



# Lisinopril Can Reduce Genotoxicity of L-Asparaginase in Bone Marrow Stem Cells

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## Abstract

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**BACKGROUND:** Lisinopril is a medication used to treat elevated blood pressure, where it acts as an inhibitor of the angiotensin-converting enzyme (ACE) and moderates the myeloid inflammatory response in bone marrow stem cells. L-asparaginase (ASNase), a chemotherapeutic agent, is used for the treatment of patients suffering from acute leukemia by increasing the genotoxicity of bone marrow stem cells.

**AIM:** This study aims to understand the effect of lisinopril on the genotoxicity of ASNase in bone marrow stem cells.

**METHODS:** Male albino Swiss mice were split into three groups. The mice in the first group were treated with lisinopril (10 mg/kg/day) for 14 days. The mice in the second group were injected intraperitoneally with ASNase (3000 IU/kg). The mice in the third group were treated with lisinopril for 14 days followed by an intraperitoneal injection of ASNase at the end of the 13<sup>th</sup> day. Genotoxicity was evaluated by calculating the percentage of the micronucleus (MN) and the mitotic index (MI).

**RESULTS:** ASNase significantly increased the genotoxicity in bone marrow stem cells by raising the %MN and by lowering the %MI. No significant effect was observed when 10 mg/kg/day of lisinopril was administered. However, a significant decline in the genotoxicity (decreasing %MN and increasing %MI) was observed when the mice were treated with lisinopril and ASNase.

**CONCLUSION:** Using lisinopril, a blood hypertension medication, with the anticancer therapeutic agent, ASNase, decreased the genotoxicity of ASNase in bone marrow stem cells.

## Introduction

Lisinopril is one of the medications used for the treatment of high blood pressure. It is an angiotensin-converting enzyme (ACE) inhibitor, which prevents the conversion of angiotensin I (ANGI) to angiotensin II (ANGII). ANGI, a powerful vasoconstrictor, stimulates the production of aldosterone, thereby minimizing the release of sodium in the urine. Lisinopril is a suitable treatment for reducing hypertension, where it decreases the reabsorption of sodium and potassium excretion from the kidneys [1], [2]. An ACE inhibitor usually targets the precursor bone marrow stem cells by modulating the myeloid inflammatory response [3]. Furthermore, ANGI leads to cell cycle arrest [4] by inducing DNA strand breaks [5]. Several studies have previously demonstrated the correlation of hypertension with cancer and have reported increased cancer mortality in patients with high blood pressure [6]. A similar relationship between high blood pressure patients and an increasing probability of cancer developing in the kidneys has also been observed [7], [8].

Complex rearrangements of DNA patterns, DNA double-strand breaks, and mitotic errors are some of the

major characteristics related to carcinomas [9], [10], [11]. In this study, micronuclei (MN) and mitotic index (MI) were regarded as indicators of DNA instability and mitotic abnormality. MN is a biological marker that is commonly used to detect DNA damage induced by physical and chemical factors [12]. They are DNA fragments that are not incorporated into the nucleus after cell division [13] and are produced from chromosome fragmentation following DNA damage. Compared to the nuclear membrane, MN membranes are fragile, leading to an increased probability of disintegration and release of DNA into the cytoplasm [14], [15]. On the other hand, MI (percentage of dividing cells in metaphase) serves as a good indicator of persistent cell proliferation and steady-state cell division [16].

L-asparaginase can hydrolyze asparagine to aspartic acid. It is used in treating leukemia as its cells require asparagine for their survival and this enzyme deprives them of it leading to increased genotoxic effects [17]. Asparagine cannot be produced by the tumor cells. Therefore, these cells can be killed due to asparagine deficiency. Deprivation of asparagine causes cell cycle arrest in the G<sub>1</sub> phase and promotes its suicidal activity, all of which lead to cell death [18]. As a result, bone marrow suppression is induced due to the inhibition of normal stem cell division by ASNase [19]. ASNase also

enhances MN formation in normal and cultured cancer cells and leads to genotoxicity and DNA breaks [17].

The present study aims to understand the effect of lisinopril on genotoxicity induced by ASNase in normal bone marrow stem cells.

