Comparative studies of calcium antagonizing effect on acute toxicity of streptomycin and gentamicin in mice.

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Summary

Comparative studies about the characteristics of the acute toxicity of streptomycin and gentamicin and the antagonization effect of calcium to their toxicity were performed in mice. This was made by comparing the time of appearance and disappearance of toxicity symptoms of this aminoglycoside before and after calcium therapy and also by comparing the LD_{50} value of both agents. It was concluded that streptomycin is less potent but more efficacious as toxic agent than gentamicin and that calcium had a competitive inhibitory effect to the toxicity of aminoglycoside perhaps because of the similarity in their charges and binding sites.

Calcium therapy seems to offer quantitatively the same protective level for both agents (nearly one time) but qualitatively better protective level against acute toxicity of streptomycin in mice than for gentamicin.

دراسات مقارنة للتأثير المعاكس للكالسيوم في السمية الحادة للستربتومايسين والجنتامايسين في الفئران

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الخلاصة

تمت دراسة الخواص السمية الحادة الستربتومايسين و الجنتامايسين في الفئران والتأثير المضاد للكالسيوم عليها و ذلك بمقارنة وقت ظهور و اختفاء أعراض التسمم بهذه

الامينوكلايكوسايدات قبل و بعد المعالجة بالكالسيوم و كذلك مقارنة الجرعة المميتة الوسطية لكلا العقارين. تم الاستنتاج بأن الستربتومايسين تأثير سمي أقل قوة ومعدل استجابة أكثر بالمقارنة مع الجنتامايسين وأن للكالسيوم تأثير تنافسي تثبيطي للفعالية السمية للامينوكلايكوسايدات ربما بسبب تشابهها بالشحنات ومواقع الارتباط.

ظهر من دراستنا بأن المعالجة بالكالسيوم تعطي كمياً نفس المستوى من الحماية لكل من العقارين (تقريباً مرة واحدة) في الفئران و لكن نوعياً تعطي مستوى أعلى من الحماية ضد التسمم الحاد بالستربتومايسين عنه بالجنامايسين .

Introduction

Streptomycin and subsequently introduced aminoglycoside (neomycin, gentamicin, Kanamycin, sisomicin, tobramycin and netlimicin), have remained in clinical use till this days. In fact, aminoglycosides are such efficacious and inexpensive broad-spectrum antibiotic that they are one of the most commonly used antibiotic groups worldwide. [1,2]

All aminoglycoside antibiotics display toxic side effects to the kidney, inner ear and neuromuscular junction. [3,4]
Recent studies indicate that calcium intake by ingestion [5] or as injection [6] offer protection from toxicity of aminoglycoside. Clinically, it was reported that neuromuscular blockade caused by aminoglycoside therapy was treated by calcium and not by cholinesterase inhibitors. [7]

Our study aim to explore the characteristics of acute toxicity of streptomycin and gentamicin and the antagonizing capability of calcium against this toxicity in mice.

Materials and Methods

The first experiment was designed to compare the acute toxicity of streptomycin with and without calcium therapy.

Sixty Swiss Webster albino mice were of both sexes (average body weight 20± 2g.) were used, they are randomly divided into two groups of 30 mice, each of which subdivided into 5 subgroups of 6 animals and housed in special cages under controlled temperature (22 ± 2°C) and 14/10 light/dark cycle. Animals of group (I) served as control and were injected subcutaneously (S.C.) with sterilized distill water, followed after 30 minutes by I.P. injection of streptomycin sulfate (Panstreptomicn-1g./Panpharma-France) at doses of 300,350,400,450 and 500 mg/kg , the volume of the injection was 1 ml /100 g. body weight.

Animals of group (II) were injected S.C. with calcium as calcium glugunate (Calcium glugunate 10% - Fugisawa-USA, Inc.) at a dose of 22.5 mg/Kg. body weight. Thirty minutes later, the sub groups were injected intraperitonealy (I.P.) with 700,750,800,850, and 900 mg/kg body weight of streptomycin.

The percentage of lethality was reported 24 hours after treatment. The LD50 was calculated by applying probit method [8].

Same experiment with same number of animals were used for acute toxicity of gentamicin in which the serial doses of gentamicin sulphate (Cidomycin 40 mg/ml-Roussel) injected I.P for the animal of the five subgroups (Group I) were respectively (250, 300, 350, 400,450) while that for group II were (550, 600,650,700, 800) mg/kg body weight, after half an hour of calcium gluconate dosing. Gentamicin LD_{50} was calculated and compared with that of streptomycin.

The second experiment was designed to compare the response to calcium therapy after selected lethal doses of streptomycin and gentamicin that gave nearly same mortality percent in mice.

Gentamicin sulfate (Cidomycin 40mg/ml–Roussel) and streptomycin were injected in two groups, each of 10 mice at dose of

400 mg/Kg and 450 mg/Kg respectively, the number of animals and the time at which they showed the most obvious toxic symptoms were recorded.

