STUDY THE INTERACTION BETWEEN CIPROFLOXACIN AND FERROUS SULPHATE ON BIOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY IN RATS

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ABSTRACT

Ciprofloxacin was evaluated as antibacterial agent for albino rats in vitro testing. The antibacterial effect was observed against Staphylococcus aureus, Streptococcus pneumoniae, E. coli, Bacillus subtilis and Salmonella typhimurium with a minimum inhibitory concentration (MIC) level of 0.28, 0.56, 0.14, 0.56 and 1.12 mg/ml, respectively. On the other hand the antibacterial activity of ciprofloxacin was reduced when combined with ferrous sulphate.

Oral administration of rats with ciprofloxacin and ferrous sulphate at doses of 9 mg ciprofloxacin and 1.8 mg ferrous sulphate as elemental iron /100 g body weight, respectively for 7 successive days show significant increase in serum enzymatic activities of AST, ALT and ALK phosphatase also level of serum creatinine.

The administration of ferrous sulphate with ciprofloxacin reduced serum concentration of ciprofloxacin from 14.5 to 5.4 ug/ml.

INTRODUCTION

Ciprofloxacin is broad spectrum antibiotic effective against both Gram +ve and Gram -ve bacteria **(Siebert, 1983)**. Ciprofloxacin is one of fluorinated quinolone which is more effective in Mycoplasma pneumoniae **(Osada, 1983)**. The pharmacokinetics, toxicity and residue in tissue were previously studied by **(Walker et al., 1989, Hildebrand et al., 1993, Shungu et al., 1993, Anadon et al., 1995 and Fujita et al., 1995)**.

In the field ciprofloxacin may be used together with ferrous sulphate, so the effect of ferrous sulphate on the antibacterial activity of ciprofloxacin and its concentration in serum must be taken in consideration. Moreover, the effect of ferrous sulphate on the antibacterial activity of

ciprofloxacin and its coneentration in serum of rats. Moreover, the effect of ciprofloxacin alone and in combination with ferrous sulphate on enzymatic activities and level of serum creatinine were lake and requires more investigation.

Accordingly this work was planned to elarify the effect of ciprofloxacin with or without ferrous sulphate on antibacterial activity in vitro, biochemical analysis and concentration of used drugs in the serum of rats.

MATERIAL AND METHODS

Animals :

40 mature albino rats of an average weight of 180-200 g b.wt. were used. Rates were fed on ordinary ration and water ad-libtium.

Drugs:

- Ciprofloxacin: was obtained from Alexandria pharmaceuticals Company and standard solution was prepared.
- Ferrous sulphate as elemental iron was obtained from El-Nasr Pharmaceutical Chemical Company, Egypt.

Bacterial strains:

The bacteria used in this study (Staphylococcus aureus, Streptococcus pneumoniae, E. coli, Bacillus subtilis and Salmonella typhimurium) were obtained from Microbiological Department of Animal Health Research Institute, Dokki.

I. Antibacterial activity in vitro:

Tests were performed by the agar diffusion method with 1 ml of inoculum, counting 109 bacterial cell per plate. Ciprofloxacin alone and in combination with ferrous sulphate were put in pores at the concentrations of 0.14, 0.28, 0.56, 1.12, 2.24 and 4.4 mg/ml with 1.8mg/ml, respectively and incubated at 37°C over night **(Finegold and Martin, 1982)**.

II. Biochemical analysis:

Four groups of 10 albino rats each (180-200g b.wt.) were used for this purpose. The first group was kept as a control group, while the second, third and fourth groups were administered orally with ciprofloxacin and ferrous sulphate at doses of 9, 1.8 (as elemental iron) mg/100g b.wt., respectively for 7 successive days.

Individual blood samples were obtained from rats, left to clot and sera were separated for bio-

chemical analysis. The enzymatic activities of AST, ALT and AP were determined by **Reitman and Frankel (1957) and Roy (1970)** while creatinine level was estimated by **Husdan and Rapoport (1968).**

III. Serum concentration of ciprofloxacin with or without ferrous sulphate in rats:

Ciprofloxacin alone and in combined with ferrous sulphate concentration in serum of rats were measured by agar diffusion method **(Bennett et al., 1966).** Standard curve of ciprofloxacin was prepared.

The residue was used directly to measure ciprofloxacin alone and in combination with ferrous sulphate concentrations **(Scheer, 1987).**

The results were subsequently analyzed by following the statistical methods established by **Snedecor (1969).**

RESULTS

I. Antibacterial activity in vitro:

The results of antibacterial activity tests of ciprofloxacin alone and in combination with ferrous sulphate are tabulated in table (1).

