Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Investigation of genotoxic effects of doripenem using cytogenetic and molecular methods

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Received March 30, 2017; **Accepted** October 18, 2017; **Published** October 31, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.10.14

Abstract: The main aim of this study was to investigate the genotoxic effects of doripenem (DRP) using both cytogenetic and molecular test systems. Although there have been some studies reporting the effects of DRP, none of them has shown the genotoxic effects of DRP. In order to achieve the main aim of the study, the human peripheral lymphocytes were treated with 100 µg/ml, 200 µg/ml, and 400 µg/ml concentrations of DRP for 24 and 48 hours, and the chromosome aberration (CA) and micronucleus (MN) methods were used as the cytogenetic tests and RAPD-PCR method was used as the molecular test to determine the genotoxic effects of DRP. DRP did not induce the chromosome aberrations and micronucleus frequencies at all concentrations and at all treatment periods. So, it was concluded that DRP did not show any cytotoxic effect. However, DRP increased the number of polymorphic bands and decreased the ratio of genomic template stability, especially at the 48-hour treatment period. In this study, according to the obtained results, it was determined that DRP failed to show any genotoxic risk at the therapeutic doses. This result also indicates that DRP could be a reliable antibiotics according to its rapid metabolism.

Key words: Chromosome aberration; Micronucleus; Genotoxicity; RAPD-PCR; Doripenem.

Introduction

Infectious diseases have been one of the greatest health problems that threaten the life of human being for centuries. For thousands of years, people looked for a way to be able to cure infectious diseases. For example a birch mushrooms (*Piptoporus betulinus*) were found in the bag of Ötzi (Iceman) that lived 5300 years ago who was found at the Ötztal Alps at Austrian-Italian border in 1991. Capasso (1998) reported that this fungus has anti-bacterial and anti-hemorrhagic effects (1). Before the discovery of synthetic antibiotics, some plants such as garlic and onion were also used by humans for their antibiotic features. Allicin, an organosulfur compound in garlic, was isolated for use as an antibacterial substance against infectious diseases (2).

The unnecessary use of antibiotics led to the development of antibiotic resistant strains of a wide variety of bacteria and resistant microorganisms such as parasites, viruses, and fungi (3-4). Antibiotic resistance has become a threat in the hospital environments, as well. A study was suggested that gram-negative microorganisms were a dominant killer among bacterial pathogens in intensive care units (5). World Health Organization (WHO) reported that many people most probably be dead at the 21st century, because of an infection resulted from a minor injury due to the growing antibiotic resistance in microorganisms (6).

Over the past few decades, new antibiotics are produced against microorganisms which developed resistance. One of them is doripenem (DRP), which was used as the test material in this study. Doripenem is a β-lactam class 2 carbapenem group antibiotic (1β-metilcarbapenem) that developed for hospitalized patients with systemic bacterial infections by Peninsula Pharmaceuticals, Inc. (Alameda, California, USA) (7). Carbapenems are used as last antibiotics resources for heavily ill patients with serious infectious diseases or for patients with suspected multi-resistant bacterial infections (5,8-10). Additionally, DRP has an anti-pseudomonal activity and used for treatment of complicated intra-abdominal infections, complicated urinary tract infections, and nosocomial pneumonia (11-12).

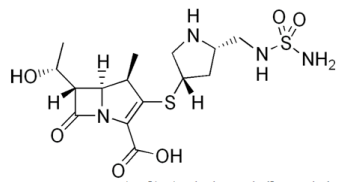
The anti-microbial activity of DRP is similar to imipenem, meropenem and ertapenem. Therefore, DRP has a significant and strong activity against Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Bacteroides fragilis*, streptococcus, methicillin-sensitive staphylococcus (13), penicillin-resistant streptococcus (14), *Streptococcus pneumoniae*, viridans (green) streptococcus group and β-hemolytic streptococcus (15). In addition, DRP is preferred for treatment of nosocomial pneumonia (16-17). Although DRP has these benefits, it can also lead to undesirable side effects such as headache, seizures, rash, pruritus, Stevens-Johnson syndrome, nausea, diarrhea, oral candidiasis, vulvo mycotic infection, renal dysfunction, renal failure, anemia, leukopenia, neutropenia, thrombocytopenia, anaphylaxis, elevated liver enzymes (18).

