Comparative Toxicological Studies between Amikacin and Tobramycin Given Alone or with Calcium Gluconate in Rabbits

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Abstract

This study consists of two experiments conducted to explore a toxicological comparison between tobramycin sulphate and amikacin sulphate, and to explore the role of calcium gluconate in antagonizing or reducing the main toxic side effects of tobramycin and amikacin like nephrotoxicity, ototoxicity and neuromuscular blockade. The first experiment was designed to study and measure the acute toxicity of these drugs by using the Up and Down method, which showed that the value of intravenous LD₅₀ of both drugs increased almost twice as much from 154.8 to 294.8 mg/kg for tobramycin and from 437.1 to 737.1 mg/kg for amikacin after the pretreatment with calcium gluconate. The second experiment was performed on rabbits injected with selected toxic dose considered as LD₅₀ of tobramycin and amikacin given by intravenous IV route for the observation of the neurotoxicity signs and recording their appearance time, until the observation of certain definite neurotoxic signs such as loss of righting reflex. At that time, calcium gluconate was given by slow intravenous infusion (over 5-10 minutes) at a high range of therapeutic dose 45mg/kg and the time of disappearance of neuromuscular blockade signs with the number of life and dead animal were recorded for each treatment. The results of this experiment showed that although tobramycin was more potent in inducing neurotoxic effects according to the dose used but amikacin is seems to be more efficacious in developing neurotoxic signs response according to it is short appearance and disappearance time of toxic signs after amikacin and calcium treatment, in comparison with that of tobramycin and calcium treatment.

Introduction

Aminoglycoside (AG) antibiotics are highly potent bactericidal antibiotics with many desirable properties that have been used in hospital settings for many years, alone or in combination with other antibiotics, to combat severe aerobic gram-negative bacterial infections caused by resistant microorganisms to less toxic antibiotics. Amikacin has the broadest spectrum of activity and was developed from kanamycin in 1976 and it is the first less toxic alternative to the older aminoglycosides. Tobramycin is an aminoglycoside obtained from cultures of *Streptomyces tenebrarius* it was approved by the FDA in 1975(1,5). There are abundant evidences that repeated administration of all aminoglycoside such as amikacin and tobramycin to experimental animals results in increased urinary excretion of divalent cations.

Hypercalciuria is the most frequently reported finding, (2,9,16).

Neuromuscular Blockade:

Amikacin and tobramycin are capable of causing neuromuscular blocking effect and result from interfering with acetylcholine release (presynaptic) and/or reducing response to acetylcholine (postsynaptic) (4). Death occurs due to neuromuscular blocking and respiratory paralysis has been reported in cats receiving high dose of amikacin, this indicate presence of strong relation-ship between neuromuscular blocking effect and acute toxicity represented by LD₅₀. (7)

Mechanism of Neuromuscular Blockade:

These drugs interfere with calcium ions movement through the calcium channels of the membrane of motor nerve ending inhibiting acetylcholine release at the synaptic cleft.(16). In a study done by (3)

they did a comparative evaluation of neuromuscular blocking effect between amikacin and tobramycin by using of sciatic nerve-gastronemius muscle and the results showed that tobramycin potency was higher than amikacin in inducing this effect. Clinical signs of intoxication included convulsions, prostration, polydipsia, dyspnea and ataxia, dogs exhibited muscle tremors, salivation, and anorexia (9). In a study undertaken by (8), indicated the variation in the tolerance of mice to a single lethal dose of amikacin. Female mice placed in cages providing constant thermal conditions and lighted for 8-20 hrs were given a single intraperitoneal injection of 1.6 to 1.9 g/kg amikacin in different times over the 24 hours (8h, 14h, 20h, and 2 h). The number of the dead mice was determined every day, for seven consecutive days. The mortality rate was influenced by time of administration of amikacin and toxicity exhibited a peak at 2 h (mean 60%) and (mean 47.7%) at 14 hr. Clinical signs of intoxication in rodents included convulsions, prostration, polydipsia, dyspnea and ataxia. Dogs exhibited muscle tremors, salivation, and anorexia. Histopathological examination of kidneys from dogs died up to 13 days after dosing revealed necrosis of the proximal convoluted tubule (9, 15).