As shown in the table, the tested antibiotic was effective against some common bacteria Streptococcus pneumoniae, Staphylococcus aureus, E. coli, Salmonella typhimurium and Bacillus subtilis) but when ferrous sulphate was added to ciprofloxacin it reduced its antibacterial activity.

II. Biochemical analysis:

Oral administration of ciprofloxacin and ferrous sulphate at doses 9, 1.8 (as elemental iron) mg/100 g b.wt., respectively for 7 successive days, significantly increased the serum enzymatic activities of AST, ALT and AP and the level of serum creatinine when compared with control group (table, 2).

III. Serum concentration of ciprofloxacin and ferrous sulphate in rats.

After repeated administration of ciprofloxacin alone and in combination with ferrous sulphate, the recorded high concentration in serum were 14.5 and 5.4 ug/ml, respectively which indicated that ferrous sulphate reduced the concentration of ciprofloxacin in serum of tested rats.

DISCUSSION

Ciprofloxacin is effective and widely used as new broad spectrum antibiotic against Gram +ve

and Gram —ve bacteria, also Mycoplasma pneumonia **(Sato et al., 1982; Osada, 1983; Siebert et al., 1983; Walser et al., 1993; Luna et al., 1991 and Minta et al., 1990)**. The toxicity and residue of the tested antibiotic in tissue was studied by **(Walker et al., 1989 and Hildebrand et al., 1993)**. However, the interaction between ciprofloxacin and ferrous sulphate on the bacterial activity and biochemical analysis have not been investigated where it was the aim of the current study.

The present study revealed that ciprofloxacin was effective against some common bacteria but much less effective when combined with ferrous sulphate. This result was agreed with **Polk et al. (1989); Smith (1989) and Lehto et al. (1994)** whom recorded the same results.

The oral administration of ciprofloxacin alone or combined with ferrous sulphate at doses 9 and 1.8 (elemental iron) mg/100g b.wt. for 7 successive days induced significant increase in the activities of AST, ALT, AP and in the level of serum creatinine. Similar results were previously obtained by **(Niki et al., 1987; Hanan, 1997)**. No available literature about the effect of combination of ciprofloxacin and ferrous sulphate on biochemical analysis. The increase of AST, ALT and AP activities reflects the degree of tissue damage. A good deal of researchers **(Cantarow and Trumper, 1962 and Duncan and Prasse, 1981)** claimed that the aetivity of AST increased in inflammatory and degenerative changes in liver because of increased liberation of enzymes from hepatic eells. In additiou, concomitant administration of tested ciprofloxacin alone or in combination with ferrous sulphate increased serum creatinine level which is specific indicator of renal damage **(Hoe and O'Shea, 1965)**.

When ciprofloxacin administered combined with ferrous sulphate, the maximum concentration of ciprofloxacin in serum was reduced, this results agree with **Kara et al., 1991** who recorded that formation of a ferric ion ciprofloxacin complex is probably the cause of the reduction in ciprofloxacin bio-availability in the presence of iron.

Finally we concluded that ferrous sulphate reduced the antibacterial activity and serum concentration of ciprofloxacin. For this reason we must avoid the usage of ciprofloxacin with ferrous sulphate.

Table (1): Antibacterial activity of ciprofloxacin and ciprofloxacin combined with ferrous sulphate.

Microorganisms	Concentration of ciprofloxacin mg/ml	Zone of inhibition	
		Without ferrous sulphate	With ferrous sulphate 1.8 mg/ml
Staphylococcus aureus	0.14	-	-
	0.28	+	-
	0.56	++	+
	1.12	+++	++
	2.24	+++	+++
	4.48	++++	+++
Streptococcus pneumoniae	0.14	-	-
	0.28	-	-
	0.56	+	-
	1.12	++	+
	2.24	+++	++
	4.48	++++	++
E. coli	0.14	+	-
	0.28	++	-
	0.56	+++	+
	1.12	+++	++
	2.24	++++	+++
	4.48	++++	+++
B. subtilus	0.14	-	-
	0.28	-	-
	0.56	+	-
	1.12	+	-
	2.24	++	+
	4.48	+++	++
S. typhimurium	0.14	-	-
	0.28	-	-
	0.56	-	-
	1.12	+	-
	2.24	++	+
	4.48	+++	

