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GENTAMICIN AND NETILMICIN RESIDUES IN TISSUES AND ORGANS OF TREATED RABBITS

By

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SUMMARY

Thirty Balady rabbits of both sexes (Weighing 1.5-2 kg.B.Wt.) were used for studying gentamicin and netilmicin residues. The effects of heat treatment and freezing on the presence of these residues were also studied. The drugs were injected 1/M (6 mg/kg B. Wt.) daily for 7 successive days. Animals were slaughtred at different intervals, samples from shoulder, thigh and back muscles, heart, liver and kidneys were examined microbiologically for detecting the residues. Parts from samples of the animals containing the highest amount of residues (slaughtered at 12 hours after treatment) were boild for 45 minutes and tested for the presence of residues, while the rest of these samples (12 hours) were freezed and examined weekly for the presence of the tested drugs.

It was achieved that traces of gentamicin or netilmicin residues could be detected in the muscle samples up to 24 hours post last injection (0.17, 0.13 ppm respectively). The residues also disapeared by boiling the samples for 45 minutes and also by freezing for one week . The least amount of residues were detected in the heart (0.07 ppm). Liver samples of both drugs contained higher levels of residues that disappeared after 48 hours following drug injection (0.3 ppm). Kidney samples of both drugs showed the highest level of residues which disappeared after 4 days for gentamicin (0.75 ppm) and 5 days for netilmicin (0.9 ppm). Moreover, boiling of kidney or liver samples for 45 minutes did not destroy the residues completely. Furthermore, freezing for 3 months also failed to destroy the residues in the kidneys.

It is concluded that gentamicin and netilmicin were highly concentrated in the kidneys followed by the liver while taces were present in the muscles which disappeared after 24 hours of injection and by boiling.

INTRODUCTION

Antibacterial agents are widely used for prophylactic and treatment of various diseases in animals. Gentamicin and netilmicin are aminoglycoside broad-spectrum antibiotics from species of Actinomycete micromonospora (Goodman and Gilman's 1985).

Gentamicin is active against a large number of gram-negative and gram-positive bacteria specially Pseudomonas, Proteus and Klebsiella-Enterobacter species which are not sensitive to other antimicrobial agents. Therefore, gentamicin is used for treatment of urinary tract infections in man (Howard et al., 1971) and aniamls (Ziv et al., 1980).

Netilmicin is one from the latest aminoglycosides to be marketed. Its antibacterial activity is broad against aerobic gram-negative bacilli. It is not metabolised by the majority of the aminoglycoside inactivating enzymes, and it may be active against bacteria that are resistant to gentamicin (Panwalker et al., 1978).

Residues of antibacterial drugs in edible tissues of food producing animals represent serious problem for human being consuming such tissues. Hypersensitivity and development of bacterial resistant strains are among the hazards of antibiotic residues (Williams, 1974).

The purpose of the present work was thus initiated to through a light on gentamicin and netilmicin residues in the rabbit tissues. The effects of heat treatment and freezing on these residues were also investigated.

MATERIAL AND METHODS

Drugs:

Gentamicin sulphate (Garamycin)® and netilmicin sulphate (Netromycin)® injections obtained from Schering corporation- New Jersy U.S.A.

Animals and experiments:

Thirty clinically healthy rabbits (Balady bread) of both sexes weighing about 1.5-2.0 kg. B.wt. were used as experimental animals throughout this study. They were kept for 15 days under well hygienic conditions and fed on barseem barley, concentrated mixture in a pellet from free from any antibacterial agents and water ad-lib. Two rabbits (control) were slaughtered and tested from the abscence of any antibacterial residues, then the rest of the rabbits were classified into two equal groups. The 1st group was given gentamicin, whereas, the 2nd was administered netilmicin. Both drugs were given intramuscularly at a dose of 6mg/kg B.wt. in two divided doses every 12 hours intervals for 7 successive days. Two rabbits from each group were slaughtered at 12, 24, 48, 72, 96, 120 and 144 hours after the last injection. Shoulder, thigh and back muscles, heart, liver and kidneys were obtained from each rabbit at slaughtering time. The samples were kept in plastic marked containers at -10°C in freezer for assaying the residues.

Detection of the residues:

The method discribed by Bogaerts and Wolf Brussels (1980), was used for detection of the antibiotic residues in rabbit tissues and organs using Bacillus subtilis BGA strain on nutrient agar media pH 6,8.

Interpretation of the resutls:

Inhibition zone ≥ 2 is considered + ve
Inhibition zone 1: <2 is considered suspecious
Inhibition zone < 1 is considered -ve.

Effect of heat treatment:

Part of the samples containing the highest amou of residues (12 hours post treatment) were boile in distilled water for 45 min. and used for detec ing the drug residues.

Effect of freezing:

The rest of all samples (12 hours post treatme were kept at -10°C in freezer and examined wee ly for detecting residues for 3 months.

RESULTS

Table (1) and (2) revealed that gentamicin and tilmicin residues in the muscles and the heart ne ly disappeared after 24 hours post administratio in liver after 48 hs, while in kidneys residues gentamicin and netilmitin were present up to 4 5 days, respectively post last injection.

