

(RESEARCH ARTICLE)



Evaluation of cytogenetic effects induced with ciprofloxacin and metronidazole as antibacterial agents on mouse bone marrow cells *in vivo*

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Abstract

Two of antibacterial agents were selected to evaluate the possibility of induction of chromosomal aberrations in mice bone marrow cells *in vivo*. The two agents, ciprofloxacin considered as antibacterial while metronidazole as antibacterial and antiparasitic agent. Our data revealed that both two agents ciprofloxacin (7 mg/kg b.w.) and metronidazole 30 mg/ kg b.w. induced a highly significant effect of chromosomal aberrations. We can concluded that both antimicrobial agents "metronidazole" and "Ciprofloxacin" have a marked mutagenic effects, and caution should be used in prescribing the drugs, especially in multiple courses or over long periods. The patients with severe liver disease may accumulate the drug.

Keywords: Chromosomal aberration; Ciprofloxacin; Metronidazole; Mice; *in vivo*

1. Introduction

A number of organic compounds obtained by chemical synthesis have useful antibacterial activity for the treatment of local, systemic, and/or urinary tract infections. Some chemical classes of synthetic antibacterial agents include the sulfonamides, certain nitro-heterocyclic compounds (for example, the nitrofurans and metronidazole), and the quinolones. Some antibacterial agents that fail to achieve adequate concentrations in the plasma or tissues for the treatment of systemic infections following oral or parenteral administration are concentrated in the urine, where they can be effective for eradicating urinary tract infections. Nitrofurantoin (a nitrofuran), nalidixic acid (a quinolone), and methenamine are examples of such urinary tract anti-infectives [1].

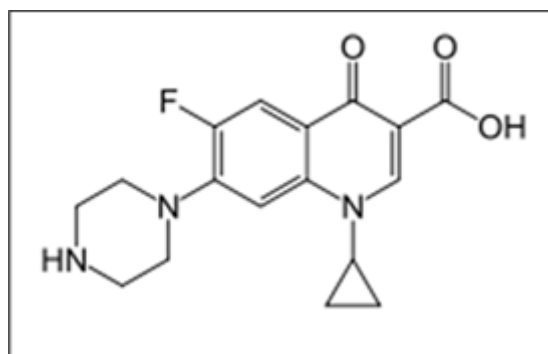


Figure 1 Ciprofloxacin

Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl) -3-quinolin carboxylic acid (Cipro, Cipro IV) is supplied in both oral and parenteral dosage forms. The hydrochloride salt is available in 250, 500, and 750 mg tablets for oral

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administration. Intravenous solutions containing 200 mg and 400 mg are provided in concentrations of 0.2% in normal saline and 1% in 5% dextrose solutions [1].

Ciprofloxacin is an agent of choice for the treatment of bacterial gastroenteritis caused by gram-negative bacilli, such as enteropathogenic *Escherichia coli*, *Salmonella* (including *S. typhi*), *Shigella*, *Vibrio*, and *Aeromonas hydrophilia*. It is widely employed for the treatment of respiratory tract infections, and is particularly effective for controlling bronchitis and pneumonia caused by gram-negative bacteria. Ciprofloxacin is also used for combating infections of the skin, soft tissues, bones, and joints. Both uncomplicated and complicated urinary tract infections caused by gram-negative bacteria can be effectively treated with ciprofloxacin. It is particularly useful for the control of chronic infections characterized by renal tissue involvement. The drug also has important applications in controlling venereal diseases [1]. Ciprofloxacin is a broad-spectrum antibiotic of the fluoroquinolone class. It is active against some Gram-positive and many Gram-negative bacteria. It functions by inhibiting DNA gyrase, and a type II topoisomerase, topoisomerase IV, necessary to separate bacterial DNA, thereby inhibiting cell division [2,3].