There have been many studies done about genotoxic effects of diverse antibiotics. These studies reported different observations on genotoxic effects of different

antibiotics. According to these studies, some of the antibiotics have genotoxic effects while the others have no genotoxic effects (19-25). However, according to our knowledge, no research has been conducted on investigating the genotoxicity of DRP. The main aim of this study was to investigate the genotoxic effects of DRP using chromosome aberration (CA) and micronucleus (MN) tests and using RAPD-PCR test in human lymphocytes in vitro.

Materials and Methods

In the present study, DRP was used as the test substance and the human peripheral lymphocytes was used as the material. Doripenem was purchased from Fluka (98%, CAT. NO: 32138). The structure and the properties of DRP was shown in Figure 1.



Chemical name :(4R, 5S, 6S)-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-3-[(3S, 5S)-5-[(sulfamoylamino) methyl] pyrrolidin-3-yl]sulfanyl-1-azabicyclo[3. 2. 0] hept-2-ene-2-carboxylic acid.
Molecular formula : $C_{17}H_{24}N_4O_6S_2.H_2O$
Molecular weight : 438.52 g/mol
CAS No : 364622-822-2

Figure 1. The structure and the properties of DRP.

Chromosome aberration (CA) and micronucleus (MN) tests

The method of Evans (1984) was used to prepare the cell culture for detection of chromosome aberrations (26). Two-hundreds µl of heparinized (1/10) peripheral blood from four healthy donors (two males, two females, non-smokers, age 23-25) was added to 2.5 ml chromosome medium (PbMax, Gibco, 12552-013) at sterile conditions (27). Then, the cultures were incubated at 37°C for 72 hours. Three non-toxic concentrations (100, 200, and 400 µg/ml) of DRP were added to the culture tubes and the cells were incubated with DRP for 24 and 48 hour treatment periods. An untreated control and a positive control Ethyl methanesulfonate (EMS, 0.2 µg/ml) (Sigma M0880) were also used to determine the difference between treated and untreated samples. The cells were exposed to colchicine (0.06 µg/ml, Sigma C9754) 2 hours before harvesting. The cells were harvested by 0.4% KCl as hypotonic solution and methanol: glacial acetic acid (3:1) as fixative. The air dried slides were stained with 5% Giemsa (Merck, 1.09204.0500), which prepared in Sorensen buffer, and covered with Entellan® (Merck, 107961).

To determine the structural and numerical chromosomal alterations, 100 well-spread metaphases were evaluated for each concentrations and treatment periods (totally 400 metaphases per concentration of 4 donors). The CA was classified according to the ISCN (International System for Human Cytogenetic Nomenclature) (28). Gaps were not evaluated as CA according to Mace et al. (1978) (29). The mitotic index (MI) was also determined by scoring 3000 cells from each donor. The MI explained the effects of the chemicals on G2 stage of cell cycle (30).

The modified methods of Fenech (2000) and Kirsch-Volders et al. (2003) were used to determine the micronucleus (31-32). Two-hundreds µl of heparinized (1/10) peripheral blood from 4 healthy donors (two males, two females, non-smokers, age 23-25) was added to 2.5 ml PbMax chromosome medium at sterile conditions and the cell cultures were incubated at 37°C for 68 hours. The cells were treated with 100, 200, and 400 µg/ml concentrations of DRP for 24 and 48 hours. An untreated control and a positive control of EMS (0.2 µg/ml) were also used. 6 µg/ml of Cytochalasin B (Sigma, C6762) was added to the cultures for 24 hour treatment period to block cytokinesis. The cells were harvested after hypotonic solution treatment (0.4% KCl, for 2 minutes) and fixative solution as described (31). The air dried slides were stained and covered as described above.