Biological Relationship between Aminoglycoside and Calcium:

The aminoglycoside antibiotics, such as amikacin and tobramycin, have the potential to induce disturbances of electrolyte homeostasis characterized by hypomagnesaemia and hypocalcaemia (11). These studies have all documented inappropriately high levels of urinary electrolyte excretion for the prevailing low plasma concentrations, indicating a failure of the kidneys to adequately conserve cations, especially calcium and magnesium. There is abundant evidence that repeated administration of gentamicin to experimental animals results in increased

urinary excretion of divalent cations. Hypercalciuria is the most frequently reported finding (4, 6, 13), although hypermagnesiuria has also been reported. Some reports have focused on the interaction between aminoglycosides and calcium in the kidney. (12 and 11) reported that calcium inhibits the binding of aminoglycosides to the renal plasma membranes, and that oral calcium loading protects against aminoglycoside nephrotoxicity. (14) also found that dietary calcium loading attenuates the severity of gentamicin nephrotoxicity in rats. In addition, it has been shown that the hypercalciuria induced by administration of gentamicin occurs with accompanying nephrotoxicity. In those studies that carried out by (4) to explore the role of calcium in reducing the toxic side effect of gentamicin in rats, he found that acute toxicity minimized to the half when calcium gluconate was intraperitoneally administered before the drug because the median lethal dose (LD50) changed from 345 to 645 mg/kg. He concluded that there was a parallel competitive inhibitory effect between gentamicin and calcium. In studies by (9) he concluded from all his experiments that calcium play important role in antagonizing the side effects of gentamicin when given alone or mixed with gentamicin, and had no effect on gentamicin antibacterial activity.

(15) gave calcium gluconate by intravenous route to a child (weight 20 kg and age 30 month) suffering from loss of consciousness due to neuromuscular blockade after administration of neomycin. The child completely recovered after several doses of calcium gluconate. (10) Found that the injection of calcium at a dose of 45 mg/kg subcutaneously caused increase in serum calcium level to 9.96 mmole in comparison with control group 8.39 mmole, and resulted in reduced accumulation of antibiotic in the renal cortex. In another experiment by (2) to treat

the signs of neuromuscular blockade induced by intravenous injection of gentamicin at a dose level of 100 mg/kg in rabbits, he found that calcium gluconate by IV at a dose of 45mg/kg lead to reversion of this effect absolutely within minutes in comparison with the control group which were untreated with calcium and died within minutes.(3) Worked on rats isolated sciatic nerve- gastronemis muscle. They showed that the neuromuscular blocking

potency of tobramycin greater than amikacin and this side effect can be reversed by calcium.(14) Carried out a study on guinea pig intestinal smooth muscle by using much kind of aminoglycosides such as gentamicin, amikacin, tobramycin and neomycin. They showed that all aminoglycoside antagonize in a competitive manner the contractile response elicited by calcium ions on guinea pig ileum.

Materials and Method

Drugs:

1. Amikacin Sulphate:

Manufactured and marketed by Kee Pharma Ltd. New Delhi (India) 500 mg/2ml.

2. Tobramycin Sulphate:

Manufactured by Ibn Hayyan Pharma Ltd. Homs - Syria. 80 mg/2ml.

3. Calcium Gluconate:

Manufactured by Sina Darou Pharma LTD. Tahrán (Iran). Concentration 10% and each ampoule contain 10 ml.

First Experiment:

Acute Toxicity of Amikacin and Tobramycin and their Combination with Calcium in Rabbits:

Thirty-two male rabbits were used. They were divided according to drug treatment (8 rabbits in each treatment), in which one rabbit was used for each dose. Different lethal doses of both amikacin and tobramycin were used by intravenous route, while calcium administration was carried out by intraperitoneal route about 15 minutes prior to amikacin or tobramycin administration. Lethality was recorded for each group after 24 hrs and LD₅₀ were calculated according to the Up and Down method (Dixon 1980).

Up and Down Method:

This test calls for dosing individual animals in sequence singly at 24 hrs intervals, with the initial dose set at (the toxicologist best estimate of LD₅₀) following each death or moribund state, the dose is lowered, following each survival, it is increased according to pre specified dose progression

factor. If death follows an initial direction of increasing doses of 10-20% or a survival follows an initial direction of decreasing dose with the same ratio. These additional animals are tested by the Up and Down following the dose adjustment pattern.

The LD₅₀ is calculated using the following equation (17)

$$LD_{50} = XF + Kd$$

XF= last dose administered

K= value from Dixon table in appendix (1)

d= difference between dose levels.

This experiment consists of 4 groups divided according to the type of treatment:

1. Amikacin sulphate: given by intravenous infusion, one rabbit used for each dose in sequence.

2. Tobramycin sulphate: given by intravenous infusion, one rabbit used for each dose in sequence.

3. Amikacin and calcium: pretreatment with therapeutic dose of calcium gluconate (22.5 mg/kg) by intraperitoneal route and then given amikacin sulphate by intra venous infusion.