The discovery of azomycin (2-nitroimidazole) in 1955 and the demonstration of its trichomonocidal properties by Horie [4] led to the chemical synthesis and biological testing of many nitroimidazole. One compound, 1-(β -hydroxyethyl)-2-methyl-5-nitroimidazole, now called metronidazole (Flagyl), was found to have particularly high activity *in vitro* and *in vivo* against *T. vaginalis* and *E. histolytica* [5,6]. Durel and associates [7] reported that oral doses of the drug imparted trichomonocidal activity to semen and urine and that high cure rates could be obtained in both male and female patients with trichomoniasis. Metronidazole has an extremely broad spectrum of antiprotozoal and antimicrobial activity, which is used to clinical advantage. Other clinically effective 5-nitroimidazoles closely related in structures and activity to metronidazole are available outside the United States. These include tinidazole (Fasigyn), nimorazole (Naxogin), and ornidazole (Tiberal). Benznidazole (Rochagan) is another 5-nitroimidazole derivative that is unusual in that it is effective in acute Chagas' disease. Metronidazole has the following chemical structure:

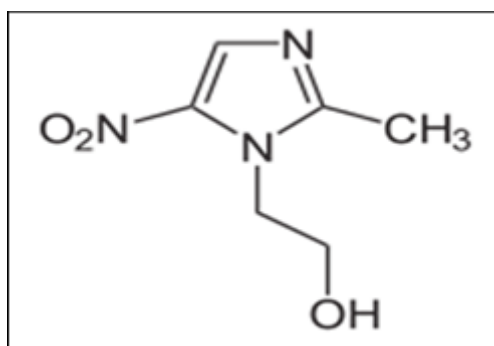


Figure 2 Metronidazole

Metronidazole is active against a wide variety of anaerobic protozoal parasites and anaerobic bacteria. The compound is directly trichomonocidal. Sensitive isolates of *T. vaginalis* are killed by $< 0.05 \mu\text{g/ml}$ of the drug under anaerobic conditions; higher concentrations are required when 1% oxygen is present or to affect isolates from patients who display poor therapeutic responses to metronidazole [8]. The drug also has potent amebicidal activity against *E. histolytica* grown in culture by itself or in mixed culture conditions [9]. Trophozoites of *G. lamblia* probably are directly affected by metronidazole at concentrations of 1 to $50 \mu\text{g/ml}$ *in vitro* [10].

The pharmacokinetic properties of metronidazole and its two major metabolites have been investigated intensively [11]. The drug usually is completely and promptly absorbed after oral administration, reaching concentrations in plasma of about $10 \mu\text{g/ml}$ for 1 hour after a single 500 mg dose. (Mean effective concentrations of the compound are $8 \mu\text{g/ml}$ or less for most susceptible protozoa and bacteria). A linear relationship between dose and plasma concentration pertains for doses of 200 to 2000 mg. Repeated doses every 6 to 8 hours result in some accumulation of the drug. The half-life of metronidazole in plasma is about 8 hours, and its volume of distribution is approximately that of total body water. About 10% of the drug is bound to plasma proteins. Metronidazole penetrates well into body tissues and fluids, including vaginal secretions, seminal fluids, saliva, and breast milk. Therapeutic concentrations also are achieved in cerebrospinal fluid.

Metronidazole is extremely useful for treatment of serious infections due to susceptible anaerobic bacteria, including *Bacteroides*, *Clostridium*, *Fusobacterium*, *Peptococcus*, *Peptostreptococcus*, *Eubacterium*, and *Helicobacter*. The drug also may be used along with appropriate antimicrobial agents for concomitant infections with aerobic microorganisms.

Metronidazole should be used with caution in patients with active disease of the CNS because of its neurological toxicity. The dosage should be reduced in patients with severe obstructive hepatic disease, alcoholic cirrhosis, or severe renal dysfunction. [11].

The present work was constructed to assess and evaluate the cytogenetic impacts of the two antibiotics; the antiprotozoal and antibacterial agent “metronidazole” and the antibacterial agent “ciprofloxacin”. Both antibiotics chosen to be tested *in vivo* with special emphasis on their possibility to produce chromosomal abnormalities.

To achieve these targets, the two drugs were planned to be examined for their ability in the induction of chromosomal abnormalities, in mice bone-marrow-cells, through oral treatment by gavage for short-period experiment and recovery period.

