A Comparison Between The Pharmacokinetics Of Gentamicin And Its Mixture With Calcium In Rabbits

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Summary

Pharamcokinetic studies were performed for gentamicin sulphate at single dose of 5 mg/kg compared with formullae of same dose of gentamicin with calcium gluconate at a dose of 7.5 mg/kg injected i.v. and i.m. to four groups of 5 rabbits. The results of i.v. injection showed elimination of first order two compartment model with lower plasma gentamicin concentration, lower inhibition bacterial period and increase of gentamicin clearance in mixture group may be due to its competition with calcium for albumin which increase the free drug available for excretion and distribution while after i.m. injection absorption of gentamicin from formullae was lower but its plasma elimination (β) was slower with nearly same inhibition bacterial period as in gentamicin dosed group. These results explain kinetically why calcium lower gentamicin toxic side effect after i.m. and i.v. injection.

دراسة مقارنة للحركية الدوائية للجنتامايسين ومزيجه مع الكالسيوم في الأرانب

مروج عبد الستار العبيدي
مركز ابن البيطار – وزارة الصناعة

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الخلاصة

تم إجراء دراسة مقارنة للحركية الدوائية لكبريتات الجنتامايسين بجرعة مفردة مقدارها 5ملغم/كغم من وزن الجسم ومزيجه مع الكالسيوم بجرعة 7.5 ملغم/كغم ثم حقنها في الوريد والعضلة لاربع مجاميع مكونة كل منها من 5 حيوانات من ذكور الارانب الهجينة.أظهرت نتائج الحقن الوريدي ان اختفاء العقار يتبع الحركية الدوائية من الطراز الاول ومن نوع الفجوة الثنائية مع نقصان في تراكيز الجنتامايسين في البلازما ونقصان في مدة التثبيط الجرثومي وزيادة في الطرح الكلوي، قد يعود لسبب ذلك إلى التنافس بين الجنتامايسين والكالسيوم للإرتباط مع الألبومين الامر الذي سبب زيادة في تراكيز الجزء الحر للعقار في البلازما وبالتالي زيادة الطرح والانتشار السريع، أما الحقن العضلي فإن درجة امتصاص العقار من المزيج تناقصت ولكن سرعة اختفاءه من البلازما (β) كان أبطيء مع فترة تثبيط بكتري متقاربة مع العقار المفرد.إن هذه النتائج تفسر حركياً دور الكالسيوم في تقليل التأثيرات السمية الجانبية للجنتامايسين بعد الحقن الوريدي والعضلي.

Introduction

Gentamicin is abroad spectrum aminogeycoside antibiotic derived from the *actinomycete Micromonospora* [1]. Gentamicin is still widely used in the whole world to treat infect of gram negative and some gram-positive bacteria [2].

Gentamicin is available for parental and topical use; it is not used orally for systemic treatments since only small amounts are absorbed from the gastrointestinal tract [1]. In veterinary medicine, gentamicin is widely used to treat infections of cats, dogs, horses and poultry which are caused by gram negative aerobic bacteria [3].

Gentamicin is not metabolized to a measurable extent and is eliminated from the body principally by renal excretion (Glomerular Filtration) and there are a remarkable extrarenal route of elimination (e.g. bile) [4]. The kinetics of gentamicin has been studied in several species, including the rabbit [5].

Previous and recent studies showed that calcium reduce the reported toxic side effect of gentamicin (nephrotoxicity, ototoxicity and neuromuscular blockade) in animals[3;6;7;8]

New formula from therapeutic doses of gentamicin and calcium gluconate have been tested and showed less toxic side effect and same antibacterial activity against *E. coli.* infection in mice in comparison with gentamicin alone.

The kinetic of gentamicin with calcium has not been reported before in any animals or human being. Calcium salts usually supplemented as food additive for domestic animals and poultry taken in consideration it may interact with gentamicin when used as therapeutic drug against bacterial infection.

