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Effect of Bezafibrate on Deoxyribonucleic Acid in the Presence of Iron and Copper

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ABSTRACT

Aim: Bezafibrate is generally used to treat hyperlipidemia and diabetic patients. Analysis of the effect of chemicals and drugs on DNA in the presence of iron and copper is a very important issue in medical research. Therefore this research aimed to analyze the effect of bezafibrate on DNA in the presence of iron and copper.

Material and Methods: Supercoiled pUC19 plasmid DNA was treated with different concentrations of bezafibrate (2.6, 1.3, 0.13, 0.013 and 0.0013 mM) in the presence of copper, iron and copper plus hydrogen peroxide followed by analyzing in agarose gel (1%) electrophoresis.

Results: Any of the bezafibrate concentrations did not break DNA in the presence of FeSO4 and CuCl2. There was no difference in the density and sharpness of the bond of supercoiled DNA in the treated samples compared to the related bond in the control samples. Although 0.13, 0.013 and 0.0013 mM of bezafibrate could not protect the DNA against the hydrogen radicals, 2.6, 1.3 mM of the drug could protect DNA by concentration dependent manner.

Conclusion: Our study shows that the harmful effect of bezafibrate on DNA fragmentation may be indirect.

Key Words: *Bezafibrate, Diabet, Supercoiled, Obesity, Copper*

Bezafibrat'ın Demir ve Bakır Varlığında Deoksiribonükleik Asit Üzerine Etkisi

ÖZET

Amaç: Bezafibrat genellikle hiperlipidemi ve diyabetik hastaları tedavi etmek için kullanılır. Kimyasal ve ilaçların demir ve bakır varlığında DNA üzerindeki etkilerinin analizi, tıbbi araştırmalarda çok önem arz etmektedir. Bu nedenle, bu araştırma bezafibratın DNA üzerindeki etkisini demir ve bakır varlığında analiz etmeyi amaçlamıştır.

Gereç ve Yöntemler: Süpersarmal pUC19 plazmid DNA bakır, demir ve bakır artı hidrojen peroksit varlığında Bezafibrat'ın farklı derişimleri (2,6 - 1,3 - 0,13 - 0,013 ve 0,0013 mM) ile muamele edildi, ardından agaroz jeli (% 1) elektroforezinde analiz edildi.

Bulgular: Bezafibrat derişimlerinin hiçbirinin FeS04 ve CuCl2 varlığında DNA'yı kıramadığı gözlemlendi. Muamele edilen Süpersarmal DNA'nın band yoğunluğu ve parlaklığı kontrol örnekler ile karşılaştırıldığında hiçbir fark görülmedi. Her ne kadar 0,13 – 0,013 ve 0,0013 mM Bezafibrat DNA'yı hidrojen radikallerine karşı koruyamasa da, ilacın 2,6 – 1,3 mM'si derişimine bağlı olarak DNA'yı koruyabildiği gözlemlendi.

Sonuç: Bizim Çalışmamız bezafibratın DNA fragmantasyonu üzerindeki zararlı etkisinin dolaylı bir şekilde olabileceğini göstermektedir.

Anahtar Sözcükler: *Bezafibrat, Diyabet, Süpersarmal, Obezite, Bakır*

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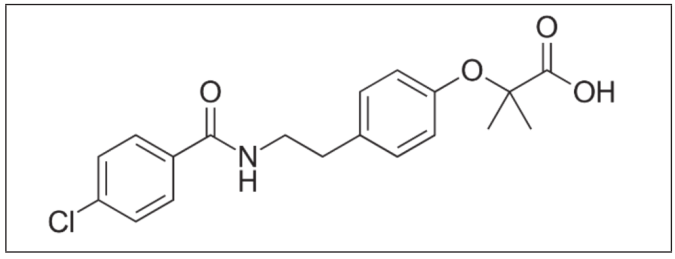
INTRODUCTION