2. Material and methods

2.1. Animals

Male mice were used in the present investigation, being obtained from the animal house of Benghazi University. Animals were selected of similar age and weight (6-8 weeks old and about 20 gm body weight).

Animals were housed in suitable cages under controlled laboratory conditions, supplied with rat chaw and water was kept *ad libitum*.

2.2. Drugs

The two antibiotics, utilized in the present experiment, comprised metronidazole and ciprofloxacin, which were obtained from Gulf pharmaceutical industries; Ras Alkhaimah, U.A.E, and Micro labs limited, India, respectively.

2.3. Chromosomal aberration test

Animals were orally treated by gavage with gastric tube, and administered with the tested doses of the two drugs dissolved in distilled water. Samples were collected after 24 hours; mice were treated for 5 consecutive days, and after 5 days of treatment, and after two weeks as a recovery period. Five mice were examined for each treatment. The applied doses were:

30 mg “metronidazole” / kg body weight,

7 mg “ciprofloxacin” / kg body weight.

Control (untreated) mice which orally with distilled water were also used as negative control.

Mice for each experiment (treated with “metronidazole” or “ciprofloxacin”) were orally gavaged one dose a day for 5 consecutive days. For sampling, animals were intraperitoneally injected (i.p.) with colchicine (0.6 mg/kg b.w.) to arrest mitosis at the metaphase stage, and then they were sacrificed 2h later. The bone marrow was rapidly removed from their femurs in a hypotonic solution (0.075 M KCl), and remained for 15 min. at 37 oC. Centrifugation at 1500 rpm, the resuspended cells (pellet) were fixed twice in ethanol: glacial acetic acid (3:1 v/v), and the slides were prepared by transferring these cells onto clear and day slides, stained with Giemsa dye (7 % in phosphate buffer, pH 6.8) [12].

At least 50 well-spread metaphases were analyzed per animal. By using t-test, the statistical analysis was carried out.

3. Results

The two antibiotics used in this study, metronidazole and ciprofloxacin, have an ability to induce chromosomal aberration in mice bone marrow cells.

The antibacterial agent "metronidazole" at the concentration of 30 mg/kg body weight induced a highly significant effect of chromosomal aberration reached 11.6% after 24 h of a single dose. The incidence of chromosomal aberrations percentage decreased after 5 consecutive doses which reached 8.8% where as it was statistically significant. On the contrary, this percentage decreased to a non- significant after 14 days as a recovery period recorded 6.4% comparing to 4.23% as in untreated controls (Table 1 and Figure 3)

The same manner recorded with the treatment of "ciprofloxacin" at the concentration of 7 mg/kg body weight with a little differences. The highly statistically significant effect of chromosomal aberration induction occurs after a single dose, 24 h, the percentage reached 10.8% and the decreasing level recorded as 10.4% and 6.8% after 5 consecutive doses and 14 days as recovery, respectively (Table 2 and Figure 3).

Normal metaphases appear as illustrated in (Figure 4) composed of 20 pairs of telocentric chromosomes without any abnormalities.

Types of chromosomal aberrations which were recorded with treatment of mice by "metronidazole" as demonstrated in table 3 are chromatid gaps, breaks (Fig. 5), deletions and fragments (Fig. 6). The gaps were the most dominant aberration types, other types of aberration were recorded as polyploidy and robertsonian translocation. Table 4 demonstrated the same types of aberrations induced with the treatment of "ciprofloxacin" with slight differences.

Table 1 Effect of metronidazole on the incidence of chromosomal aberration in mice bone marrow for 5 days.

Treatment	Time exposure	Counted metaphase	Abnormal metaphase	% of chromosomal aberration
Control (untreated)	---	260	11	4.23
Metronidazole (30 mg/kg b.w.)	24 hours	250	29	11.6 **
	5 days	250	22	8.8 **
	14 days (recovery)	250	16	6.4

** High significant at level (p<0.01)

Table 2 Effect of ciprofloxacin on the incidence of chromosomal aberration in mice bone marrow for 5 days.

