BENHA VETERINARY MEDICAL JOURNAL, VOL. 25, NO. 1:256-264, SEPTEMBER 2013

EFFECT OF SPECTINOMYCIN ON ISOLATED SMOOTH AND CARDIAC MUSCLES AND NEUROMUSCULAR JUNCTIONS

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A B S T R A C T

The pharmacodynamic effects of spectinomycin on smooth muscles were investigated in isolated organs (gastrointestinal, cardiovascular, uterine and tracheal smooth muscles). As the effect of graded increased concentrations of spectinomycin on isolated smooth muscles showed inhibitory effect. Spectinomycin in the tested concentrations produced a dose-dependent negative inotropic effect on isolated rabbit's heart and guinea pig's auricles. Spectinomycin in all tested concentrations did not induce any effects on the isolated guinea pig's tracheal chain and rabbit's aortic strips. Neuromuscular blockade effect of spectinomycin was investigated on isolated frog's gastrocnemius muscles and rectus abdominis muscle preparations. It was concluded that, spectinomycin directly inhibited smooth muscles of gastrointestinal tract and depressed those of uterus in various stages of sex cycle as well as cardiac muscle. Spectinomycin might act directly to induce neuromuscular blockade. It was concluded that spectinomycin scarcely any pharmacological properties which might be lead to severe adverse reaction in clinical use.

Keywords: Cardiac muscles, neuromuscular junctions, smooth muscles, spectinomycin.

(BVMJ-25(1):256-264, 2013)

1. INTRODUCTION

Spectinomycin is an aminocyclitol antibiotic. In veterinary medicine, it is used therapeutically for bacterial respiratory and enteric infections [1]. It is a broad spectrum antibiotic with a bactericidal activity which based on its ability to inhibit protein synthesis in the 30s ribosomal sub-unit of the cell. It is administered to cattle, pigs, sheep and poultry as injectable solutions, orally as aqueous solutions or in feed. Spectinomycin is an antibiotic that is active against a variety of aerobic Gram-negative and Gram-positive organisms as well as Mycoplasma species [2]. Therefore, the purpose of this study was to investigate the pharmacodynamic effects of spectinomycin on smooth, cardiac and skeletal muscles.

2. MATERIALS AND METHODS

2.1. Materials:

2.2. Drug:

Spectinomycin (Spectam®, Amon) is an aminocyclitol antibiotic produced by Streptomyces spectabilis. It is used in human medicine for the treatment of uncomplicated gonorrhoea. In veterinary medicine, spectinomycin is used therapeutically for bacterial respiratory and enteric infections. Spectinomycin is administered singly or in combination with other antibiotics to cattle,

pigs and poultry by injectable solutions and orally as aqueous solutions or in feed [3].

2.3. Laboratory animals:

Guinea pigs of both sexes and different weight (300-450 gm) were used for investigating the effect of spectinomycin on the isolated ileum, auricles and tracheal chain smooth muscle. Rabbits of both sexes and different weight (1500-4000 gm) were used for studying the effect of spectinomycin on isolated duodenum, heart, aortic strip. Rats of both sexes and different weights (150-220 gm) were used for studying the effects of spectinomycin on isolated colon, fundic strip, uterine muscle in different stage of sex cycle and phrenic nerve hemidiaphragm. Egyption toads were used for studying the effect of spectinomycin on isolated rectus abdominis muscle and sciatic nerve gastrocnemius muscle preparations.

2.2. Methods:

2.2.1. Studying the effect of spectinomycin on the isolated ileum of guinea pigs [4].
2.2.2. Studying the effect of spectinomycin on isolated rabbit duodenum, rat's colon and uterine muscle of rats in various stages of sex cycles [5].
2.2.3. Studying the effect of spectinomycin on rat's fundic strip [6].
2.2.4. Studying the effect of spectinomycin on isolated guinea pig's tracheal smooth muscle using the glass jar bath apparatus [7].
2.2.5. The glass jar bath was used for studying the effect of spectinomycin on isolated guinea pig's auricles [8].
2.2.6. Gunn's apparatus (heart infusion assembly) was used for studying the effect of spectinomycin on rabbit's heart [9].
2.2.7. Studying the effect of spectinomycin on rabbit's aortic strip [10].
2.2.8. The effect of spectinomycin on the frog's gastrocnemius muscle-sciatic nerve preparation and on the isolated frog's rectus abdominis muscle were investigated [11].