Fibrates are used worldwide to prevent ischemic vascular diseases because they are an effective agent in lowering triglyceride and low-density lipoprotein cholesterol levels in the blood (1). Bezafibrate, one of the fibrate drugs intervenes in insulin sensitivity and lipid metabolism by activating all known peroxisome proliferator-activated receptors (PPARs) (2). The drug is generally used to treat hyperlipidemia and diabetic patients (3). Obesity is a serious problem worldwide. Bezafibrate is prescribed as tablets (200mg and 400mg). In our country, Turkey, in 1990, 18.8% of the adult population (28.5% in women and 9% in men) were obese, while in 2010 this number increased to 36% (44% in women and 27% in men) (4). There are many drugs that have been withdrawn and abandoned after their toxic effect has been confirmed by researchers (5). Although bezafibrate is known to be nongenotoxic carcinogens, some study has shown that fibrate group drugs exert their genotoxic effects by reactive oxygen species (ROS) (6, 7). Researches are very interested to study Drug-DNA interactions because DNA (Deoxyribonucleic acid) is an important material in maintaining cell life. DNA (deoxyribonucleic acid) including all genetic information of living things. Interactions of drugs and chemicals with DNA and the resulting DNA damage are often associated with cancer (8). DNA damaging drugs promote the growth of cancerous cells. Genotoxic drugs used by many women have increased the risk of breast cancer more than twice (9). The changes in genetic information can be transmitted to future generations. Cooper and iron are essential elements of life. Both of them are important elements in the maintenance of chromosome structure (10). They are transition elements and show two oxidation states, oxidized states (Cu2+ and Fe+++) or reduced states (Cu+ and Fe2+). In a reduced state, they catalyze the generation of damaging reactive oxygen species (ROS) that has a toxic effect (11). There are many studies that show the presence of correlation between iron intake and the risk of multiple cancers such as colorectal cancer (12), Breast cancer (13), Esophagus cancer (14) and Lung cancer (15). In addition, there are a lot of reports showing the association between serum copper level and cancers such as lung cancer (16) and cervical cancer (17). Therefore, this study aimed to investigate whether bezafibrate can be transformed into a destructive substance against DNA in the presence of iron and copper.

MATERIAL and METHODS

Reagents

In this research, Bezafibrate (catalog No: 1042-1) was purchased from Sigma-Aldrich and used as the test substance. The molecular weight of bezafibrate was 361.82 and its chemical structure was as follows:



Chemical structure of bezafibrate

Preparation of Plasmid DNA

pUC19 plasmid DNA was purified from Escherichia coli NEB5α previously transformed with the plasmid. The plasmid purification was conducted by EZ-10 Spin Column Plasmid DNA Miniprep Kit (bio basic) using the kit instructions. Purity and amount of the DNA were analyzed by beer-lambert law and agarose gel (1%) electrophoresis (14, 15).

Effect of Bezafibrate on DNA in the Presence of Iron

Bezafibrate solutions were prepared by using DMSO as a solvent. 49μM bp of pUC19 plasmid DNA were treated with 1.5 mM of FeSO4 plus different concentrations of bezafibrate (2.6, 1.3, 0.13, 0.013, and 0.0013 mM) by using the procedure used by Yi et al. (18). It was then incubated at 37°C for 1 hour followed by analyzing in agarose gel (1%) electrophoresis at 90 volts for 55 minutes and staining with ethidium bromide (19). A sample containing only plasmid DNA and the other with DNA plus FeSO4 were used as control samples. All tests were performed in PBS (Phosphate Buffered Salt) (pH, 7.4) buffer.

Effect of Bezafibrate on DNA in the Presence of Copper

In order to study the effect of bezafibrate on DNA-copper interaction, 49 μM bp of pUC19 plasmid DNA were treated with 60 μM of CuCl2 plus different concentrations (2.6, 1.3, 0.13, 0.013, and 0.0013 mM) of the drug at 37°C for 45 minutes. The reaction mixture was then subjected to analysis on a 1% agarose gel electrophoresis for 55 minutes at 90 volts. A plasmid DNA treated with no substance and DNA sample treated with only CuCl2 were used as control samples. All tests were performed in PBS (Phosphate Buffered Salt) (pH, 7.4) buffer.