Treatment	Time exposure	Counted metaphase	Abnormal metaphase	% of chromosomal aberration
Control (untreated)	---	260	11	4.23
Ciprofloxacin (7 mg/kg b.w.)	24 hours	250	27	10.8 **
	5 days	250	26	10.4 **
	14 days (recovery)	250	17	6.8

** High significant at level (p<0.01)

Table 3 Percentage of the chromosomal aberration and types of aberration induced with metronidazole on mice bone-marrow cells.

Treatment	Time	Chrom. aberr. No.	Types of abnormalities						
			Cht. gap	Cht. br.	Chr. gap	Chr. br.	Del.	Frag.	Others
Control	--	11	5	-	-	-	4	2	-
	24 hr	29	10	4	3	1	6	3	2
Metronidazole	5 days	22	8	3	3	1	4	2	1
	14 days recovery	16	5	2	2	-	4	3	-

Table 4 Percentage of the chromosomal aberration and types of aberration induced with ciprofloxacin on mice bone-marrow cells.

Treatment	Time	Types of abnormalities
-----------	------	------------------------

		Chrom. aberr. No.	Cht. gap	Cht. br.	Chr. gap	Chr. br.	Del.	Frag.	Others
Control	--	11	5	-	-	-	4	2	-
	24 hr	27	10	4	3	1	5	3	1
Ciprofloxacin	5 days	26	8	3	5	1	6	3	-
	14 days recovery	17	5	2	3	-	4	3	-

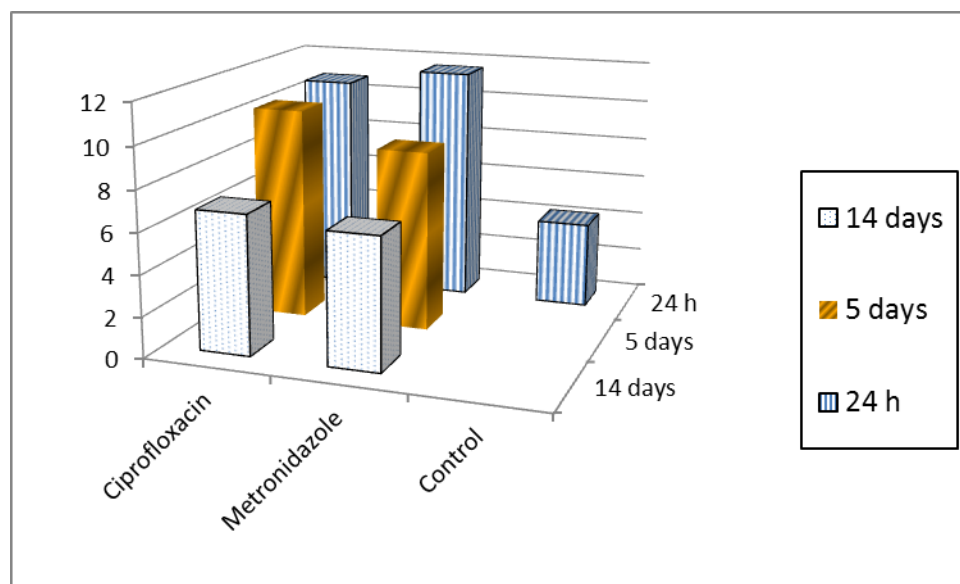


Figure 3 The effect of ciprofloxacin and metronidazole on the chromosomal aberration after 1, 5 days and 14 days as recovery.