Two-thousands binucleated cells were scored for each donor (8000 binucleated cells were scored per concentration) to evaluate the micronucleus frequency. To evaluate the nuclear division index (NDI), the cells with 1, 2, 3 and 4 nuclei were scored by calculating 1000 cells for each concentration and treatment period. The NDI was calculated using formula: NDI = (M1) + (2xM2) + (3xM3) + (4xM4)/N; where M1–M4 represent the number of cells with one to four nuclei and N is the total number of viable cells scored (31).

RAPD-PCR test

Ten oligonucleotide primers were used to determine the molecular genotoxicity of DRP in human peripheral lymphocytes with different GC base pair proportion (Table 1) as described by Atienzar and Jha (2006) with minor modification (33). 330 µl non-heparinized peripheral blood from two healthy donors (a male and

Table 1. Oligonucleotide primers.

Primers	Sequences (5'-3')	G+C %	Cat.no
PM1	5'-GTTTCGCTCC- 3'	60	OPB-01
PM2	5'-GTAGACCCAT- 3'	50	Buyukleyla, 2013
PM3	5'-AAGAGCCCGT- 3'	60	Buyukleyla, 2013
PM4	5'-TTGGCACGGG- 3'	70	OPD-07
PM5	5'-AACGCGCAAC- 3'	60	Buyukleyla, 2013
PM6	5'-GGTGACGCAG- 3'	70	OPB-07
PM7	5'-GGGTAACGCC- 3'	70	OPA-09
PM8	5'-CCCGTCAGCA- 3'	70	Buyukleyla, 2013
PM9	5'-TCCGATGCTG- 3'	60	OPS-07
PM10	5'-CTGCGCTGGA- 3'	70	OPU-16

Table 2. Content of RAPD-PCR mixture.

PCR mixture	Volume (µl)
ddH_2O	11.5
10X Taq Buffer	2.5
$MgCl_2$ (25 mM)	3.0
dNTP mix (25mM) (Fermantas, RO191)	0.5
Primer (5 pmol)	4.0
Taq DNA Polimerase(500 Unit/µl) (Fermantas, SB38)	1.0
DNA (5 ng/ml)	2.5
Total Volume	25

a female, non-smokers, age 24) was added to PbMax medium at sterile conditions and incubated at 37±0.5°C for 72 hours. Then, the cultured cells were treated with 100, 200, and 400 µg/ml concentrations of DRP for 24 and 48 hours. An untreated negative control and two positive controls (EMS 0.2 µg/ml and sodium azide, SA, 2 µg/ml) (Sigma, S2002) were also added to the analysis. EMS was used to cause frameshift mutation and SA was used for detection of a single base substitutions (single-point mutations). At the end of the incubation, the culture cells were washed twice with sterile 0.9% NaCl and DNA was isolated using the High Pure PCR Template Preparation Kit (Roche Diagnostics, GmbH, Mannheim, Germany) according to the manufacturer's protocol.

Thirty-five RAPD reactions were performed using a Verity 96-well thermal cycler (Applied Biosystems, USA) using the 10 oligonucleotide primers. The content of PCR reaction mixtures and PCR conditions were presented in Table 2 and Table 3.

After final extension PCR products were electrophoresed on %1.8 agarose gel (Thermo Scientific, SM0321) at 150 V for 75 minutes. Next, the gel was stained with ethidium bromide (5 µg/ml) for 30 seconds and it was kept in distilled water for 30 minutes and then the gel was visualized under UV illumination (Vilber Lourmat, Infinity 1100).

To determine the polymorphic bands, all amplified bands in untreated control were scored as "0" and new or missing bans in treated group were scored as "1". The bands in treated groups were scored "0" as the same in control group. The genomic template stability (GTS) rate was calculated using the following formula: GTS% = 100 - 100.(a/n), where "a" is the polymorphic bands in a treated group (total number of new and missing bands) and "n" is the total number of bands in untreated control group (34). Changes in RAPD profiles decreased the GTS rate, which indicates to genotoxic effects.