Calcium was injected I.P as calcium gluconate at a dose of 22.5 mg/Kg after the appearance of slow abdominal respiration. The time at which toxic response developed and disappeared after treatment was recorded.

Statistical analysis: -

Student T-test was used for statistical comparison between times of appearance of toxic symptoms after administration of streptomycin and gentamicin both before and after calcium treatment and also between time of disappearance of toxic symptoms after calcium therapy (table-3).

Results

The results of acute toxicity (LD_{50}) of streptomycin and gentamycin without and with calcium as calculated from (figure 1 and 2) were 397 & 794 mg/kg for streptomycin, 363 and 661 mg/kg for gentamicin correspondingly.

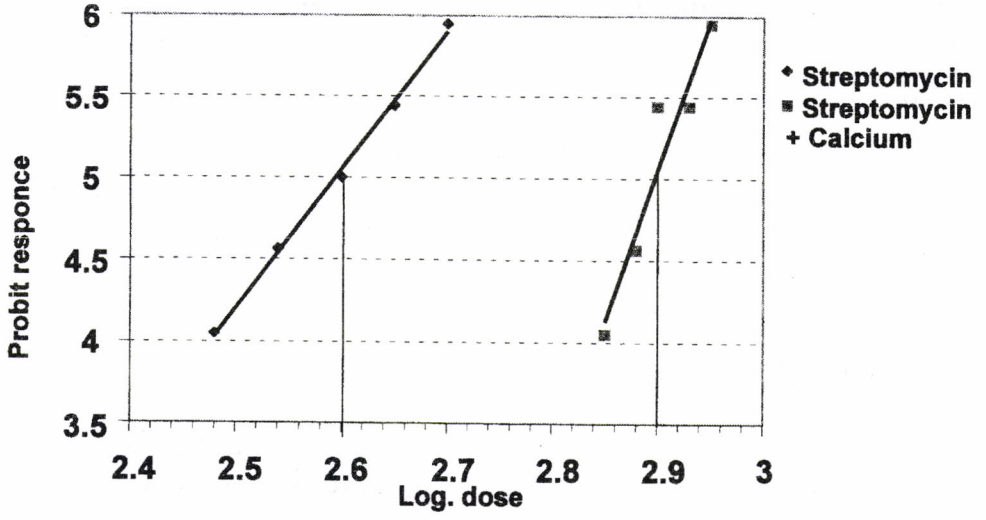

Figure 1: Acute toxicty of Streptomycin with and without calcium

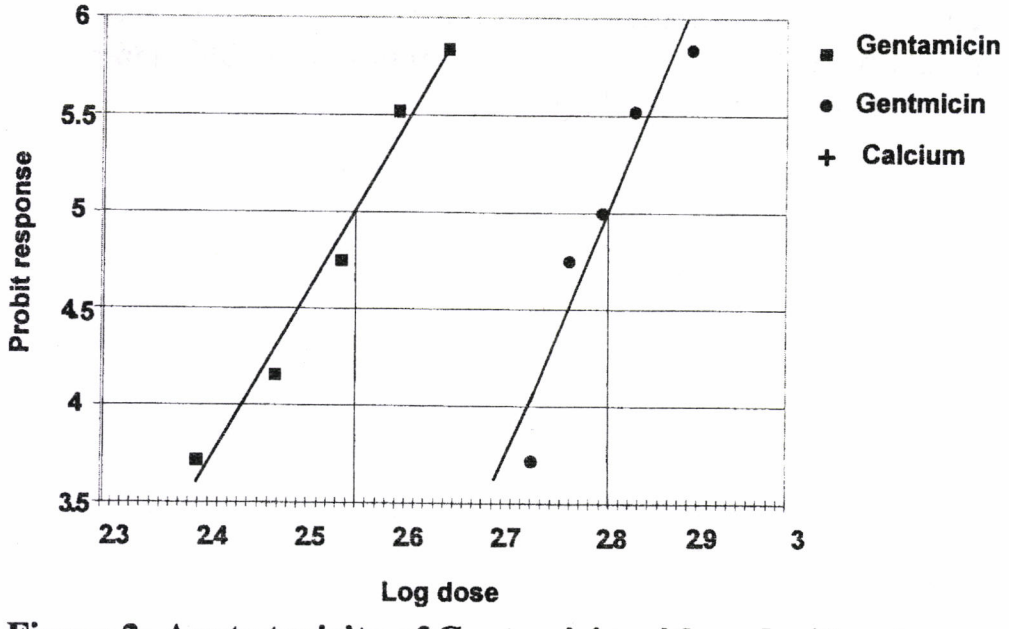

Figure 2: Acute toxicity of Gentamicin with and without Calcium

The number of animals that showed acute toxicity symptoms with their appearance and disappearance time after calcium tretment for both antibiotics are listed in table (1,2, & 3).

Table (1): Antagonizing effect of calcium for cute toxicity symptoms of streptomycin in mice.