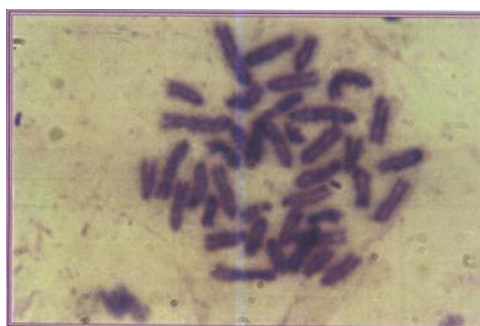


Figure 4 Normal metaphase in mouse bone marrow cell (untreated control)

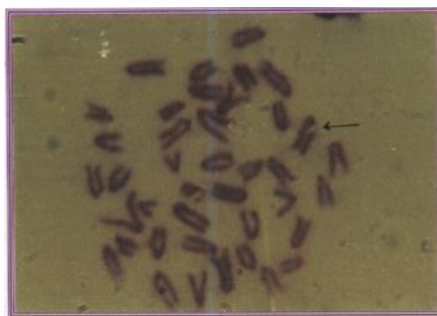


Figure 5 Metaphase with chromatid break in mouse bone marrow cell after treatment with 30 mg "metronidazole"/kg body wt.

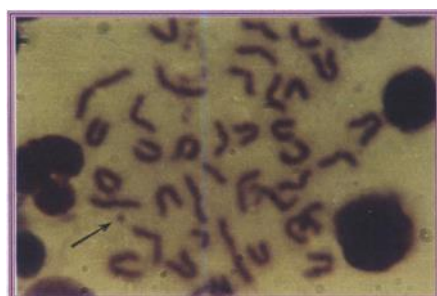


Figure 6 Metaphase with fragment in mouse bone marrow cell after treatment with 7 mg "ciprofloxacin "/kg body wt.

4. Discussion

Our experimental data revealed that the two antibacterial agent have a potent cytogenetic effect on the induction of chromosomal abnormalities, which clearly appears 24 h after single treatment of either two agents. It is noticed that "metronidazole" has a higher effect than "ciprofloxacin" which recorded 11.6% and 10.8% respectively.

Both of the agents affect the bone marrow cells of mice as a high percentage of chromosomal aberration, which mean that the agents were well absorbed often oral administration and widely distributed in body tissues, that agrees with the opinion of Winer [13] which reported "ciprofloxacin" well absorbed and distributed in body tissue within 12h, while Katzung [14] reported that "metronidazole" has a small molecular size, and permeates all tissues by simple diffusion, oral metronidazole is rapidly absorbed (80% within 1 hour).

Through the 14 days after last administration of either "metronidazole" or "ciprofloxacin", the two drugs were eliminated from the mice body which translated as a decrease of the incidence of chromosomal aberration percentage reached to the levels so closely to the untreated control or the normal level. That explanation of rapid elimination was agreed with the opinion of Katzung [14] and in Drug.com [15] who reported that 10% of ciprofloxacin is excreted in urine as metabolites. Urinary excretion is virtually complete 24 hours after administration.

Hollaender [16] pointed out that the aberrations in metaphase cells are basically of two sorts distinguished on the basis of the unit of the of breakage/exchange. These in which structural changes involve the whole chromosome.

Many types of structural chromosomal aberrations were observed in the mouse bone-marrow cells which treated either with "ciprofloxacin" or "metronidazole" even after the recovery period (7 days after the last treatment). Tables 3 and 4 illustrates these types where chromatid types was the highest percentage of located followed by chromatid breaks and deletion types. Fragments and chromosome gap were also recorded.

In publication provided by Deway et al [17], they postulated that the type of chromosomal structural alterations, produced by either physical or chemical agents, depend essentially on the lesions induced in the DNA and, therefore, upon the chemical structure of the genotoxic substance. In this regard, structural chromosomal aberrations result basically from breakage and rearrangement of the whole chromosome into abnormal forms. These are most efficiently

induced by such substances that directly break the backbone of the chromosomes, mainly DNA (e.g. ionizing radiation and radiomimetic chemicals) or these which significantly distort the DNA helix such as the intercalating agents.

Finally, we can conclude that the two drugs metronidazole and ciprofloxacin with the oral treatment of the used dose have mutagenic effects. Katzung [14] also pointed the same conclusion where reported that metronidazole and its metabolites recovered from the urine of patients taking the drug have been proved by *in vitro* assays to be mutagenic in certain strains of *Salmonella typhimurium* (Ames test). Chronic oral administration of very large doses to mice has produced a statistically significant increase in the number of lung and liver tumors. This effect has not been found, however, in any non-rodent species. Although the drug has been used in humans for 20 years, no increase in congenital abnormalities, stillbirths, or low birth weight has been reported. Culture of human lymphocytes with metronidazole, up to 10,000 µg/mL, has revealed no toxic activity.