Statistical analysis

The t-test was used for the statistical significance of all parameters. Dose response relationships were determined from the correlation and regression coefficients for the percentage of total CA and abnormal cells (AC), mean MI, MN, and NDI.

The mean number of polymorphic bands and the percentage of GTS value were also analyzed using t-test.

Results

Doripenem did not significantly induce neither the percentage of CA nor AC at all concentrations and treatment times with the exception of 200 µg/ml concentration for 24 hour treatment period (Table 4). Additionally, no dose-dependent effect was observed. It also did not induce the percentage of MN at all concentrations at any treatment period (Table 4). Doripenem was not cytotoxic in cultured human peripheral lymphocytes. Additionally, DRP did not decrease neither the MI nor the NDI at all concentrations at both treatment periods as shown in Table 5.

A significant increase was observed in total poly-

Table 3. RAPD-PCR protocol.

Program No	Operation	Temperature (°C)	Time (min.)	Number of Cycles
1.	Initial denaturation	95	5	1
2.1	Denaturation	95	1	35
2.2	Annealing	34	1	
2.3	Extension	72	2	
3.	Final extension	72	10	1

Table 4. The percentage of CA, AC and MN in human peripheral lymphocytes treated with DRP for 24 and 48 hours[+].

Test substa.	Time (hour)	Concent. (µg/ml)	Abnormalities B'	B"	P	Structural CA±SE (%)	Abnormal Cells±SE (%)	MNBC±SE (%)
Control	-	-	5	1	3	1.50±0.64	2.00±0.70	0.39±0.10
EMS	24	0.2	64	26		22.50±4.85	18.75±3.81	1.02±0.12
DRP	24	100	7	1	5	2.00±0.57	3.25±1.03	0.76±0.13
"	24	200	20	1	3	5.25±0.47**	5.50±0.86*	0.84±0.24
"	24	400	11	2		3.25±1.79	3.25±1.79	1.08±0.29
EMS	48	0.2	100	75		43.75±9.89	26.25±6.75	1.42±0.24
DRP	48	100	11	3		3.50±1.84	3.50±1.84	0.81±0.19
"	48	200	19	2		5.25±1.65	4.50±1.50	0.90±0.28
"	48	400	12	7		4.75±1.43	4.25±1.18	1.08±0.23

[+] MNBC, micronucleated binuclear cells; B', chromatid type break; B", chromosome type break; P, polyploidy.

Table 5. The MI and NDI in human peripheral lymphocytes treated with DRP for 24 and 48 hours.

Test substances	Time (hour)	Concentrations (µg/ml)	MI±SE (%)	NDI±SE
Control	-	-	7.89±0.71	1.62±0.12
EMS	24	0.2	5.13±0.48	1.31±0.08
DRP	24	100	7.60±0.49	1.43±0.09
"	24	200	7.80±0.19	1.51±0.09
"	24	400	7.08±0.31	1.42±0.08
EMS	48	0.2	5.21±1.16	1.59±0.08
DRP	48	100	8.18±0.40	1.67±0.05
"	48	200	7.50±0.64	1.56±0.05
"	48	400	7.18±0.27	1.61±0.10

Table 6. The RAPD bands profile in female and male DNA in human cells exposed to DRP.

Test Substan.	Time (hour)	Concent. (µg/ml)	Mean number of polymorphic bands		Total polymorphic bands mean ±SE
			Male DNA±SE	Female DNA±SE	
Control	--	--	0.000±0.000	0.000±0.000	0.000±0.000
EMS	24	0.2	0.094±0.028***	0.113±0.034***	0.103±0.021***
SA	24	2.0	0.056±0.022*	0.056±0.024**	0.056±0.012***
DRP	24	100	0.009±0.008	0.011±0.010	0.010±0.007
DRP	24	200	0.009±0.006	0.022±0.015	0.015±0.008
DRP	24	400	0.018±0.013	0.056±0.024**	0.036±0.013**
EMS	48	0.2	0.056±0.022*	0.113±0.034***	0.082±0.019***
SA	48	2.0	0.056±0.021*	0.147±0.038***	0.097±0.021***
DRP	48	100	0.018±0.013	0.068±0.027**	0.041±0.014**
DRP	48	200	0.037±0.018*	0.056±0.024**	0.046±0.015**
DRP	48	400	0.066±0.024**	0.090±0.030***	0.077±0.019***

