The Effect of Dimethyl Sulfoxide on Chromosomal Abnormalities in Human Peripheral Blood Lymphocytes

Geliş Tarihi: 09.10.2020, Kabul Tarihi: 05.12.2020

İlyas YÜCEL1,*, Mahir BINİCİ2 Fikriye Fulya KAVAK3 Diclehan ORAL4 Selahattin TEKEŞ5 Mahmut BALKAN6

Department of Medical Genetics, Faculty of Medicine, Dicle University, Diyarbakir, Turkey.

1. ORCID: 0000-0002-4446-0469,
2. ORCID: 0000-0003-1039-9361,
3. ORCID: 0000-0003-0278-7188,
4. ORCID: 0000-0002-0074-0602,
5. ORCID: 0000-0001-6505-1112,
6. ORCID: 0000-0003-0138-6622

Abstract

Objective: The main aim of this study was to examine the effects of dimethyl sulfoxide (DMSO) on chromosomal abnormalities in human peripheral blood lymphocytes.

Methods: Peripheral blood samples were collected from two healthy men and two healthy women. Then, in vitro studies were conducted with these blood samples, and the results were cytogenetically analyzed. There were two groups: a DMSO group and a control group. DMSO and control medium were added to the samples at 24 hours and 48 hours.

Results: A total of 800 metaphases were examined in this study. Depending on the increase in the number of groups and the time of application, an increase in chromosomal abnormalities was observed, and these were recorded.

Conclusion: In many previous studies, the effects of DMSO have been examined in various tissues and the body, but there are fewer studies about the effects on chromosomes. In this study, we researched the effects of DMSO, except for negative effects and toxicity, on chromosomal abnormalities.

Key Words: DMSO, Cytogenetics, Chromosomal abnormality

Corresponding author: E-mail: yucel-ilyas@hotmail.com

© 2020 Published by International Archives of Medical Research. All rights reserved.
Introduction
Dimethyl sulfoxide (DMSO) is an amphipathic molecule with a highly polar domain and two nonpolar groups. These properties make it soluble in both aqueous and organic media. Therefore, DMSO is a remarkably efficient solvent for water-soluble compounds and disrupts hydrogen bonds (1).

DMSO, which is commonly used in several human therapeutic situations, such as drug-delivery systems, cryopreservation of autologous peripheral blood stem cells, and embolization of cerebral aneurysms or arteriovenous malformations (AVMs), has a variety of biological actions that have made it a focus of numerous studies (2, 3).

Although DMSO has occasionally been proposed to be neuroprotective and oxidative and induce behavioral alterations, its mechanisms of action remain unclear (4).

It has been reported to alter the permeability of the cell wall and facilitate the transport of substances across membranes (5).

Studies conducted by Chaloupka showed that DMSO tends to be angiotoxic and neurotoxic (6, 7).

DMSO is an organic compound that has a plethora of biological actions, including antioxidant, anti-inflammatory, antinociceptive, and radioprotective effects (8, 9).

It has also been shown to modify enzyme activity, change the secondary structure of both DNA and RNA, affect the mitotic cycle of normal dividing cells, and impede cell membrane-bound electron transfer systems (10-12).

Based on this information, the effects of DMSO on chromosomal abnormalities have to be strictly examined.

Method
In this study, we used peripheral blood samples, which were collected from two 25-year-old men and two 25-year-old women. Before the blood was collected, the individuals had not smoked or taken any drugs in the previous six months. The individuals who gave blood were healthy and had no chromosomal abnormalities.

The lymphocytes from the samples were analyzed in vitro. The concentration of DMSO that was added to the tubes was calculated at 3.7 μl DMSO for every 1 ml. For the control group, a nutrition medium was added instead of DMSO (13, 14).
Blood microculture

Medium 5 ml
Blood 0.25 ml
Phytohemagglutinin 0.10 ml
Total 5.35 ml

According to that, the amount of DMSO used for each tube was as follows: 3.7x5.35=20 μl. The amount of DMSO added to the tubes at a specified time was calculated. In the control group, the nutrition medium that was used instead of DMSO was HAM’s F-10, and this medium was added to the tube at the same time that was specified.

Normally, the in vitro blood culture period is 72 hours. In our study, two different times were chosen: 24 hours after starting the blood culture (A) and 48 hours after starting the blood culture (B). DMSO and medium were added to the tubes at these times. For every four tubes that were created, a total of 16 tubes were prepared.