Effect of Bezafibrate on Radical Hydroxyl-Mediated DNA Breakage

To examine the protective effects of bezafibrate on DNA against the *radical hydroxyl*, the pUC19 plasmid DNA (49 μM bp) was treated with 60 μM CuCl2 plus 6mM H2O2 plus different concentrations of bezafibrate (2.6, 1.3, 0.13,

0.013, and 0.0013 mM) and followed by incubation at 37°C for 1 hour. After that, the reaction mixture was subjected to analysis on agarose gel (1%) electrophoresis for 55 minutes at 90 volts (20).

RESULTS

Preparation of Plasmid DNA

pUC19 Plasmid DNA was purified as a concentration of 0.247 mM bases. After purification, the DNA solution showed 0.08 absorbance value at 260 nm wavelength and the ratio of A260/A280 obtained as >1.8, showing that the DNA solution was sufficiently pure. In agarose gel electrophoresis it showed three bonds; related to supercoiled, linear and relaxed form of the plasmid DNA. The supercoiled form having faster migration than the other two forms made thicker and sharper bond in comparison with the other two bands (Figure 1).

Effect of Bezafibrate on DNA in the Presence of Iron

As it was shown in figure 2, any of the bezafibrate concentrations (2.6, 1.3, 0.13, 0.013 and 0.0013 mM) plus FeSO4 could not break the pUC plasmid DNA. There was no difference in the density and sharpness of the bond of supercoiled DNA in the treated samples compared to the related bond in the control samples.

Effect of Bezafibrate on DNA in the Presence of Copper

2.6, 1.3, 0.13, 0.013 and 0.0013 mM of bezafibrate did not convert copper to cuprous breaking double helix DNA. There was no difference in the density and sharpness of the bond of supercoiled DNA in the treated samples compared to the related bond in the control samples (Figure 3).

Effect of Bezafibrate on Radical Hydroxyl-Mediated DNA Breakage

In the presence of H2O2 and CuCl2, DNA damaging hydrogen radicals were produced. as shown at figure 3, although 0.13, 0.013 and 0.0013 mM of bezafibrate could not protect the DNA against the hydrogen radicals, 2.6, 1.3 mM could protect DNA by concentration dependent manner (Figure 4).

DISCUSSION

One of the inexpensive, fast and valuable methods to study the DNA-breaking activity of chemicals, is the treatment of plasmid DNA with the chemicals and analyze it in agarose gel electrophoresis (21, 22). The native conformation of pUC-19 plasmid DNA is supercoil. Any breakage in one of the double strands converts the supercoiled form to open circular (relaxed) form while breakage in the same position on both strands makes linear plasmid DNA. Migration of the linear form through agarose gel is slower than the supercoiled form and faster than the open circle form (23). Therefore the pUC19 plasmid DNA was selected as a DNA sample in this research. The normal serum ferritin range for men is 15-320 mg/mL and for women is 6-155mg/mL (11). Human physiological plasma iron and copper total