Significant compared to control; *: P≤0.05; **: P≤0.01; ***: P≤0.001.

morphic bands at all concentrations of 48 hour treatment period and only for the highest concentration (400 µg/ml) and for 24 hour treatment period without a dose-dependent effect (Table 6). The GTS rate was also significantly decreased at the highest concentration for 24 hour, and in all concentrations for 48 hour treatment periods without a dose-dependent manner (Table 7).

Discussion

This study was conducted to evaluate the genotoxic effect of the DRP by using both cytogenetic and molecular methods. The chromosomal aberrations (CA) and micronucleus (MN) tests were used as cytogenetic methods and RAPD-PCR technique was used as a molecular method. Doripenem did not induce the mean of CA and the frequency of MN, and also did not cause cytotoxicity in peripheral blood cells. However, DRP significantly induced the total polymorphic bands especially at 48 hours treatment period at all concentrations, and significantly decreased the GTS rate. So, it can be concluded that DRP did not cause any chromosomal damage while it caused nucleotide alterations such as SA which could cause nucleotide changes.

The methods used in this study served us as determinants of both the structure and the number of chromosome abnormalities and DNA base changes that have been caused by xenobiotics. Chromosom aberrations and MN methods allow us to detect the microscopic levels of chromosome and chromatid type abnormalities originated from other chromosomal damages such as

Table 7. Genomic template stability rate of the male and female DNA in human cells exposed to DRP.

Test Substances	Time (hour)	Concentrations (µg/ml)	Genomic template stability mean±SE
Control	--	--	100,00±0.00
EMS	24	0.2	90.09±1.77***
SA	24	2.0	93.50±1.79**
DRP	24	100	98.54±1.07
DRP	24	200	98.04±1.15
DRP	24	400	96.00±1.34**
EMS	48	0.2	91.03±2.88**
SA	48	2.0	89.26±2.71***
DRP	48	100	95.42±1.71*
DRP	48	200	95.04±1.49**
DRP	48	400	91.94±2.41**

Significant compared to control; *: P≤0.05; **: P≤0.01; ***: P≤0.001.

fragment translocation, sister chromatid union, deletion etc. (35,40). On the other hand, RAPD-PCR method allows us to detect the base changes (base substitutions, base deletions or additions) that changed the primer binding sites (33,41).

Previously, DRP was used for therapy at the dose of 500 mg/person/day intravenously (11-12). Normally, if a young person is assumed to be 60 kg and is exposed to the dosage mentioned above, this corresponds to dosage of 0.0083 mg/day. This dose is much lower than the dose which was used in this study. Hence, DRP decreased GTS rates at the lowest dose (100 mg/ml) only for 48 hours treatment period. When all of these are taken into consideration as a whole and considering the therapeutic dose of DRP cannot be used in such a high dose, it can be said that DRP is unlikely to have any genotoxic and mutagenic risks. Doripenem has no cytotoxic effect because it did not decrease the MI and NDI. Therefore, it can be concluded that DRP did not create any stress conditions on cell division and DNA replication.

The experimental doses, which were used in this study, were much higher than the therapeutic doses. The main purpose of using these much high concentrations of DRP was to determine the genotoxic effects of non-cytotoxic chemical that are highly soluble in water. In addition to that, to provide information about the effects of higher doses than the therapeutic doses in case of taken accidentally. Similarly, Madle et al. (1993) reported that non-toxic chemicals could be used over 5 mg/ml concentration in in vitro systems (42). In the present study, DRP was tested at 400 µg/ml as a maximum dose for its genotoxic and cytotoxic effects while the maximum daily dose for human was 0.0083 µg/mg. So, it is clearly understood that DRP cannot be used at such high doses. On the other hand, DRP has a fast metabolic way that its half-life is about only an hour. It is metabolized to β-lactam ring and eliminated via kidneys while approximately 80% of DRP is unchanged and excreted in the urine (43).