3. RESULTS

The effect of graded increased concentrations of spectinomycin on the contractility of guinea pig's ileum, rabbit's duodenum, rat's colon and rat's fundic strip and guinea pig's tracheal chain are recorded in the table 1. The effect of spectinomycin on the uterine motility of rats at various stages of sex cycle was presented in table 2. Trials were performed to locate the site of action of spectinomycin on the gastrointestinal motility and the results showed that spectinomycin had a direct intestinal smooth muscles inhibition effect and had an antihistaminic like effect on rat fundic strip. Spectinomycin exerts its depressant effect on uterine muscles in non estrous, estrous, early and late pregnancy stages of sex cycle which revealed to a direct effect of spectinomycin on uterine motility as shown in figure (1 A,B). Spectinomycin depressed the isolated guinea pig's auricles, rabbit's heart figure (2) and this a negative inotropic effect of spectinomycin was not referred to β1 adrenergic blocking effect as adrenaline (1 µg/ml bath) was able to produce its cardiac stimulatory effect in the presence of spectinomycin (1280 µg/ml bath). Spectinomycin (1280µg/ml bath) was able to produce its inhibitory effect in the presence of atropine sulfate (0.1 µg/ml bath). Spectinomycin had a dose-dependent neuromuscular blocking effect on isolated frog's gastrocnemius muscle, which was nearly similar to the action of procaine. The effect of graded increased concentrations of spectinomycin on isolated frog's rectus abdominis muscle had a neuromuscular blockade in the presence of acetylecholine.

Table (1): The effect of spectinomycin on isolated guinea pig's ileum, rabbit's duodenum, rat's colon, rat's fundic strip and guinea pig's tracheal chain.

Concentrations µg/ml bath	Responses of			
	Guinea pig's ileum	Rabbit's duodenum	Rat's colon	Guinea pig's tracheal chain
10	No effect	No effect	No effect	No effect
20	No effect	No effect	No effect	No effect
40	No effect	No effect	No effect	No effect
80	No effect	Slight inhibition in the force	Slight inhibition in the force	No effect
160	Slight inhibition in the force	Slight inhibition in the force	Slight inhibition in the force	No effect
320	Slight inhibition in the force	Marked inhibition in the force of contractions	Marked inhibition in the force of contractions	No effect
640	Marked inhibition in the force and rate of contractions	Marked inhibition in the force and rate of contractions	Marked inhibition in the force and rate of contractions	No effect
1280	Marked inhibition in the force contractions	Complete relaxation	Complete relaxation	No effect
2560	Complete relaxation	*----------	----------	No effect

* (----) = Not done.

Table (2): Effect of spectinomycin on uterine motility of rats at various stages of sex cycle.

Concentrations (µg/ml bath)	Response of uterine motility			
	Non estrus	Estrus	Early pregnant	Late pregnant
10	No effect	No effect	No effect	No effect
20	No effect	No effect	No effect	No effect
40	Slight inhibition in the force	No effect	Slight inhibition in the force	Slight inhibition in the force
80	Moderate inhibition in force and frequency	Moderate inhibition in force and frequency	Marked inhibition in force and frequency	Marked inhibition in force and frequency
160	Complete relaxation	Complete relaxation	Complete relaxation	Complete relaxation

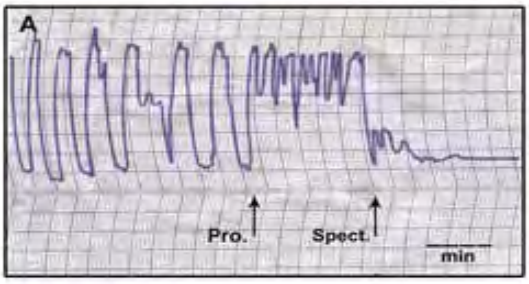

Figure 1 (A): Site of action of spectinomycin (Spect.) on isolated rat's uterus during early pregnant stage, 1 µg/ml bath propranol (Pro.) followed by 160 µg/ml bath spectinomycin (Spect.).

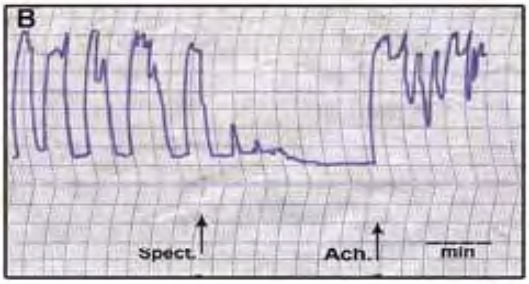

Figure 1 (B): Site of action of spectinomycin (Spect.) on isolated rat's uterus during early pregnant stage. 160 µg/ml bath of spectinomycin (Spect.) followed by 0.25 µg/ml bath of acetylcholine (Ach.).