Toxicity symptoms	Animal No. showed symptom	Appearance time (min.)	Disappearance time after treatment(min.)
General weakness	All	4-5	20-25
Incordination	All	10-12	16-19
Rapid respiration	All	12-15	12-15
Loss of righting reflex	All	16-18	6-10
Convulsion	All	19-20	-
Slow abdominal respiration	All	20-23	3-5
Paralysis	1	28	-
Death	1	30	-

Table 2 antagonizing effect of calcium for acute toxicity of gentamicin in mice

Toxicity symptoms	Animal No. showed symptom	Appearance time (min.)	Disappearance time after treatment(min.)
General weakness	All	1-3	45-50
Incordination	All	3-4	35-40
Rapid respiration	All	4-5	30-35
Loss of righting reflex	All	5-7	15-25
Convulsion	All	6-8	-
Slow abdominal respiration	All	7-9	10-17
Paralysis	1	10	-
Death	1	12	-

Table 3: Statistical comparison for times of appearance and disappearance of toxicity symptoms of streptomycin and gentamicin.

Toxicity symptoms	Appearance time (X ± SE)		disappearance time (X ± SE)	
	Streptomycin	gentamicin	Streptomycin	gentamicin
General weakness	4.4 ± 0.75	2.0 ± 1.0	22.6±2.7	47.0 ± 2.8c
Incordination	11.0 ± 1.42	3.4 ± 0.78	17.4±1.63^{B}	37.4 ±2.96c
Rapid respiration	13.4 ± 1.6	4.6 ± 0.8	13.2±1.4^{B}	32.6 ±3.1c
Loss of righting reflex	16.8 ± 1.2	6.2±1.2	7.8±2.0^{C}	20.0 ±5.7
Convulsion	19.4 ± 0.78	6.8±1.1	-	-
Slow abdominal respiration	21.6 ±1.62	8.0±1.0	3.8 ±1.1^{C}	14.4 ±3.8a
Paralysis		-		
Death		-		

(A) P< 0.05 (B) P< 0.01 (C) P< 0.001

Discussion

The result of experiment I showed that there was a shift in lethality slope of calcium treated group from that of streptomycin alone, this indicate a decline nearly one time in acute toxicity since LD_{50} value of the second group increase to 794 mg/Kg in comparison with that of the first group 397 mg/kg (figure 1).

The shifting to the right of the second group slope indicates a competitive inhibition of streptomycin by calcium which could be attributed to the similarity in charges of both substance (both are cationic) and sharing the same binding site which creates competition

for transport through the cell membrane of target organs and tissues [8, 9].

Nearly similar results were obtained for gentamicin sulfate acute toxicity as that reported by Ahmed J.N.[10] . Both experiment showed a decline in gentamicin acute toxicity nearly to the half, since LD_{50} value of the calcium treated group was 661 mg/Kg and for the gentamicin control group was 363 mg/Kg (figure 2). While Ahmed [10] who used the same dose and treatment technique for gentamicin but used calcium borogluconate instead of calcium gluconate ate same dose reported LD_{50} of 654 and 355 mg/kg body weight for the same groups respectively.

It was noticed by comparing acute toxicity slopes of the two aminoglycoside with and without calcium (figure 1&2), that higher dose was needed for streptomycin to begin lethally but with steeper lethality slope in comparison with that of gentamicin. Which make us conclude that streptomycin is less potent but more efficacious as a toxic agent than gentamicin and calcium offer quantitatively same protective level (nearly one) for both aminoglycosides.

It was reported that the use of aminoglycoside in toxic doses [11] as well as therapeutic doses [1,7] lead to hypercalcuria and hypercalcemia in human and animals [1,7,11].

Calcium antagonizing effect was attributed to the compensation for its deficiency at the cellular site of the kidney, ear and muscles and the repair of the damage caused by aminoglycoside toxic effect [12].

The results of the experiment II showed that toxicity symptoms of streptomycin appeared with delay but this disappear after calcium therapy earlier than that of gentamicin with significance differences ($P<0.05$, $P<0.01$, $P<0.001$) taking in consideration the lethal doses of both drugs were used that gave similar mortality percentage in mice. This result come in agreement with those of other studies that reported lesser accumulative affinity of streptomycin in kidney cortex and inner ear resulting in lower nephrotoxic and ototoxic affect than gentamicin [13,14].

The present study showed that streptomycin acute toxicity was qualitatively more responsive to calcium therapy than gentamicin probably because of its lower binding affinity than gentamicin making it more easily displaced by calcium, thus decreasing it cellular concentration and toxicity in target organs and tissues. From these results, one can conclude that calcium offer more qualitative, but not quantitative, protection against streptomycin acute toxicity than against gentamicin by providing faster recovery time for streptomycin toxicity symptoms.

Further studies are required to enable comparison of antagonizing effect of calcium against the side effect of different amionglycosides making it possible to formulate new amionglycosides-calcium preparations with lower toxicities.

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