Tanimoto et al. (2008) used *Pseudomonas aeruginosa* in order to determine the genotoxicity of DRP (44). Authors found that DRP inhibited the mutant formation. Likewise, in the present study, it was observed that DRP did not have any genotoxic and cytotoxic effects on human peripheral lymphocytes.

This is the first report on genotoxicity and mutagenicity of DRP on human blood lymphocytes. During preparation of the project proposal, we did not find any study that have been done on the genotoxicity of DRP in literature. This is also an opportunity to compare the genotoxic risk of DRP in in vivo animals or in another different test system. Previous studies have been shown that β-lactam antibiotics had no genotoxic and cytotoxic effects on human peripheral lymphocytes (19,45-47). Likewise, we observed similar results in the present study and the results suggested that DRP caused mutations only at doses much higher than the therapeutic doses.

Consequently, DRP did not show any genotoxic risk at the therapeutic doses. This may also indicate that DRP is a safe antibiotics according to its rapid metabolism. However, further studies should be done to exactly determine the possible genotoxicity of DRP. For example, multiple test systems and bacterial reverse mutation tests could be included in in vivo researches.

Ethics

This study was confirmed by Ethics Committee of Non-Interventional Research of the Fırat University, Elazığ/Turkey. Date and number of the ethics committee approval is following: 13.01.2015 and 2015/06, respectively. All of the volunteers provide their written informed consent to be included in the study regarding the use of their whole blood samples for research studies. The study is conducted in agreement with the statement on the Declaration of Helsinki approved by the World Medical Association meeting in Edinburgh.

Conflict of interest

The authors report no conflict of interest and are responsible for the content and writing of the paper.

Acknowledgments

This work was supported by Adiyaman University Research Fund: FEFYL/2014-0004. This study consists of a part of Onur Bozkurt M.Sc. Thesis. Additionally, authors would like to thank all volunteers who participated in this study.

References

1. Capasso L. 5300 years ago, the Ice Man used natural laxatives and antibiotics. Lancet 1998; 352:1864.

2. Cavallito CJ, Bailey JH. Allicin, the antibacterial principle of Allium sativum. I. Isolation, physical properties and antibacterial action. J Am Chem Soc 1944; 66:1950-1951.

3. Goossens H, Ferech M, Stichele RV, Elseviers M. Outpatient antibiotic use in Europe and association with resistance. Lancet 2005; 365:579-87.

4. WHO (World Health Organization). Global action plan on microbial resistance. http://www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf (Last accessed: December 2016)

5. Torres JA, Villegas MV, Quinn JP. Current concepts in antibiotic-resistant gram-negative bacteria.Expert Rev. Anti-Infect Ther 2007; 5:833-43.

6. WHO (World Health Organization). Antimicrobial Resistance Global Report on Surveillance. http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf (Last accessed December 2016)

7. Iso Y, Irie T, Nishino Y, Motokawa K, Nishitani Y. A novel 1β-methylcarbapenem antibiotic, S-4661: Synthesis and structure-activity relationships of 2-(5 substituted pyrrolidin-3-ylthio)-1βmethylcarbapenems. J Antibiot (Tokyo) 1996; 49:199-209.

8. Bradley JS, Garau J, Lode H, Rolston KV, Wilson SE, Quinn JP. Carbapenems in clinical practice: a guide to their use in serious infection. Int J Antimicrob Agents 1999; 11:93-100.

9. Paterson DL. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs). Clin Microbiol Infect 2000; 6:460-3.

10. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev 2005; 18:657-86.

11. Bhavnani SM, Hammel JP, Cirincione BB, Wikler MA, Ambrose PG. Use of pharmacokinetic-pharmacodynamic target attainment analyses to support phase 2 and 3 dosing strategies for doripenem. Antimicrob Agents Chemother 2005; 49:3944-7.