The aim of present study to explore the kinetics of new formulae and its reflection on the toxicity and antibacterial activity of gentamicin in rabbit.

Materials and Methods

Twenty adult male hybrid rabbits weighing (1300-1600) gram were allowed to accommodate in special cage under controlled condition for four weeks. The animals were divided into two equal groups and that subdivided into two subgroups (A & B). Animals of the first group were injected intravenously with gentamicin alone at a dose of (5 mg/kg) for subgroup A and a mixture of same dose of gentamicin with calcium gluconate at a dose (7.5 mg/kg) while the second group injected intramuscular with same drug and doses for both subgroup (A, B). The drug injection and blood collection by a

sterile syringe (2 ml) was inserted into the lateral marginal auricular vein for injection and from the same vein and the main auricular artery and heart for blood collection in time (5, 15, 30, 60) min. (2, 3, 6, 8, 12, 24) hrs.

Clotted blood samples (1 ml) were centrifuged at 3500 PPM for (10 min.). Serum samples were frozen at (-16) °C until they were analysed. Gentamicin serum conc. were determined by applying at biological assay, using *Staphylococcus aurous* (ATCC) as test bacteria and media A as tested culture. The tested bacteria was activated by transmission into brain heart infusion broth (BHI) (Mast – Diagnostic/ Biolife) every month.

The method of punching and filling wells of standards gentamicin concentration or serum samples in seeded agar (USP, 1995; B.P., 1993) was used to determine the size of inhibition zone using zone meter instrument (Antibiotic Zone Reader Fisher-Lilly, USA). The minimum inhibitory concentration of gentamicin (MIC) in seeded agar was (0.05 μg/ml).

Results

In case of i.v. injection, the mean gentamicin concentration after 5 min. were (17.4 ± 0.4) μg/ml for subgroup A and (7.92 ± 0.707) μg/ml for subgroup B. These concentration declined to the level of (0.018 ± 0.0012) μg/ml after 8 hrs. and (0.0368 ± 0.003) μg/ml after 6 hrs. for subgroup A & B respectively (table 1), while their ($\beta t_{1/2}$) & (AUC) were (0.963 ± 0.02) hr And (10.918 ± 0.39) μg/ml. hr. for subgroup A, (o.788 ± 0.001) hr. and (8.414 ± 0.02) μg/ml. hr. for subgroup B. These results were significantly different ($P<0.05$). The results showed also significant difference between renal clearance, K_{12} rate and not significant one in Vd and K_{21} values in comparison between subgroups A & B of i.v. injection (table 3).

In case of i.m. injection, the peak gentamicin serum conce. observed after one hr. and (0.5) hr. were (4.6 ± 0.089) μg/ml and (2.3 ± 0.158) μg/ml for subgroup A & B respectively (table 2), while their ($\beta t_{1/2}$) were (0.627 ± 0.022) hr. for subgroup A and (0.735 ± 0.003) hr. for subgroup B. The (Vd) of gentamicin measured for subgroup A was (o.630 ± 0.002) L/kg and (0.415 ± 0.01) L/kg for subgroup B, while its renal clearance was (0.458 ± 0.0002) L/hr. kg and (0.5942 ± 0.00001) L/hr. kg for subgroup A & B respectively. The bioavailibility and AUC recorded for gentamicin alone were (76.828 ± 0.017)%, (8.388 ± 0.19) μg/ml. hr. and (39.28 ± 0.006)%, (3.306 ± 0.052) μg/ml. hr. for drug mixture respectively. These results were significantly different ($P<0.05$) in comparison between both subgroups A & B (table 4).

