test, the product sustained within 2 hours in 0.1 M HCl (pH 1.2); disintegration in the phosphate buffer (pH 6.8) was 1.5 within 3 min. Dissolution kinetics of the received tablets was researched. The enteric-coated tablets could sustain the influence of 0.1 M solution of hydrochloric acid within 2 hours and fully released the active ingredient in a buffer solution with pH 6.8 within 1 hour.

Assessment of the mutagenic properties of Arglabin native in tablets was carried out in the Ames test on *Salmonella typhimurium* in a microplate format (Ames MPF™ Penta I kit, Xenometrix, Switzerland) [8-10]. The strains of bacteria included in a kit are in compliance with the Organization for Economic Cooperation and Development (OECD) 471 Guideline for Testing of Chemicals [11].

About 10^7 bacteria of *Salmonella typhimurium* were exposed to the tested sample in 6 concentrations (as well as positive and negative controls) within 90 minutes (adequate timing for two cell divisions) in the medium containing histidine. After 90 minutes the exposed cultures were introduced into pH-indicator histidine-free medium and poured into 48 wells of a 384-well microplate. Within two days bacteria that underwent reverse mutations to a prototrophic state on these amino acids formed colonies. Products of bacteria metabolism caused pH drop of the indicator medium and changed color in wells. Wells with the revertant colonies were calculated for each concentration of the tested sample; the obtained data were compared to the negative control (solvent). Each

concentration was tested in triplicates to provide a statistical robustness.

The investigated doses of Arglabin native in tablets ranged (in terms of Arglabin substance) from 5.0 to 100 μg/mL (5, 10, 15, 25, 50 and 100 μg/mL) for strains TA100 and TA1535; from 5.0 to 250 μg/mL (5, 10, 25, 50, 100 and 250 μg/mL) for strains TA98 and TA1537.

Calculations were made by means of Excel spreadsheet (Ames_Calculation_Sheet_Ver2_03.xls). The following parameters have been calculated: "the average number of positive wells per a concentration" which is equal to the average value of positive wells after triplicates per one tested concentration; "the standard deviation of positive wells number per a concentration" which represents a standard deviation of the average number of positive wells per a tested concentration; "frequency rate exceeding a zero line" which is defined as the ratio of positive wells average per a tested concentration to the zero line of negative control (solvent). The zero line is calculated by addition of the positive wells average for negative control and the standard deviation value. The frequency rate of revertants exceeding a zero line less than 2.0 has not been considered as a positive result because differences are deemed unreliable. Sample for which the concentration dependence of effect has been revealed, or frequency rate exceeding a zero line has been over 2.0, is classified as a mutagen. Student's *t*-test (unilateral, unpaired) was used to check the reliability of differences at significance level $p = 0.05$.

The increase in number of revertant colonies under influence of the tested sample in comparison with the negative control confirms that the sample exhibits a mutagenic activity in the Ames test MPF™ Penta I.

3. Results and Discussion

The investigated doses of new original immunomodulator ranged (in terms of Arglabin substance) from 5.0 to 100 μg/mL (5, 10, 15, 25, 50 and 100 μg/mL) for strains TA100 and TA1535; from 5.0 to 250 μg/mL (5, 10, 25, 50, 100 and 250 μg/mL) for strains TA98 and TA1537. At higher concentrations Arglabin reveals a cytotoxic effect on *Salmonella typhimurium*. No growth of *Salmonella typhimurium* implied the decrease in a number of revertant colonies against the baseline of natural mutations which makes it impossible to research its mutagenic potential at higher concentrations.

Based on the obtained data the following values have been calculated:

(1) "The average number of positive wells per a concentration" which is equal to the positive wells average after triplicates per one tested concentration;

(2) "The standard deviation of positive wells number per a concentration" which represents a standard deviation of positive wells average per a tested concentration;

(3) "The zero line" is calculated by addition of the positive wells average for negative control (solvent) and the standard deviation value;

(4) "Frequency rate exceeding a zero line" which is defined as a ratio of positive wells average per a tested concentration to the zero line of negative control (solvent).

The frequency rate of revertants exceeding a zero line less than 2.0 has not been considered as a positive result because differences are deemed unreliable. Sample for which the concentration dependence of effect has been revealed, or frequency rate exceeding a zero line has been over 2.0, is classified as a mutagen.

Research results of mutagenic properties of Arglabin native in tablets in the Ames test on *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 are presented in Tables 1-4.

Tables 1 and 4 demonstrate that within the investigated dose range from 5.0 to 250 μg/mL for strains TA98, TA1537 tablets of Arglabin native decrease the number of revertant colonies against the baseline of spontaneous mutations. In Tables 2 and 3, it is shown that Arglabin native in tablets in the studied dosage range from 5.0 to 100 μg/mL for strains TA100,

TA1535 also does not increase the number of revertant colonies against the baseline of spontaneous mutations.

Under the effect of Arglabin native tablets in the Ames test on *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 the average number of positive wells does not differ from the corresponding value in the negative control. The frequency rate exceeding a zero line within the studied dose ranges of Arglabin native tablets on *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 is less than 2.0; therefore, distinctions are not considered reliable (significance level $p > 0.05$) in Tables 1-4.

It is obvious from the presented results that tablets of Arglabin native do not increase the number of revertant colonies against the baseline of spontaneous mutations in the predefined range.

Table 1 Research results of mutagenic properties of Arglabin native in tablets in the Ames test on *Salmonella typhimurium* strains TA98.