12. Mandell L. Doripenem: a new carbapenem in the treatment of nosocomial infection,Clin Infect Dis 2009;49:1-3.

13. Paterson DL, Depestel DD. Doripenem, Clin Infect Dis 2009; 49:291-8.

14. Jones RN, Huynh HK, Biedenbach DJ. Activities of doripenem (S-4661) against drug-resistant clinical pathogens. Antimicrob Agents Chemother 2004; 48:3136-40.
15. Fritsche TR, Stilwell MG, Jones RN. Antimicrobial activity of doripenem (S-4661): a global surveillance report 2003. Clin Microbiol Infect 2005; 11:974-84.
16. Merchant S, Gast C, Nathwani D, Lee M, Quintana A, Ketter N, Friedland I, Ingham M. Hospital resource utilization with doripenem versus imipenem in the treatment of ventilator-associated pneumonia. Clin Ther 2008; 30:717-33.
17. Kongnakorn T, Mwamburi M, Merchant S, Akhras K, Caro JJ, Nathwani D. Economic evaluation of doripenem for the treatment of nosocomial pneumonia in the US: discrete event simulation. Curr Med Res Opin 2010; 26:17-24.
18. Gold HS, Pillai SK. Antistaphylococcal agents. Infect Dis Clin North Am 2009; 23:99-131.
19. Jaju M, Jaju M, Ahuja YR. Evaluation of genotoxicity of ampicillin and carbenicillin on human lymphocytes in vitro: chromosome aberrations, mitotic index, cell cycle kinetics, satellite associations of acrocentric chromosomes and sister chromatid exchanges. Hum Toxicol 1984; 3:173-91.
20. Rencuzogullari E, Ila HB, Topaktas M, Kayraldiz A, Budak S, Arslan M. No significant increase in chromosome aberrations and sister chromatid exchanges in cultured human lymphocytes treated with spiramycin. Teratogen Carcin Mut 2002; 22:51–8.
21. Agarwal SK, Bhatnagar U, Rajesh N. Acute and genotoxic profile of a dimeric impurity of cefotaxime. Int J Toxicol 2004; 23:41-5.
22. Venkatesh P, Shantala B, Jagetia GC, Rao KK, Baliga MS. Modulation of doxorubicin-induced genotoxicity by Aegle marmelos in mouse bone marrow: a micronucleus study. Integr Cancer Ther 2007; 6:42-53.
23. Rencuzogullari E, Azirak S, Canimoglu S, Parlak S, Buyukleyla M. Effects of natamycin on sister chromatid exchanges, chromosome aberrations and micronucleus in human lymphocytes. Drug Chem Toxicol 2009; 32:47-52.
24. Rocco L, Peluso C, Stingo V. Micronucleus test and comet assay for the evaluation of zebrafish genomic damage induced by erythromycin and lincomycin. Environ Toxicol 2012; 27:598-604.
25. Cui J, Zhang Z, Ran Y, Zhao W, Kang X, Deng W. Study on the genotoxicity of cefuroxime lactone--an impurity substance in antibiotics. Wei Sheng Yan Jiu 2012; 41:717-22.
26. Evans HJ. Handbook of Mutagenicity Test Procedures. In: Kilbey BJ, Legator M, Nichols W, Ramel C, eds., Human peripheral blood 63 lymphocytes for the analysis of chromosome aberrations in mutagen tests. 2nd ed. 1984; Amsterdam, Elsevier Science Publishers, Netherlands.
27. Rencuzogullari E, Topaktas M. The relationship between quantities of bromodeoxyuridine and human peripheral blood with determination of the best differential staining of sister chromatids using chromosome medium-B. J Nat Appl Sci Ins 1991; 5:19-24.
28. Paz-y-Miño C, Bustamante G, Sánchez ME, Leone PE. Cytogenetic monitoring in a population occupationally exposed to pesticides in Ecuador. Environ Health Perspect 2002; 110:1077-80.