Table 1: Gentamicin mean concentration (μg/ml) in rabbit blood serum after IV injection for gentamicin alone or as a mixture with calcium gluconate

Time sampling/ hr.	Drug concentration in blood serum (μg/ml) Mean ± SE			
	First group (A)		First group (B)	
0.08	0.400 17.4+	A	7.92 ± 0.07070	B
0.25	7.9+ 0.361	A	6.246 ± 0.0014	B
0.5	5.4+0.158	A	4.404 ± 0.0043	B
1	3+ 0.214	A	2.79 ± 0.028	B
2	1.4 + 0.187	A	1.295 ± 0.001	B
3	0.65+ 0.035	A	0.492 ± 0.0021	B
6	0.081+ 0.0037	A	0.0368 ± 0.003	B
8	0.018+ 0.0012	A		B

Different letters mean there is a significant different at ($P<0.05$)

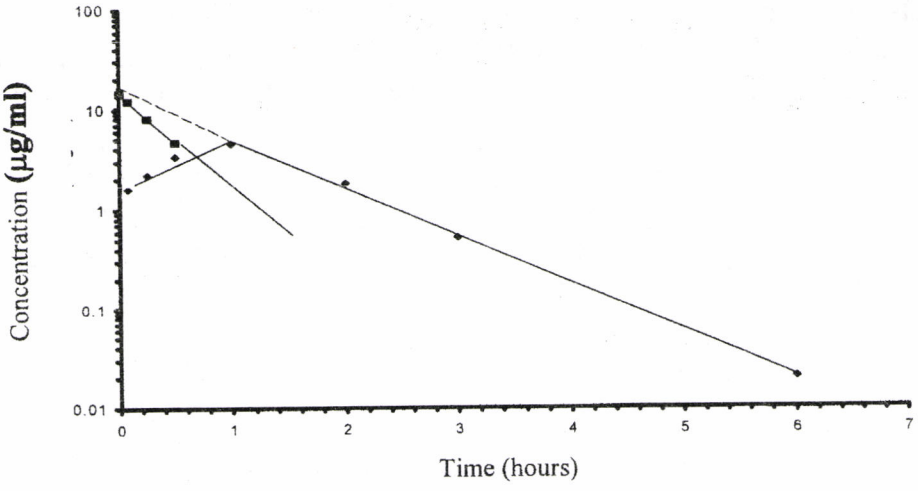

Figure 1: Gentamicin mean concentration in Rabbit blood serum (µg/ml) after (i.v.) injection of gentamicin sulphate.

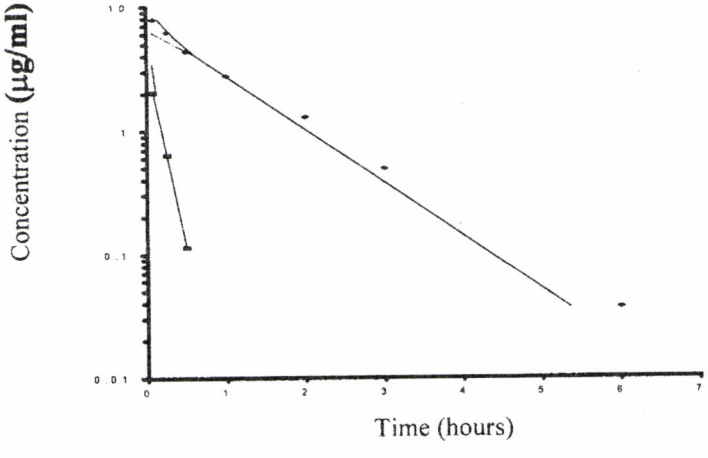

Figure 2: Gentamicin mean concentration in Rabbit blood serum (µg/ml) after (i.v.) injection to the mixture of the drugs.