4. Tobramycin +calcium: pretreatment with therapeutic dose of calcium gluconate 22.5mg/kg by intraperitoneal route and then given tobramycin sulphate by intra venous infusion.

Procedure of Intravenous Infusion:

One of the rabbit's marginal ear veins was made clearly obvious and prominent by shaving off the hair covering it and pressing on the vein down stream to block the flow of blood temporarily. A 27 gauged butterfly

canulla was attached to polyethylene tube and a syringe, was inserted in to the marginal ear vein and secured in the place

by tape. This procedure of cannulation was used in acute toxicity and in neuromuscular blockade experiment.

Appendix (1): Table of K value according to the Up and Down acute toxicity results.

Second part of serial	+ K represents serial results as follows				
	O	OO	OOO	OOOO	
XOOO	-0.157	-0.154	-0.154	-0.154	OXXX
XOOX	-0.878	-0.861	-0.860	-0.860	OXXO
XOXO	0.701	0.737	+0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.186	OXOO
XXOO	0.305	0.372	0.380	0.381	OOXX
XXOX	-0.305	-0.169	-0.144	-0.142	OOXO
XXXO	1.288	1.500	1.544	1.549	OOOX
XXXX	0.555	0.896	0.985	1.007	OOOO
	X	XX	XXX	XXXX	Second part of serial
	- K represents serial results as follows				

$$LD_{50} = X_f + K_d$$

(Dixon, 1980)

Second Experiment: Neuromuscular Blockade

This experiment was performed on rabbits, and both drugs, amikacin and tobramycin, were given by intravenous infusion (slow infusion) until the signs of neuromuscular blockade which are represented by the head and ears dropping as critical signs of paralysis. Five rabbits were used for each treatment with selected toxic dose of amikacin and tobramycin according to their LD₅₀ and route used, (437.1 mg /kg) and (154.8mg/kg) for both

drugs respectively. These treatments were used for observation of the of neurotoxic signs and recording their appearance time, until the observation of certain definite neuromuscular toxic sign represented by loss of righting reflex at that time calcium gluconate were given by intravenous infusion at high range of therapeutic dose 45 mg/kg. The time of appearance and disappearance of neuromuscular blockade signs with the number of life and dead animals were recorded for each treatment.

Results

First Experiment:- Acute Toxicity

Table (1) Result of acute toxicity of amikacin sulphate given by IV alone and in combination with calcium gluconate in rabbits according to the Up and down method

Treatment	Animal used	Dose Range	Difference between doses	Results after 24 hrs	LD50 Mg/kg
Amikacin sulphate	8	250-450	50	ooooxoxo	437.1
Amikacin sulphate+Calcium gluconate	8	600-750	50	ooooxoxo	737.1

O = Survival X = Death

Table (2) Result of acute toxicity of tobramycin sulphate given by IV alone and in combination with calcium gluconate in rabbits according to the Up and Down method

Treatment	Animal used	Dose Range	Difference between doses	Results after 24 hrs	LD50 Mg/kg
Tobramycin sulphate	8	80-160	20	ooooxoxo	154.8
Tobramycin sulphate+Calcium gluconate	8	220-300	20	ooooxoxo	294.8

O = Survival X = Death

Second experiment: Neuromuscular blockade:-

Table (A) Time of appearance and disappearance of neuromuscular blockade signs after amikacin sulphate and calcium treatment in rabbits.

Treatment	Animal Number	Toxic Signs	Time of Appearance	Time of Disappearance
Amikacin sulphate IV	5 rabbits	Abdominal respiration	1.60 min	3.1 min
		Head shaking	2.10 min	4.8 min
		Strong body muscle Contraction	3.11 min	6.5 min
		Head and ear dropping	4.58 min	8 min
Calcium gluconate	2 Animals recovered	Loss of right reflex	5.30 min	10min
	3 animals died	Respiratory difficulty and death		Total Recovery after 11 min

Table (B) Time of appearance and disappearance of neuromuscular blockade toxic signs after Tobramycin sulphate and calcium gluconate treatment in rabbits.