Table (2): Effect of oral administration of ciprofloxacin with and *without ferrous sulphate on serum enzymatic* activities and level of creatinine in rats, mean ± SE (n=10)

Group	Dose mg/100 g b.wt.	ALT u/l	AST u/l	AP u/l	Creatinine mg%
Control	-	22.83 ± 0.87	68.8 ± 0.15	16.6 ±1.82	26.3 ± 0.07
Ciprofloxacin	9	129.5*** ± 1.29	114.25*** ± 4.36	41.5*** ± 1.55	35.79*** ± 0.52
Ferrous sulphate	1.8	22.78 ± 0.99	68.5 ± 2.85	16.66 ± 0.06	26.95 ± 0.2
Ciprofloxacin and ferrous sulphate	9+1.8	118.75*** ± 5.33	93.75*** ± 2.56	30.5*** ± 0.75	30.52* ± 0.2

* Significant at (P<0.05) *** Significant at (P<0.001)

REFERENCES

Anadon, A.; Marinez-Larranaga, M. R.; Diaz, M. J.; Fernandez, R.;Martinez, M. A. and Fernandez, M. C. (1995) : Pharmacokinetics and tissue residues of norfloxacin and its N-desethyl and oxometabolities in healthy pigs. J. Vet. Ph. and Therap., 18: 220-225.

Bennett, J. V.; Brodie, J. L.; Benner, E. J.; Kirby, W. M. (1966) : Simplified, accurate method for antibiotic assay of clinical specimens. Appl Microbiol., 14 (2):170-177.

Cantarow, A. and Trumper, M. (1962) : Clinical biochemistry. 6th Ed. W. B. Saunders Co. Philadelphia.

Duncan, J. and Prasse, W. (1981) : Liver in veterinary laboratory medicine. The Iowa State Univ. Press. USA.

Finegold, S. M. and Martin, W. T. (1982) : Diagnostic microbiology. 6th Ed. The C. V. Mosby Company. USA.

Fujita, K.; Matanoka, N.; Takenoka, T.; Hiraishi, K.; Yokota, T.; Asahi, T.; Tatara, K.; Yuasa, M. and Kaifu, Y. (1995) : Pharmacokinetics of ofloxacin- measurement of drug concentration in saliva of patients with impaired renal function. Durg (Kagawa Medical School), 29 (Suppl.2), 312-313.

Hanan, M. Sobhy (1997) : Comparative investigation of ofloxacin and doxycycline on cytogenetic and biochemical analysis. Egypt. J. Comp. Path. Clin. Path., 10 (2): 105-117.

Hildebrand, H.; Kempka, G.; Schluter, G. and Schmidt, M. (1993) : Chondrotoxicity of quinolones in vivo and in vitro. Arch of Toxic., 67 (6): 411-415.

Hoe, C. M. and O'shea, J. D. (1965) : The correlation of biochemistry and histopathology in liver disease in the dog. Vet. Rec. 77: 1164-1171.

Husdan, H. and Rapopart, A. (1968): Chemical determination of creatinine with deproteinization. Clin. Chem., 14: 222;

Kara, M.; Hasinoff, B. B.; Mckay, D. W. and Campbell, N. R. (1991) : Clinical and chemical interactions between iron preparations and ciprofloxacin. Br. J. Clin. Pharmacol., 31 (3): 257-61.

Lehto, P.; Kivist, K. T. and Neuvonen, P. J. (1994) : The effect of ferrous sulphate on the absorption of norfloxacin, ciprofloxacin and ofloxacin. Br. J. Clin. Pharmacol., 37 (1): 82-5.

Luna, R. D.; Crescenzo, G.; Amorena, M.; Oliva, G.; Consalvo, F. and Ciaramella, P. (1991) : Plasma kinetics and mammary excretion of enrofloxacin administered by intrauterine

infusion in water buffalo.

Minta, Z.; Bugajak, P. and Karczewski, W. (1990) : The evaluation of the effectiveness of baytril in the treatment of Salmonellosis in chicken broilers. Medycyna Weterynaryjna, 46 (9): 525-528.

Niki, Y.; Soejima, R. and Kawane, H. (1987) : New synthetic quinolone antibacterial agents and serum concentration of theophylline. Chest., 92: 66-669.

Osada, Y. H. (1983) : Activity vs. clinical isolates of Mycoplasma pneumoniae. Ogawa Ibid, 23: 509.