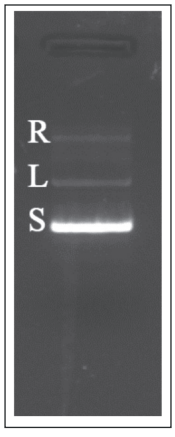
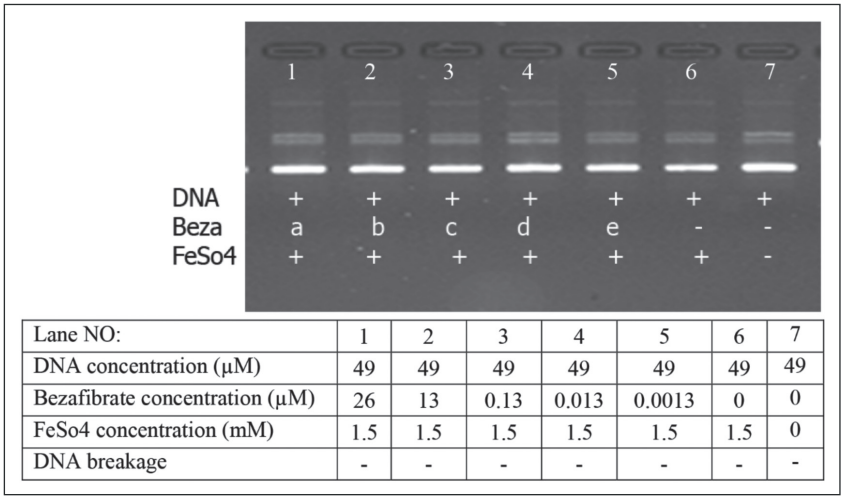


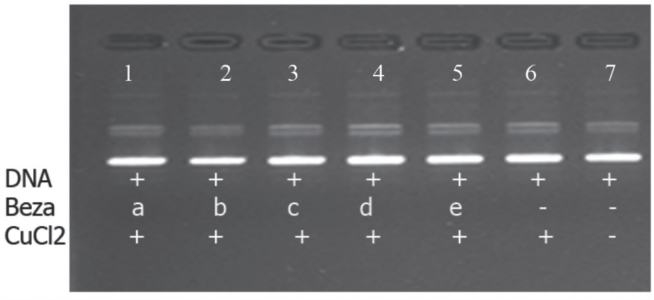
Figure 1: Analysis of the purified plasmid in agarose gel electrophoresis. R, L, and S represent relaxed, linear and supercoiled form, respectively. 3 µl of 0.247 mM bases of pUC19 plasmid DNA were loaded in well.



Lane NO:	1	2	3	4	5	6	7
DNA concentration (µM)	49	49	49	49	49	49	49
Bezafibrate concentration (µM)	26	13	0.13	0.013	0.0013	0	0
FeSo4 concentration (mM)	1.5	1.5	1.5	1.5	1.5	1.5	0
DNA breakage	-	-	-	-	-	-	-

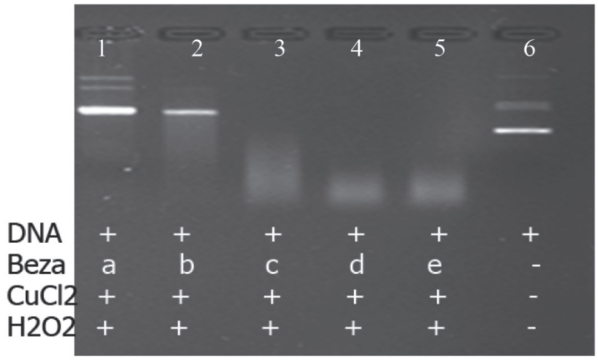
Figure 2: Treatment of PUC19 plasmid DNA (49 µM bp) with bezafibrate plus iron. a, b, c, d and e represent 2.6, 1.3, 0.13, 0.013 and 0.0013 mM respectively. Plus and minus signs represent presence and absence, respectively.
The concentration of FeSo4 was 1.5 mM.