Arglabin concentration, μg/mL	Number of occurrences, n	Positive wells average, m	Standard deviation, SD	Zero line	Frequency rate exceeding a zero line	*t*-test, *p*-value (unilateral, unpaired)
0	6	6.33	1.75	8.08		
5	3	5.33	1.53		0.66	0.2152
10	3	4.00	2.65		0.49	0.0755
25	3	4.33	2.52		0.54	0.1001
50	3	7.33	3.79		0.91	0.2952
100	3	5.00	3.00		0.62	0.2081
250	3	4.33	2.08		0.54	0.0852
Positive control (2-nitrofluorene)	3	47.00	1.00			

Table 2 Research results of mutagenic properties of Arglabin native in tablets in the Ames test on *Salmonella typhimurium* strains TA100.

Arglabin concentration, μg/mL	Number of occurrences, n	Positive wells average, m	Standard deviation, SD	Zero line	Frequency rate exceeding a zero line	*t*-test, *p*-value (unilateral, unpaired)
0	3	5.00	1.73	6.73		
5	3	5.33	0.58		0.79	0.3838
10	3	4.67	1.53		0.69	0.4075
15	3	5.33	1.53		0.79	0.4075
25	3	5.33	2.08		0.79	0.4208
50	3	4.33	1.53		0.64	0.3217
100	3	3.67	1.15		0.54	0.1647
Positive control (4-nitroquinoline N-oxide)	3	46.00	1.00			

Table 3 Research results of mutagenic properties of Arglabin native in tablets in the Ames test on *Salmonella typhimurium* strains TA1535.

Arglabin concentration, μg/mL	Number of occurrences, n	Positive wells average, m	Standard deviation, SD	Zero line	Frequency rate exceeding a zero line	*t*-test, *p*-value (unilateral, unpaired)
0	6	1.67	1.03	2.70		
5	3	1.33	1.53		0.49	0.3525
10	3	1.00	1.00		0.37	0.1938
15	3	1.33	1.15		0.49	0.3363
25	3	1.67	0.58		0.62	0.5000
50	3	0.67	1.15		0.25	0.1137
100	3	1.33	0.58		0.49	0.3131
Positive control (N4-aminocytidine)	3	47.00	1.00			

Table 4 Research results of mutagenic properties of Arglabin native in tablets in the Ames test on *Salmonella typhimurium* strains TA1537.

Arglabin concentration, μg/mL	Number of occurrences, n	Positive wells average, m	Standard deviation, SD	Zero line	Frequency rate exceeding a zero line	*t*-test, *p*-value (unilateral, unpaired)
0	3	4.33	3.21	7.55		
5	3	4.00	1.00		0.53	0.4361
10	3	4.67	1.53		0.62	0.4395
25	3	4.33	2.52		0.57	0.5000
50	3	2.00	1.00		0.26	0.1481
100	3	2.33	0.58		0.31	0.1743
250	3	2.67	0.58		0.35	0.2133
Positive control (9-aminoacridine)	3	47.67	0.58			

The mutagenic potential of the immunomodulator Arglabin native has been investigated in the Ames test used to determine the existence of gene mutations. It is based on the identification of revertant colonies of *S. typhimurium* strains, which are histidine auxotrophs, to a prototrophic state, i.e. capable of survival and growth on the histidine-free medium. In our research we have used four strains of *S. typhimurium* TA98, TA100, TA1535, and TA1537 to define mutations of two types: a base pair substitution and a frameshift. Tests involving these strains help carry out a comprehensive analysis of possible mutagenic effects of the medicine under study.

The results were conclusive and confirmed that the investigated preparation does not have a mutagenic activity. There was no increase in the number of revertant colonies of *Salmonella typhimurium* neither in terms of strains TA100, TA1535 nor when using strains TA98, TA1537. Consequently, Arglabin native in tablets does not cause gene mutations neither of a base pair substitution or a frameshift type.

Therefore, based on the negative response received in the Ames test, we have ascertained that the new original immunomodulator Arglabin native in tablets does not reveal a mutagenic effect on *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 in the studied dosage range.

Further research suggests that the preclinical safety studies of Arglabin native in tablets should be carried on to ensure its application as a new original immunomodulator.

5. Conclusions

In the given research, mutagenic properties of Arglabin native in tablets have been investigated using the Ames test on *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537. The investigated doses of Arglabin native in tablets ranged (in terms of Arglabin substance) from 5.0 to 100 μg/mL (5, 10, 15, 25, 50 and 100 μg/mL) for strains TA100 and TA1535; from 5.0 to 250 μg/mL (5, 10, 25, 50, 100 and 250 μg/mL) for strains TA98 and TA1537. At higher concentrations Arglabin manifested a cytotoxic effect on *Salmonella typhimurium*, consequently, making it impossible to investigate its mutagenic potential further.

In the studied dosage range, Arglabin native in tablets did not induce a number of revertant colonies to rise against the baseline of spontaneous mutations. The average number of positive wells in all assayed concentrations of Arglabin native on *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 was similar to that in the negative control. The frequency rate exceeding a zero line in the studied dosage range of Arglabin native was less than 2.0, consequently, the differences are not considered reliable (significance level $p > 0.05$).

Accordingly, taking into account the negative results of the Ames test, it can be concluded that a new original immunomodulator Arglabin native in tablets does not express mutagenic effects on *Salmonella typhimurium* strains TA98, TA100, TA1535, and

TA1537 within the studied dosage range.

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