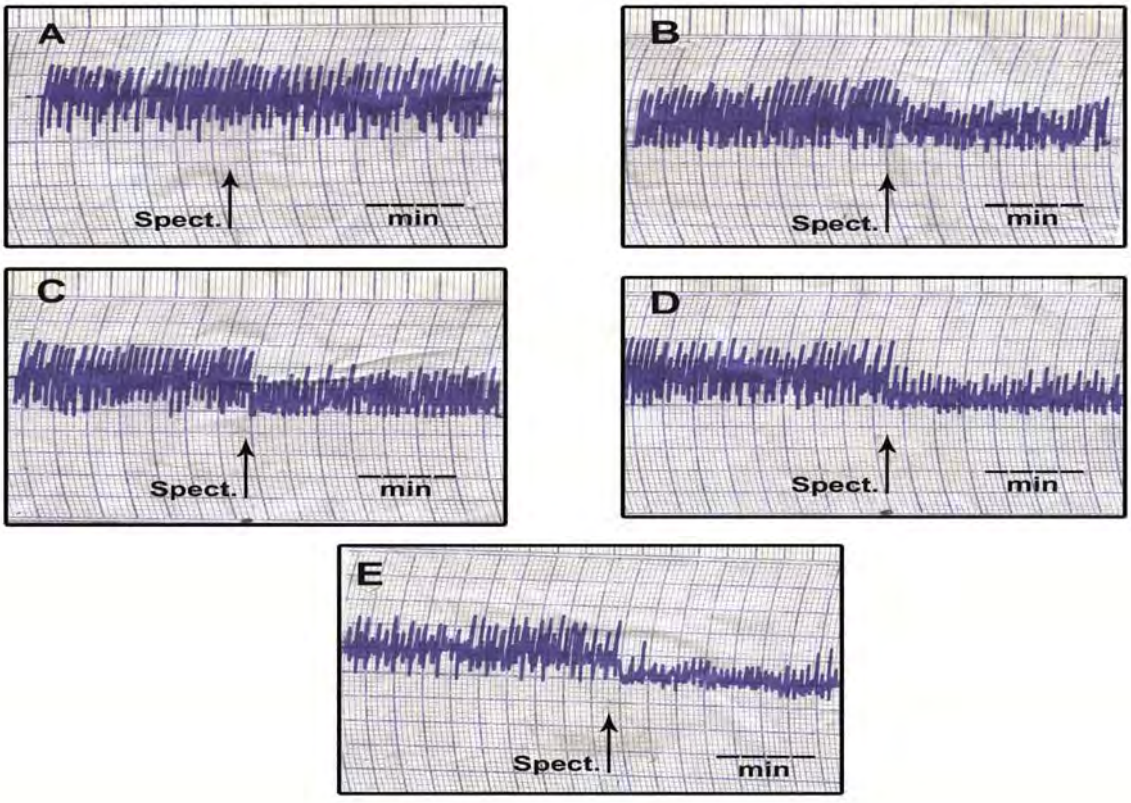

Figure (2): Effect of spectinomycin (Spect.) on isolated guinea pig's auricles.

(A) 80 µg/ml bath spectinomycin (Spect.).
(B) 320 µg/ml bath spectinomycin (Spect.).
(C) 640 µg/ml bath spectinomycin (Spect.).
(D) 1280 µg/ml bath spectinomycin (Spect.).
(E) 2560 µg/ml bath spectinomycin (Spect.).

4. DISCUSSION

The present investigation showed that spectinomycin in-vitro inhibited the contractility of guinea pig's ileum, rat's colon and rabbit's duodenum. This inhibitory effect of spectinomycin was proportional to the tested concentration. Addition of acetylcholine as a cholinergic agonist, nicotine sulfate in small concentration as ganglionic stimulant to rabbit's duodenum, guinea pig's ileum and rat's colon caused marked stimulation in the presence of spectinomycin. Moreover spectinomycin induced its intestinal relaxant effect even after complete blockage of α-adrenergic receptors by phentolamine. These results proved that the inhibitory effect of spectinomycin on such smooth muscle seemed to be mostly through a direct effect on the intestinal smooth muscle i.e myogenic effect. These obtained results were similar to those found that eight aminoglycoside antibiotics exerted inhibitory effect on the motility of the intestine and only quantitative difference existed between them [12]. Such inhibitory effect was not exerted through the adrenergic receptors and the normal pendular movements of the intestine were restored by calcium. Also, these results were explained through a competition of aminoglycosides with calcium ions for binding sites of a transport system which carries the activator ion to myoplasm which cause reduction of calcium passage to cell [13]. Spectinomycin inhibited the uterine motility during non-pregnant stages (estrus, non-estrus) and pregnant stages (early as well as late stages) and its effect was dose dependent. These effects might be attributed to the direct action of spectinomycin on the isolated uterus. This conclusion came from that addition of acetylcholine (0.25µg/ml bath) produced its stimulatory effect in the presence of spectinomycin, and spectinomycin produced its relaxant effect on the uterus after its stimulation with 1µg propranolol/ml bath. This direct effect of spectinomycin can be attributed to its modulatory effect on Ca++ which explained that aminoglycosides antibiotics affect superficially bound extracellular calcium only and calcium not only had the ability to restore the normal contractility of the uterus but also exert protective action against the inhibitory effect of aminoglycoside antibiotics of the contractility of rat's uterus where the aminoglycoside antibiotics compete with calcium ions for the specific calcium ions binding sites on the cell membrane [14]. The mechanism by which aminoglycoside antibiotics produced uterine relaxation has many similarities to the mechanism by which they produced neuromuscular blockade [15] relaxation of the intestinal smooth muscle and depression of the cardiac muscle. The obtained results in the present work are consistent with those of gentamicin sulfate inhibited spontaneous, as well as oxytocin or prostaglandin f2 α-induced contractions of myometrium isolated from non-pregnant cows in a dose-dependent manner, also, both the frequency and amplitude of concentrations were significantly inhibited by gentamicin sulfate [16].