Polk, R. E.; Healy, D. P.; Sahai, J.; Drawl, L. and Racht, E. (1989) : Effect of ferrous sulphate and multi-vitamins with zinc on absorption of ciprofloxacin in normal volunteers. Antimicrob. Agents Chemother., 33 (11): 1841-4.

Reitman, S. and Frankel, S. (1957) : Colorimetric method for the determination of serum transaminase activity. Am. J. Clin. Path., 28: 56-58.

Roy, A. V. (1970) : A rapid method for alkaline phosphatase estimation. Clin. Chem., 16: 431;

Sato, K.; Matsuura, Y. and Inoue, M. (1982) : In vitro and in vivo antibacterial activity of ofloxacin. Anti microb. Agnets chemother., 22: 548.

Scheer, M. (1987) : Vet. Med. Rev., 2: 104-118.

Shungu, D. L. Weinberg, E. and Gadebusch, H. H. (1993) : In vitro antibacterial activity of norfloxacin (MK-0366, AM 715) and other agents against GIF pathogens. Anti-microbial Agents and Chemotherapy, 23: 86-90.

Siebert, G. (1983) : Broad spectrum, fluorinated quinolone comparison antibacterial activity with other nalidixic acid analoges. Eur. J. clin. Microbiol., 2: 548.

Smith, J. T. (1989) : Oral iron, 4-quinolones and enteric infection. 29th Int. Conl. Antimicrob. Agent Chemother, Houston Tex. 149.

Snedecor, G. W. (1969) : Statistical methods. 4th ed. The Iowa, Ames, Iowa.

Walker, R. D.; Stein, G. E.; Budsberg, S. C.; Rosser, E. J. and Macdonald, K. H. (1989) : Serum and tissue fluid norfloxacin concentration after oral administration of the drug of healthy dogs. American J. Vet. Research, 50: 154-157.

Walser, K.; Gandorfer, B.; Steinberger, A.; Treitinger, E. and Winter, T. (1993) : Studies of the antibacterial activity and pharmacokinetics of enrofloxacin (baytril) in lactating cows. Tierarztliche Umschau, 48 (7): 414-416.

الملخص العربى

دراسة تأثير السيبروفلوكساسين وكبريتات الحديدوز على التحليل البيوكيميائى وتأثيره كمضاد بكتيرى فى الفئران البيضاء

المشتركون فى البحث

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قسم بحوث الكيمياء والنقص الغذائى معهد بحوث صحة الحيوان - الدقى

تتناول هذه الدراسة تقييم مدى كفاءة المضاد الحيوى السيبروفلوكساسين كمضاد بكتيرى باستخدام عترات من كل من البكتريا العنقودية والسبحية والميكروب القولونى والباسيلس ساتلس والسالمونيلا تيفيميوريم ووجد أن أقل تركيز (MIC) للسيبروفلوكساسين يسبب تأثيراً على البكتريا عند مستويات ٠٫٢٨، ٠٫٥٦، ٠٫١٤، ٠٫٥٦ و ١٫١٢ ملليجرام / مللى على التوالى. ومن ناحية أخرى فقد وجد أن كفاءة المضاد الحيوى السيبروفلوكساسين تقل عندما يتم أخذه مع كبريتات الحديدوز (كمصدر للحديد).

وقد تم إعطاء المضاد الحيوى السيبروفلوكساسين فى الفئران منفرداً أو متحداً مع كبريتات الحديدوز عن طريق الفم بجرعة مقدارها ٩ و ١٫٨ ملليجرام / ١٠٠جرام من وزن الحيوان على التوالى لمدة ٧ أيام متصلة، وقد تبين من التحاليل البيوكيميائية لمصل الدم وجود زيادة معنوية فى كل من إنزيمات الألنين أمينو ترانسفيريز واسبرتات أمينو ترانسفيريز وانزيم الفوسفاتيز القلوى وكذلك مستوى الكرياتينين بالدم عند إعطاء المضاد الحيوى مع كبريتات الحديدوز.

كما وجد أن أعلى تركيز للسيبروفلوكساسين بدون أو فى وجود كبريتات الحديدوز هو ١٤٫٥ و ٤٫٥ ميكروجرام / مللى على التوالى فى مصل الفئران المختبرة.

نستخلص من ذلك أنه يجب تفادى إعطاء كبريتات الحديدوز أثناء تناول السيبروفلوكساسين حيث أنها تقلل كفاءته كمضاد بكتيرى وأيضاً تقلل تركيزه فى مصل الدم وتؤثر أيضاً على مستوى إنزيمات الكبد ومستوى الكرياتينين فى مصل الدم.