Table 2: Gentamicin concentration mean (μg/ml) in Rabbit serum after i.m. injection for the drug only and as a mixture with calcium gluconate

Sampling time	Drug concentration in blood serum (μg/ml) Mean + SE			
	Second group (A)		Second group (B)	
0.08	1.59 ± 0.034	A	0.18 ± 0.0049	B
0.25	2.2 ± 0.176	A	1.5 ± 0.122	B
0.5	3.39 ± 0.044	A	2.3 ± 0.158	B
1	4.6 ± 0.089	A	1.4 ± 0.223	B
2	1.8 ± 0.083	A	0.59 ± 0.007	B
3	0.52 ± 0.083	A	0.19 ± 0.01	B
6	0.02 ± 0.003	A	0.013 ± 0.0007	B

* Different letters mean there is a significant different $P<0.05$

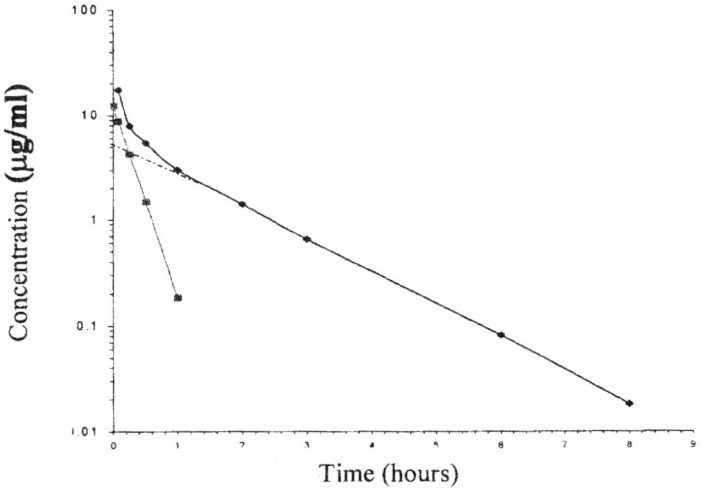

Figure 3: Gentamicin mean concentration in Rabbit blood serum (μg/ml) after (i.m.) injection of gentamicin sulphate.

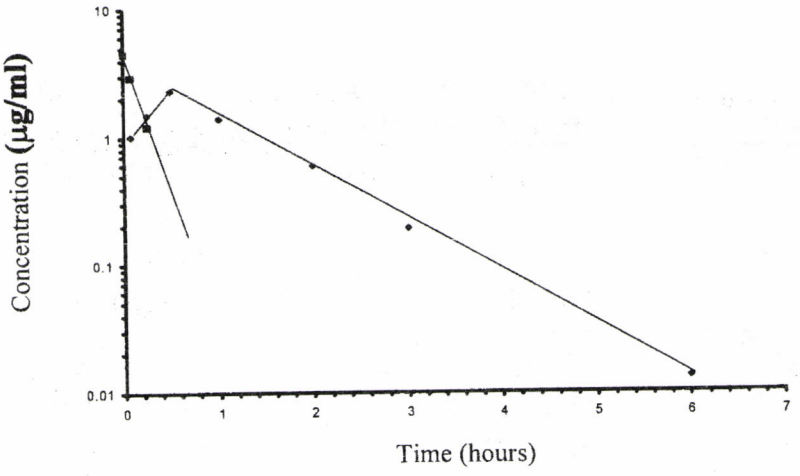

Figure 4: Gentamicin mean concentration in Rabbit blood serum (µg/ml) after (i.m.) injection to the mixture of the drugs

Table 3: Gentamicin pharmacokinitics parameters after i.v. injection in first group for the drug & its mixture with calcium gluconate ± SE

Pharmacokinitic parameters	First group A G		First group B G + Ca	
α/hr^{1-}	3.835±0.13	A	6.646±0.071	B
β/hr^{1-}	0.717±0.016	A	0.879±0.01	B
A-µg/ml	10.67±0.866	A	2.847±0.063	B
B-µg/ml	5.845±0.71	A	7.02±0.030	B
αt1/2 hr	0.182±0.006	A	0.1043±0.001	B
βt1/2 hr	0.968 ± 0.02	A	0.788±0.001	B
V_d L/Kg	0.645±0.002	A	0.676±0.03	A
AUC µg/ml.hr	10.918±0.39	A	8.414±0.02	B
Cl L/Kg/hr	0.460±0.016	A	0.5942±0.002	B
$K_{21}\ hr^{1-}$	1.894±0.26	A	4.981±0.03	B
$K_{12}\ hr^{1-}$	1.133±0.079	A	1.37±0.035	A