The result also showed that boiling of the samp containing high levels of residues (12 hs p treatment) destroy the residues completely in samples except in kidneys and to some extent the liver (Table 3 & 4).

After freezing the samples of muscles and org containing high levels of residues for 12 wee the residues of both drugs continoued only in kidneys to the end in effective concentrati (Table 5 & 6).

Table 1: Mean inhibition zones in mm. of gentamicin intramuscularly injected into rabbits (6 mg/kg. B/wt.) daily for 7 successive days.

Hours	Control		12		24		48		72		96		120		144	
PH / Samples	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8
Shoulder m.	0.0	0.0	1.5	2.5	0.5	1.5	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Thigh m.	0.0	0.0	1.0	1.5	0.5	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Back m.	0.0	0.0	2.0	2.5	1.0	1.5	0.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Heart	0.0	0.0	0.5	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Liver	0.0	0.0	3.0	3.5	2.0	2.5	1.0	2.0	0.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Kidney	0.0	0.0	8.0	10.0	6.0	9.0	5.5	6.5	3.5	4.0	2.0	2.5	0.5	1.0	0.0	0.0

m: muscles

Table 2: Mean inhibition zones in mm. of netilmicin intramuscularly injected into rabbits (6 mg/kg. B/wt.) daily for 7 successive days.

Hours	Control		12		24		48		72		96		120		144	
PH / Samples	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8
Shoulder m.	0.0	0.0	1.5	2.0	1.0	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Thigh m.	0.0	0.0	0.8	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Back m.	0.0	0.0	1.5	2.0	0.8	1.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Heart	0.0	0.0	0.0	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Liver	0.0	0.0	3.0	3.5	2.0	3.0	1.5	2.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Kidney	0.0	0.0	10.0	12.0	8.0	10.0	7.0	9.0	5.0	7.0	4.0	5.0	2.0	3.0	1.0	1.5

m: muscles

Table 3: Effect of boiling for 45 minutes on gentamicin residues in rabbits slaughtered after 12 hours post drug administration for 7 days (mean inhibition zones in mm).

Treatment	Before boiling		After boiling	
PH / Samples	6	8	6	8
Shoulder m.	1.5	2.5	0.0	0.0
Thigh m.	1.0	1.5	0.0	0.0
Back m.	2.0	2.5	0.0	0.0
Heart	0.5	1.0	0.0	0.0
Liver	3.0	3.5	1.0	2.0
Kidney	8.0	10.0	2.0	3.0

Table 4: Effect of boiling for 45 minutes on netilmicin residues in rabbits slaughtered after 12 hours post drug administration for 7 days (mean inhibition zones in mm).

Treatment	Before boiling		After boiling	
PH / Samples	6	8	6	8
Shoulder m.	1.5	2.0	0.0	0.0
Thigh m.	0.8	1.0	0.0	0.0
Back m.	1.5	2.0	0.0	0.0
Heart	0.0	1.0	0.0	0.0
Liver	3.0	3.5	2.0	2.0
Kidney	10.0	12.0	3.0	4.0

m: muscles

Table 5: Effect of freezing on gentamicin residues in rabbit samples (mean inhibition zones in m.m).

Weeks	0		1		2		3		4		5		6		7		8		9		10		11	
PH / Samples	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8
Shoulder m.	1.5	2.5	1.0	2.0	1.0	1.5	0.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Thigh m.	1.0	1.5	0.5	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Back m.	2.0	2.5	1.0	2.0	0.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Heart	0.5	1.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Liver	3.0	3.5	2.5	2.5	2.0	2.5	1.5	2.0	1.0	1.5	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Kidney	8.0	10.0	7.5	9.0	7.0	8.5	7.0	8.5	6.5	7.5	6.0	7.0	6.0	6.5	5.5	6.0	5.0	6.0	4.5	5.0	4.0	5.0	3.5	4.0

m: muscles

Table 6: Effect of freezing on netilmicin residues in rabbit samples (mean inhibition zones in m.m).

Weeks	0		1		2		3		4		5		6		7		8		9		10		11	
PH / Samples	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8	6	8
Shoulder m.	1.5	2.0	1.0	1.5	0.5	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Thigh m.	0.8	1.0	0.5	0.8	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Back m.	1.5	2.0	1.0	1.5	0.5	1.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Heart	0.0	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Liver	3.0	3.5	2.5	3.0	1.5	2.0	1.0	1.5	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Kidney	10.0	12.0	9.0	11.0	8.0	10-.5	7.5	9.0	7.0	8.5	6.0	8.0	5.5	7.0	5.0	6.0	4.5	5.5	4.0	5.0	4.0	4.5	3.5	4.0

m: muscles

DISCUSSION

Nowadays antibiotics are used extensively in food producing animals for prophylaxis and treament of various diseases as well as for growth promotion. Most of these antibiotics leave residues in the animal tissues and may cause hazards to human beings consuming such tissues.