concentrations (free and bound) range between (11–31 μM) and (14–19 μM), respectively (24). Copper is one of the most redox active metal ions found in cells and is closely associated with chromatin (25). Therefore the analysis of the effect of chemicals and drugs on DNA and proteins, in the presence of iron and copper is very important and interesting for many researchers. In contrast to the drugs such as Resveratrol (26), bleomycin (27), chloramphenicol (28), N-acetylcysteine (29) and procarbazine (30), bezafibrate, even at high concentrations, did not cause any breakage in a supercoiled plasmid in the presence of iron or copper. In contrast, bezafibrate could protect supercoiled pUC19 plasmid DNA against damaging hydroxyl radicals generated by Fenton reactions. Marina Isidori and her coworkers (31) investigated the genotoxic effect of bezafibrate with Ames test using Salmonella typhimurium strains TA98 and TA100 and showed that bezafibrate and its photoproduct had no genotoxic risk. Takayoshi Maiguma et al. (32) by determining mitochondrial enzyme activity showed that more than almost 100μM of bezafibrate reduced the human embryonal rhabdomyosarcoma cells (HRMSC) viability. The same group by Hoechst 33342 staining determined that the decrease of HRMSC viability were contributed to the chromatin condensation occurring in the cells after treatment by 500 μM bezafibrate. Chromatin condensation can result from DNA fragmentation (33). Topaktas et al. (34) reported bezafibrate to have a cytotoxic effect on cultured human peripheral blood lymphocytes. Lucia Rocco and her coworkers, using RAPD-PCR, Diffusion Assay and Comet Assay methods showed that bezafibrate (57 ng/L) led to a significant increase of DNA fragmentation in sperm cells (6). Before, some reports have been published about the relation between the decline in brain functions, myalgia, myopathy and anemia (35-37).



Lane NO:	1	2	3	4	5	6	7
DNA concentration (μM)	49	49	49	49	49	49	49
Bezafibrate concentration (μM)	26	13	0.13	0.013	0.0013	0	0
CuCl2 concentration (μM)	60	60	60	60	60	60	0
DNA breakage	-	-	-	-	-	-	-

Figure 3: Treatment of PUC19 plasmid DNA (49 μM bp) with bezafibrate plus copper. a, b, c, d and e represent 2.6, 1.3, 0.13, 0.013 and 0.0013 mM respectively. Plus and minus signs represent the presence and absence, respectively. The concentration of CuCl2 was 60 μM.



Lane NO:	1	2	3	4	5	7
DNA concentration (μM)	49	49	49	49	49	49
Bezafibrate concentration (μM)	26	13	0.13	0.013	0.0013	0
CuCl2 concentration (μM)	60	60	60	60	60	0
H2O2 concentration (mM)	6	6	6	6	6	-
DNA protection	+	+	-	-	-	+

Figure 4: Protective effect of bezafibrate against hydroxyl radical. The concentration of pUC19 plasmid DNA, CuCl2 and H2O2 were as 49 μM bp, 60 μM, and 6 mM, respectively. a, b, c, d and e represent 2.6, 1.3, 0.13, 0.013 and 0.0013 mM respectively. Plus and minus signs represent the presence and absence, respectively.

In contrast to the reports, in my previous research bezafibrate did not damage DNA even in the presence of an oxidant agent and reductive agent (related findings have not yet been published). In addition, in this research DNA was not affected with bezafibrate in presence of copper or iron. Therefore the discrepancies of these results with previously reported findings by other researchers can be related to the binding of the drug with DNA and/or with proteins involved in chromatin structure. Drug binding causes structural and conformational changes in the DNA such as DNA bending, winding double or single-strand breaks, resulting in DNA damage (38). Furthermore, as previously reported about methyl 2-benzimidazole carbamate by Banduhn and Obe (39), it may possibly be due to the binding and effect of bezafibrate on microtubules. Therefore the next research of our lab will be aimed to study the binding of the drug to DNA, histones and microtubules proteins.

In conclusion, in contrast to the drugs such as Resveratrol, bleomycin, chloramphenicol, N-acetylcysteine and procarbazine, bezafibrate did not break double helix DNA in the presence of iron and copper. Although 0.13, 0.013 and 0.0013 mM of bezafibrate could not protect the DNA against the hydrogen radicals, 2.6, 1.3 mM could protect DNA by concentration dependent manner. Therefore, our study demonstrates that the detrimental effect of bezafibrate on DNA fragmentation may be related to the indirect effect of the drug.

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