## Materials and Methods

### Animals

Male Swiss albino mice were generously provided by the "National Center for Drug Control and Research, Baghdad, Iraq." Fifty animals were housed in cages and kept on a 12 h light/dark cycle with accustomed feed and water *ad libitum* at  $24 \pm 1^\circ\text{C}$ . The mice were divided into five groups (each group consisted of 10 animals).

### Grouping of animals

In all groups, mice were sacrificed after 14 days and bone marrow slides were prepared. Bone marrow was flushed out of the bones using phosphate-buffered saline by aspiration.

### Control group

Mice were housed for 14 days without any treatments.

### Group I: Negative control group

The mice were drinking water containing 0.1% alcohol without any further treatment for 14 days [20]. Alcohol increases the solubility of lisinopril and has no effect on stem cell viability [20].

### Group II: Lisinopril

The mice were treated with lisinopril using tablets (10 mg) manufactured by AstraZeneca, UK. The tablets were dispersed in water containing 0.1% ethanol, as previously reported by Rafael-Fortney *et al.*, 2011 [20]. The water bottles were replaced 3 times every week. To calculate the average lisinopril dose, mice were weighed and the consumed water volume was recorded giving a concentration of 10 mg/kg/day; a dose that has a similar effective dose to that reported earlier [20].

### Group III: L-asparaginase

A vial containing 10,000 IU ASNase (Fehlandtstr, Germany) was obtained from Al-Karama Teaching Hospital. Untreated mice were housed for

14 days and injected intraperitoneally with ASNase (3000 IU/kg) at the end 13<sup>th</sup> day [21].

### Group IV: Lisinopril and ASNase treatment

The mice were treated with lisinopril (10 mg/kg/day) for 14 days, and ASNase (3000 IU/kg) was injected intraperitoneally at the end of the 13<sup>th</sup> day.

### Genotoxicity assay

To determine the percentage of MI and MN, five slides with bone marrow were prepared and 1000 cells from each mouse were analyzed.

#### 1. Mitotic index assay:

The percentage of MI was calculated, as described by Allen *et al.* (1977) [22]:

$$MI = \frac{\text{Number of Cells in Metaphase}}{\text{Total Cell Number}} \times 100$$

#### 2. Micronucleus assay

Bone marrow was aspirated using heat inactivated plasma, as previously described by Schmid (1975) [23]:

$$MN = \frac{\text{Number of Micronuclei}}{\text{Total Cell Number}} \times 100$$

### Statistical analysis

Social sciences statistical package version 24 (SPSS 24) was used to perform data analysis. A one-way ANOVA was used to check the significance of difference between scores and variables.  $p < 0.05$  was considered statistically significant.

## Results

The number of cells examined in each experimental group was 10,000 ( $10 \times 1000$  cells). Table 1 summarizes the numbers of MI and MN scores together with their percentages. No significant change in values of MI ( $p = 0.09$ ) and MN ( $p = 0.07$ ) and their percentages were observed in both control group and negative control group.

When mice were treated with lisinopril (10 mg/kg/day) for 14 days, a decrease in the percentage of MN ( $p = 0.057$ ) and an increase in the percentage of MI ( $p = 0.06$ ) were observed when compared to the control groups. Contrarily, a significant rise in MN ( $p = 0.007$ ) and a decline in MI ( $p = 0.009$ ) were observed when mice were injected with ASNase (3000 IU/kg) at the end of the 13<sup>th</sup> day. However, when mice were treated with

**Table 1: Percentage of micronucleus and mitotic index in the control and treatment groups**

Control and treatment groups	Number of animals used	Number. of cells examined	MI		MN	
			Number of MI	MI % + SE	Number of MN	MN % + SE
Control group	10	10,000	642	6.42 ± 0.2	268	2.68 ± 0.08
Group I: 0.1% ethanol	10	10,000	615	6.15 ± 0.07	255	2.55 ± 0.08
Group II: Lisinopril (10 mg/kg/day)	10	10,000	675	6.75 ± 0.06	205	2.05 ± 0.08
Group III: ASNase (3000 IU/kg)	10	10,000	412	4.12 ± 0.15	392	3.92 ± 0.12
Group IV: Lisinopril and ASNase	10	10,000	591	5.91 ± 0.12	286	2.86 ± 0.02

MI: Mitotic index, MN: Micronucleus, ASNase: Asparaginase.

lisinopril for 14 days and injected with ASNase at the end of the 13<sup>th</sup> day, a significant reduction in MN ( $p = 0.03$ ) and a significant rise in MI ( $p = 0.01$ ) were observed when compared to the group treated with ASNase alone.