Treatment	Total animal number	Toxic Signs	Time of appearance In minutes	Time of disappearance In minutes
Tobramycin sulphate IV	5 animals	Abdominal respiration	4.12 min	6.5min
		Heavy head	5.13 min	8.9 min
Calcium gluconate IV	2 animals recovered	Hind limb paralysis	6.1 min	12.5 min
		Head and ear dropping	7.6 min	20.8min
		Loss of right reflex	10.2 min	23.2 min
	3 animals died	Respiratory paralysis and death		Total recovery after 25 min

Discussion

The results of the **acute toxicity experiment** showed that the value of IV LD₅₀ of amikacin was more than that of tobramycin, which means that amikacin is safer than tobramycin, while a pretreatment with calcium gluconate led to decrease the acute toxicity nearly to the half since the values LD₅₀ of both drugs increased almost twice to that of their LD₅₀ values when given alone. This led to the conclusion that there is some competition between these antibiotics and calcium ions at the cellular level transport system. The results of the acute toxicity study were in accordance with studies carried out by AL-Hamiree (2) who confirmed the presence of linear and parallel-dose relationship of acute toxicity for gentamicin and calcium gluconate. In the **second experiment** the findings indicate that calcium can reverse or prevent an aminoglycoside – mediated neuromuscular blockade. Tobramycin seems to be more potent than amikacin in inducing toxic effects according to LD₅₀ results of acute toxicity. However the results of

neuromuscular blockade effect of both antibiotics showed that amikacin is more efficacious in developing response (neurotoxic signs) than tobramycin according to the time of appearance of these signs which were in short time, within few minutes, this led to the failure of calcium therapy if it is not administered at once but the successful one led to complete recovery of the rabbit and disappearance of all neurotoxic signs. The time of disappearance of these toxic signs with amikacin is much faster than tobramycin treated animal. This may be attributed to the structural differences between the two antibiotics which may lead to rapid transport of amikacin through nerve membrane that differ structurally from kidney and ear cell. In addition the mechanism of neuromuscular toxicity differ from that of nephrotoxic and ototoxic effect, since it included acetyl choline being released according to the rapidness of aminoglycoside transport through nerve cell and availability of calcium within

neuron. The results come in agreement with other studies by Al-Hamiree (2) who treated rabbits with a toxic dose of gentamicin (100mg/kg) into the ear vein by IV infusion. After one minute, the neurotoxic signs appeared which were represented by loss of balance, unsteady gait, limb paralysis, head and ear dropping, after the appearance of these signs a therapeutic dose of calcium administered and led to disappearance of these signs and the animals return to normal condition within 3 minutes. In a study by Renna *et.al.*, (3) a comparative evaluation of neuromuscular blocking effect between amikacin and tobramycin was carried out by using of sciatic nerve gastronemius muscle. They showed that tobramycin potency is higher than amikacin in inducing this effect. According to neuromuscular

blockade results, it seems that tobramycin is more potent neurotoxic drug according to the dose used but amikacin is more efficacious in developing toxic response this may be due to the differences in antibiotics and nerve cells structure as well as the mechanism of neuromuscular blockade that include acetylcholine and calcium availability. Pretreatment of these drugs with calcium gave complete protection with amikacin and complete to partial protection with tobramycin, both drugs exhibit a dose response nephrotoxicity relationship with treatment periods. From the acquired results it is recommend that calcium be given as combination treatment either alone or as new formulating drug to reduce tobramycin, amikacin or other aminoglycoside toxic side effects.

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دراسة المقارنة السمية بين الامكاسين وتوبراماسين على حده اومع كالسيوم كليكونت في الأرانب

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الخلاصة

تألفت هذه الدراسة من تجربتين:-

التجربة الأولى صممت لغرض دراسة وقياس السمية الحادة لهذه العقاقير بطريقة (up and down method) والتي اظهرت انخفاضاً ملحوظاً في السمية الحادة لكل من التوبراماسين و الاميكاسين عندما اعطيت لوحدها بالوريد او مع كلوكونات الكالسيوم المحقونة بجرعة 22.5 ملغم/كغم في الخلب ، حيث تغيرت السمية الحادة للتوبراماسين و تضاعفت تقريباً من 154.8 الى 294.8 و بالنسبة للاميكاسين من 437.1 الى 737.1 ملغم / كغم . استنتج من هذه التجربة ان للكالسيوم دور مهم في تقليل السمية الحادة الى النصف.اجريت التجربة الثانية في الأرانب حيث حقنت بجرع سامة مختارة تمثلت بالجرعة النصفية القاتلة من التوبراماسين والاميكاسين عن طريق الحقن الوريدي وبعد ظهور اعراض الغلق العصبي- العضلي المتمثلة بتدلي الاذان و الراس تم حقن كلوكونات الكالسيوم عن طريق الوريد و تم تسجيل الوقت.