29. Mace JML, Daskal Y, Wray W. Scanning-electron microscopy of chromosome aberration. Mutat Res 1978; 52:199–206.
30. Buyukleyla M, Rencuzogullari E. The effects of thymol on sister chromatid exchange, chromosome aberration and micronucleus in human lymphocytes. Exotoxicol Environ Safety 2009; 72:943-47.
31. Fenech M. The in vitro micronucleus technique. Mutat Res 2000; 455:81–95.
32. Kirsch-Volders M, Sofuni T, Aardema M, Albertini S, Eastmond D, Fenech M, Ishidate M Jr., Kirchner S, Lorge E, Morita T, et al. Report from the in vitro micronucleus assay working group. Mutat Res 2003; 540:153-63.
33. Atienzar FA, Jha AN. The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. Mutat Res 2006; 613:76-102.
34. Atienzar FA, Conradi M, Evenden AJ, Jha AN, Depledge MH. Qualitative assesment of genotoxicity using random amplified polymorphic DNA: Comparison of genomic template stability with key fitness parameters in Daphnia magna exposed to benzo[a]pyrene. Environ Toxicol Chem 1999; 18:2275–82.
35. Turpin R, Lejeune J. Human afflictions and chromosomal aberrations. 1969; Pergamon Press, Oxford.
36. Yunis JJ. ed. New chromosomal syndromes. 1977; Academic Press, Orlando, FL.
37. Wilkins LE, Brown JA, Wolf B. Psychomotor development in 65 home-reared children with cri-du-chat syndrome. J Pediatr 1980; 97:401-5.
38. Kirsch-Volders M, Elhajouji A, Cundari E, Van Hummelen P. The in vitro micronucleus test: a multi-endpoint assay to detect simultaneously mitotic delay, apoptosis, chromosome breakage, chromosome loss and non-disjunction. Mutat Res 1997; 392:19-30.
39. Norppa H, Falck GCM. What Do Human Micronuclei Contain? Mutagen 2003; 18: 221-233.
40. Kocaman AY, Rencuzogullari E, Topaktas M. In vitro investigation of the genotoxic and cytotoxic effects of thiacloprid in cultured human peripheral blood lymphocytes. Environ Toxicol 2014; 29:631-41.
41. Buyukleyla M. Detection of genotoxic effects of food additive sodium metabisulfite using RAPD/PCR method. PhD, Çukurova University, Adana, Turkey, 2013.
42. Madle S, Beek B, Nowak C. Zum Verständnis von Chromosomenmutationstests an Somazellen. (To Understand the Chromosome Mutations in Somatic Cells) In: Fahrig, R., ed. Mutationsforschung und Genetische Toxicologie (Mutation and Genetic Toxicology). Germany, Wissenschaftliche Buchgesellschaft, 1993.
43. Keam SJ. Doripenem. A review of its use in the treatment of bacterial infections. Drugs 2008; 68:2021-2057.
44. Tanimoto K, Tomita H, Fujimoto S, Okuzumi K, Ike Y. Fluoroquinolone enhances the mutation frequency for meropenem-selected carbapenem resistance in pseudomonas aeruginosa, but use of the high-potency drug doripenem inhibits mutant formation. Antimicrob Agents Chemother 2008; 52:3795-800.
45. Zavarise G, Ghiazza G, Grillo G, Morino P, Mondavio M. Evaluation of the effect of cloxacillin on human lymphocyte chromosomes through the study of karyotypic changes and sister chromatid exchanges. Boll Soc Ital Biol Sper 1984; 60:2143-8.
46. Koseoglu V, Kismet E, Soysal Y, Ulucan H, Dündaröz R, Imirzalioglu N, Gökçay E. Investigation of DNA damage in lymphocytes exposed to benzathine penicillin G Pediatr Int 2004;46:415-8.
47. Istifli ES, Topaktaş M. Cytogenetic genotoxicity of amoxicillin. Environ Mol Mutagen 2010; 51:222-8.