Group A (at 24 hours/48 hours exposure)
Group B (at 48 hours/24 hours exposure) (Table-1)

Table-1: Added material- Time table

<table>
<thead>
<tr>
<th>TIME</th>
<th>24’TH HOUR</th>
<th>48’TH HOUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEDIUM (NUTRIEN MIXTURE)</td>
<td>CONTROL</td>
<td>CONTROL</td>
</tr>
<tr>
<td>DMSO</td>
<td>DMSO</td>
<td>DMSO</td>
</tr>
<tr>
<td>ADDED MATERIAL</td>
<td>-A-</td>
<td>-B-</td>
</tr>
<tr>
<td>ADDED MATERIAL</td>
<td>-A-</td>
<td>-B-</td>
</tr>
</tbody>
</table>

A modified microculture technique was used in this study. The lymphocyte microculture technique is a method of chromosome preparation. Afterward, all phase chromosomes were obtained and prepared for examination (15, 16).

The chromosomes were stained on microscope slides after microculture. Giemsa banding was used to stain the samples. For a flat Giemsa stain, 5 ml Giemsa was added to 95 ml distilled water, and a coating buffer was prepared. Microscope slides were stained in the buffer for 5 minutes and then washed and dried.
Metaphases were examined on light microscopy, and the results were noted on a form. The first examination employed a 10X ocular lens, and the metaphases were marked. Then, an immersion was used, and the metaphases were examined at 100X magnification. Afterward, all examination results were checked and noted on the form again. A total of 50 metaphases were examined for each tube, and a total of 800 (16X50) metaphases were examined.

Results
The metaphases examined in the control and DMSO groups were compared and recorded. Existing chromosomal abnormalities included fractures, gaps, deletion, duplication, endoreduplication, fragments, dicentric chromosomes, and satellite association.

In the examined metaphases of samples to which DMSO was added, more abnormalities were found. There were 4.5% more abnormalities in the DMSO group than in the control group. According to these results, DMSO is one of the causes of chromosome abnormalities (Table-2).

Table-2: % Chromosome abnormality (Fixed time). ap<0.001 compared with the control group. The data were analyzed using the student’s t test.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examined metaphases</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Normal metaphases</td>
<td>340</td>
<td>322</td>
</tr>
<tr>
<td>Abnormal metaphases</td>
<td>60</td>
<td>78 a</td>
</tr>
<tr>
<td>% Abnormal metaphases</td>
<td>15</td>
<td>19,5</td>
</tr>
</tbody>
</table>

Chromosome fractures and gap abnormalities are more commonly found in the metaphase plate. There were more abnormalities in the A-B and C chromosome groups, compared with in execution time abnormality increments. There were few minor deletions in the metaphase plate. Abnormalities are structural and numerical, and there are more structural abnormalities than numerical abnormalities. Many of the abnormalities are structural.

In the control group, the rate of abnormalities was 15%, and after DMSO was added, the abnormalities increased to 19.5% (Graphic -1).
Graphic-1: % abnormality graphic (Fixed time)

The periods that were determined were 24 hours (for the 48-hour application) and 48 hours (for the 24-hour application). DMSO and control solution were added to tubes at the determined time. As the application time increased, the quantity of abnormalities increased. At 48 hours after application, there were 72 abnormal metaphases, whereas at 24 hours after application, there were 66 abnormal metaphases. According to the results, increasing in the application time affected abnormalities %2.5 (Table 3).

Table-3: Chromosome abnormalities % (active ingredient fixed). The data were analyzed using the student’s t test.

<table>
<thead>
<tr>
<th></th>
<th>24’th hour (48 hours application)</th>
<th>48’th hour (24 hours application)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examined metaphases</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Normal metaphases</td>
<td>328</td>
<td>334</td>
</tr>
<tr>
<td>Abnormal metaphases</td>
<td>72</td>
<td>66</td>
</tr>
<tr>
<td>% Abnormal metaphases</td>
<td>18</td>
<td>16.5</td>
</tr>
</tbody>
</table>

About the data with increasing application time, abnormality and aberrations are increased (Graphic-2).
Graphic-2: % Abnormality (active ingredient fixed)

According to the graphics, there is a linearly proportional between active ingredient-increasing application time and % abnormality (Graphic-3).

Graphic-3: % Abnormality (depend on time and active ingredient)
Discussion

In this study all data were noted to the form that was created before. preparations were examined and finding information analyzed.

Numerical chromosome aberrations were detected at levels too low to be ignored. Abnormalities of groups were calculated and compared. In control group abnormality is %15 on the other hand the application of DMSO increased abnormality and the result is %19.5. A statistically significant difference was found between patient and control groups. (\(p<0.001\)) According to the results, DMSO affected chromosome abnormality and increase percentage of anomaly.

There was no statistically significant difference between the application time groups. (\(p>0.05\)) As a result, DMSO is effective on chromosome anomalies. However, the DMSO’s application time causes anomaly, but it does not represent a statistically significant difference.

References


Figures

Figure-1: Deletion
Figure-2: Acentric chromosome

Figure-3: Chromatid gap
Figure 4: Satellite association