## Discussion

The chemical and physical changes induced in DNA lead to DNA damage, ultimately causing a loss of function. DNA damage can be produced by a variety of endogenous and exogenous factors, such as free radicals, radiation, and chemicals [24]. Reactive oxygen species (ROS) directly induce DNA damage by oxidizing nucleosides [25]. Chemotherapy drugs are also known to enhance ROS levels, contributing to genotoxicity [26]. Genotoxicity and cytotoxicity can be produced by MN induction [27]. Therefore, MN analysis is broadly used to study the aneugenic and clastogenic effects of chemicals and other agents, giving a precise analysis of the genotoxicity induced [28]. Accordingly, the MN assay is employed as a very suitable test to measure genotoxic potential in human peripheral blood lymphocytes [29]. This assay is considered a specific method for the analysis of genotoxicity for many mutagens and carcinogens. However, it is worth noting that MN formation is associated with defects and loss of genetic material and can be used together with MI to enhance the results obtained, especially when studying genotoxicity in blood cells [30].

The solvent containing 0.1% ethanol in water did not show any effect on the MN and MI formation rates, as presented in Table 1. The absence of any genotoxic effect of this concentration of alcohol is in agreement with the results given by Rafael-Fortney *et al.* (2011) [20].

Lisinopril insignificantly decreased genotoxicity by decreasing MN and increasing MI compared to the control group (Table 1). The changes in these values of MN and MI are worth considering because the mice appeared normal. This insignificant reduction in genotoxicity could be attributed to the effect of lisinopril scavenging free radicals that have the ability to cause great damage to DNA [24].

ASNase significantly induced high genotoxicity by increasing MN and decreasing MI (Table 1). ASNase is known to induce DNA damage and cause abnormalities in the cell cycle, which are manifested by cell cycle arrest and DNA breaks. Moreover, ASNase can induce micronucleus formation in normal cells and increase DNA breaks [17].

These DNA damages degrade the level of ASNase in the cell membrane, leading to depletion of its concentration followed by protein dysfunction and cell death [31]. It is worth mentioning that the process of converting asparagine into aspartic acid by ASNase is followed by an enhancement in oxidation levels and a lessening in the reduction state [32]. This oxidation state has the potential to increase ROS levels and cause DNA damage [25], [26].

When mice were treated with lisinopril for 14 days and injected intraperitoneally with ASNase at the end of the 13<sup>th</sup> day, a significant reduction in the genotoxicity of ASNase was observed, as presented in Table 1. A significant decrease in MN and an increase in MI were clearly observed when compared with the group that received ASNase alone. This reduction in genotoxicity may be due to the capability of lisinopril to inhibit ACE. It is well documented that ACE can increase DNA degradation and affect its stability. This is why ANGI is converted to ANGII, which induces DNA damage causing an increase in DNA breaks [5].

Furthermore, lisinopril decreases mitotic errors, which are the hallmark of most carcinomas and DNA double-strand breaks. Usually, high blood pressure is caused by an increase in ANGII. Lisinopril inhibits ANGII production and protects bone marrow stem cells. On the other hand, ACE inhibition can protect myeloid precursor cells from the high concentration of ANGII [3], which may justify the good relationship between kidney cancer and hypertension [7], [8]. This may also suggest that reducing blood pressure with lisinopril could diminish the possibility of cancer.

## Conclusion

Lisinopril can reduce the genotoxicity caused by ASNase in bone marrow stem cells. This effect may imply that using lisinopril to reduce high blood pressure in patients with acute leukemia receiving ASNase can compromise its effectiveness for cancer therapy.

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