National Toxicology Program [18] stated that oral exposure has been shown to cause cancer in experimental animals and has also demonstrated some mutagenic effects in bacterial cultures.

5. Conclusion

It should be stated that both antimicrobial agents "metronidazole" and "Ciprofloxacin" have a marked mutagenic effects, and caution should be used in prescribing the drugs, especially in multiple courses or over long periods. The patients with severe liver disease may accumulate the drug.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author has no conflict of interest to declare.

References

- [1] Delgado JN and Remers WA. (1998). Textbook of organic medicinal and pharmaceutical chemistry. Lippincott-Raven, New York.
- [2] Drlica K and Zhao X. (1997). "DNA gyrase, topoisomerase IV, and the 4-quinolones". *Microbiology and Molecular Biology Reviews*, 61(3), 377–92.
- [3] Pommier Y, Leo E, Zhang H and Marchand C. (2010). "DNA topoisomerases and their poisoning by anticancer and antibacterial drugs". *Chemistry & Biology*, 17(5), 421–33.
- [4] Horie H. (1956). Antitrichomonas effect of azomycin, *J. Antibiotics (Tokyo)*, 9A, 168.
- [5] Cosar C and Julou L. (1959). Activite de l'(hydroxy-2' enthyl)-1-methyl-2-nitro-5-imidazole (8.823 R.P.) vis-à-vis des infections experimentales a *Trichomonas vaginalis*. *Ann. Inst. Pasteur (Paris)*.
- [6] Cosar C, P Ganter and L Julou. (1961). Etude experimentale du metronidazole, 8823 R.P. activities trichomonacide et amoebicide. Toxicitee et proprietes pharmacologiques generals. *Presse Med*, 69, 1069-1072.
- [7] Durel P, V Roiron, H Siboulet and Lj Borel. (1960). systemic treatment of um n trichomoniasis with derivative of nitroimidazole, 8823 R.p. *Br. J. Vener. Dis*, 36, 21-26.
- [8] Johnson PJ. (1993). Metronidazole and drug resistance. *Parasitol. Today*.
- [9] Burchard GD and Mirelman D. (1988). *Entamoeba histolytica*: Virulence potential and sensitivity to metronidazole and emetine of four isolates possessing nonpathogenic zymodemes. *Exp. Parasitol.*
- [10] Jokipii L and Jokipii AMM. (1980). *In vitro* susceptibility of *Giardia lamblia* trophozoites to metronidazole and tinidazole. *J. Infect. Dis*.

- [11] Lau JYN, Bain VG, Susan ED, John GO, Alfredo A, Graeme JA and Roger Williams. (1992). High-level expression of hepatitis B viral antigens in fibrosing cholestatic hepatitis. *Gastroenterology*, 102(3), 956-962.
- [12] Yoside TH and Amana K. (1965). Autosomal Polymorphism in Laboratory Bred and Wild Rats, *Rattus Norvegicus*. *Misims Chromosoma*, 16, 658-667.
- [13] Winer BJ. (1971). In: *Statistical Principles in Experimental Design*, 2nd edn, (McGraw-Hill, New York), 201–205.
- [14] Katzung BG. (1989). *Basic and clinical pharmacology*, 5th edition, Prentice-Hall International Inc., USA, 743.
- [15] Hollander A. (1976). *Chemical mutagens, principles and methods for their detection*, Vol. 4, Pleum Press, New York and London.
- [16] Dewey WD, Furman SC and Miller HH. (1970). Comparison of lethality and chromosomal damage induced by X-rays in synchronized Chinese Hamster Cells *in vitro*. *Radiat., Res*, 43, 561-581.
- [17] National Toxicology Program. (2016). "Metronidazole". Report on Carcinogens (Fourteenth ed.). National Toxicology Program (NTP).

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