* the same letters mean there is no significant different ($P<0.05$)
- Different letters mean there is a significant different ($P<0.05$)

Table 4: Gentamicin pharamacakinitics parameters after i.m. injection in second group for the drug & its mixture with calcium gluconate ± SE

Pharmacokinitic parameters	Second group A G		Second group B G + Ca	
α/hr^{1-}	1.971±0.043	A	4.541±0.363	B
β/hr^{1-}	1.11±0.039	A	0.943±0.004	B
A-μg/ml	14.329±0.906	A	3.376±0.14	B
B-μg/ml	15.614±1.214	A	3.6042±0.056	B
αt1/2 hr	0.352±0.009	A	0.157±0.015	B
βt1/2 hr	0.627 ± 0.022	A	0.735±0.003	B
V_d L/Kg	0.630±0.002	A	0.415±0.01	A
AUC μg/ml.hr	8.388±0.19	A	3.306±0.052	B
Cl L/Kg/hr	0.458±0.0002	A	0.5942±0.00001	B
F%	76.828±0.017	A	39.28±0.006	B

* Different letters mean there is a significant different ($P<0.05$).

Discussion

The results of i.v. gentamicin injection alone or with calcium showed that elimination from the plasma follow first order two compartment open model. This agreed with results from the other studies in rabbit [5,9], or in other animals and human [10, 11, 12, 13]. It seems that adding calcium gluconate to gentamicin formulae led to decline in gentamicin plasma concentration within time, decrease in (α & $\beta t_{1/2}$, increase in its clearance (Cl), distribution rate constant (α) and constant rate of transport from the central to peripheral (K_{12}) or vise versa (K_{21}) in comparison with the results of i.v. injection of gentamicin alone (table 1&3) (fig. 1&2).

This might attributed to the fact that both gentamicin and calcium bind to plasma albumin [14], this led to competition on protein binding site and so increase in the amount of free drug that available

for excretion and distribution.Also the reported competition between calcium and gentamicin at the cellular site [15] , led to decrease in absorption of gentamicin from the formulae after i.m. injection (bioavailability (F) 39.2%) in comparison with that of gentamicin alone (F) 76.8%).

However, gentamicin plasma concentrations were lower in subgroup B than A, the bacterial inhibition period were the same.

This clear when we compare the slope of B phase which was less steeper and shown in subgroup B than A, indicating that may be there was another route of elimination other than renal since clearance was higher in subgroup B than A.

Previous studies indicate there was a remarkable rate of hepatic elimination through bile[16,17],. so it was natural to assume that hepatic elimination higher in subgroup A than B proportional with their rate of protein binding[18] , which is higher in subgroup A than B due to competition of gentamicin with calcium for binding with albumin.

It was concluded from the results of both invitro and invivo experiments that there was a competitive interaction between gentamicin and calcium ions which reflected their effect on the kinetic parameters of gentamicin and led to the decline in their plasma concentration in the mixture dosed subgroups to the levels lower than the recorded as critical values (5-12) µg/ml. This explains kinetically the decline of toxicological parameters both biochemical and histologically that reported by the study of Ubaid, K. A., [19] ,which used therapeutic doses of gentamicin and calcium in different laboratory animals.

The period of gentamicin antibacterial activity showed a little decline in case of mixture dosed group that was injected i.v., while no change noticed between the two subgroups in case of i.m. injection. This case opens the way for increasing this period either by increasing the dose of gentamicin in the mixture formulae or by administering calcium in

separate route, e.g., orally, to increase gentamicin absorption after i.m. injection.

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