Gentamicin and netilmicin are aminoglycoside antibiotics, they were injected into rabbits every twelve hours for seven successive days for studying their withdrawal time in the examined rabbit tissues.

It was achieved that gentamicin and netilmicin residues were 0.17, 0.13ppm in the rabbit muscles respectively and disappeared after 24 hours of drug administration. This result agrees with that of Davies et al. (1984) who recorded that the concentrations of aminoglycosides in the secretions and tissues were low. From the hazardous point of view this time (24 hours) will be lost during trasportation of the rabbits from the farm to the ket and in the market itself thus no hazard of suming such meat..Moreover, boiling of r meat samples showed disappearance of the dues from the muscles. "thus it is safe for hu consumption if they were given gentamicin c tilmicin within their therapeutic levels.

The liver and kidneys showed higher conce tions of the tested antibiotics (0.3 ppm for the and 0.75, 0.9 ppm for the kidneys). This is a uted to the role of the liver in the metabolism the kidney in the excretion of these antibi Out results coordinate the results of Chisho al. (1968) who recorded that gentamicin was centrated in the urine. Nunnery and Riley (and McCracken et al. (1971) reported that ge icin was excreted and concentrated in man ki Moreover Mariel et al. (1972), Luft and (1974) and Bush et al. (1981) found that this was concentrated in the kidney of differen mals. Goodman and Gilman's (1991) reporte the aminoglycosides are excreted almost en

by the glomerular filteration.

In our work gentamicin and netilmicin disappeared from the liver samples after 48 hours while in the kidney samples after 96 and 120 hours respectively.

Unfortunately the residues in the liver and kidney samples of both tested drugs did not disappear completey by boiling for 45 minutes. This may be due to the higher levels of the residues which represented by the large inhibition zones in the kidney and liver samples in comparison to muscle samples. Thus kidney and liver containing gentamicin or netilmicin residues are unfit for human consumption except after 4 days of gentamicin administration and 5 days for netilmicin.

The residues of gentamicin and netilmicin disappeared after one week of freezing in the muscle samples. This result may be due to the little amount of these drugs in the muscle (up to 0.17 ppm) thus their residues were rapidly disappeared. On the other hand the residues in the kidney samples did not disappear even after 3 months of freezing this may be attributed to the concentration of these drugs in the kidney (up to 0.9 ppm).

REFERENCES

Bogaerts, R. and Wolf Brussels, F. (1980): A standardized method for the detection of residues of anti-bacterial substances in fresh Meat. Fleischwirtsch. (4) 60.

Bush, M.; Locke, D.; Neal, L.A. and Carpenter, J.W. (1981): Gentamicin tissue concentration in various avian species following recommended dosage therapy. Am. J. Vet. Res., 42, (12) 2114-2116.

Chisholm, G.D.; Calnan, J.S. and Water Worlts, P.M. (1968): Distribution of gentamicin in body fluids. Br. Med. J., 2, 22-24.

Davies, B.D.; Brammett, R.E.; Bendrick, T.W. and Himes, D.L. (1984): Dissociation of maximum concentration of Kanamycin in plasma and perilymph from ototoxic effect. J. Antimicrob. Chemother. 14, 291-302.

Goodman, L.S. and Gilman's, A. (1985): The pharmacological basis of therapeutics. 7th edition, copyright by MacMillan Publishing Co. Printed in U.S.A.P. 1150.

Goodman, L.S. and Gilman's, A. (1991): The pharmacological basis of therapeutics. 8th edition, Maxwell MacMillan International Edition. P. 1103.

Howard, J.W.; Donoso, E.; Mimica, I. and Zilleruels, G. (1971): Gentamicin injection in infants with low birth weight. J. Infect. Dis. (124). suppl., 232-233.

Luft, F.C. and Kleit, S.A. (1974): Renal parenchymal accumulation of aminoglycosides. J. Infect. Dis., (130), 656-659.

Mariel, C.P.; Veyssier, J.C. and Decerner, P.E. (1972): Urinary pH and excretion of gentamicin. Br. med. J., (2), 206.

McCracken, G.H.; Chrane, D.F. and Thomas, M.L. (1971): Pharmacological evaluation of gentamicin in new born infants. J. Infect. Dis., (124) suppl., 214-223.

Nunnery, A.W. and Riley, H.D. (1969): Gentamicin: Pharmacolgic observation in new borns and infants. J. Infect. dis., (119), 402-405.

Panwalker, A.P.; Malon, J.B.; Zimelis, V.M. and Jackson, G.G. (1978): Netilmicin: Clincial efficacy, tolerance, and toxicity. Antimicrob. Agents Chemother., 13, 170-176.

Williams, S.H. (1974): Clinical problems of preventive Medicine, antibiotic resistant bacteria in animals and danger to human health. Veterinary, J. (130), 110.

Ziv, G.; Storper, M.; Wanner, M. and Nicolet, J. (1980): Comparative pharmacological investigations with gentamicin and tobramycin in new born calves. Schweiz. Arch. Tierheilkd., 122 (8), 417-426.