The guinea pig's tracheal smooth muscles seemed to be insensitive to the tested concentrations of spectinomycin. In presence of spectinomycin, histamine was not able to produce its stimulatory effect, thus spectinomycin blocked the action of histamine on the tracheal smooth muscles. The obtained results are consistent with results recorded that netilmicin possessed an antihistaminic effect [17]. The obtained results dissimilar with neomycin which inhibited maximal 5-hydroxytryptamine induced contraction in the guinea pig trachea and neomycin had no effect on carbamylcholine or histamine-induced contraction [18].

The obtained results in this study on the cardiovascular muscles proved that, spectinomycin had a negative inotropic effect on the isolated guinea pig's auricles and rabbit's heart. Spectinomycin produced a direct and dose dependent depression of the myocardial contractility. The inhibitory effect of spectinomycin was not referred to either ß1 adrenergic blocking effect or cholinergic stimulant effect, as adrenaline (1µg/ml bath) in guinea pig's auricles and isoprenaline (5 x 10-4 m) in rabbit's heart were able to produce their cardiac stimulatory effects in the presence of spectinomycin, and after addition of atropine sulfate, spectinomycin was able to produce its inhibitory effect. The negative inotropic effect of spectinomycin on the isolated guinea pig's auricles and rabbit's heart was similar to results stated the myocardial depressant action of aminoglycosides [19], and similar with results found that the efficacy of inhibitory action of gentamicin was reduced by increasing the external calcium ions concentration from 1.8 to 5.4 or 10.8 mmol/l, but not by the application of adrenaline and the depressant action of gentamicin was due to a blockade of slow channels, whereby gentamicin may have dislocated calcium from the binding sites at slow channels on the external surface of the membrane [20]. Also the obtained results are similar with those recorded that neomycin has cardio-depressive effects and the common action of neomycin in all systems apparently concerns the movement of calcium across cell membranes and calcium act as antagonist of the curare-like activities of neomycin and its cardio-depressive action [21]. Also the obtained results may be attributed to reduction of the cellular calcium content in a dose dependent manner. It was found that dibekacin, sisomicin and gentamicin reduced the cellular calcium content by 10-20% in a dose dependent manner where they replace calcium from lipid monolayer on the superficially bound calcium of isolated beating left atria from guinea pigs [22]. So the loss of superficially bound calcium was accompanied by a decline of the contractile force by 40-90%.The obtained results revealed that spectinomycin had no effect on the smooth muscle of aorta but it decreased contractile response produced by nor-adrenaline.

Regarding the effect of spectinomycin on skeletal muscle preparations (frog's rectus abdominus, rat's phrenic nerve hemidiaphragm and frog's sciatic nerve gastrocnemius muscle), the drug elicited a marked neuromuscular blocking activity on response to indirect muscle twitches. This refer to that, trials were performed to detect the site of action of spectinomycin on skeletal muscle preparations. The results revealed that spectinomycin did not impair the stimulatory effect of neostigmine and acetylcholine on rat's phrenic nerve hemidiaphragm preparation. Spectinomycin exhibited a local anesthetic activity on frog's sciatic nerve gastrocnemius muscle. The inhibitory effect of spectinomycin on skeletal muscle preparations might be attributed to that recorded that the inhibition of calcium ions function in nervous and non-nervous tissues has been recognized as a pharmacologic property of the aminoglycoside antibiotics where these antibiotics interfere with the uptake or binding of calcium ions by competing with cation for negative-charged and superficial binding sites on cellular membranes on fast and slow contracting muscles of cat, therefore the aminoglycoside antibiotics may be a useful pharmacological tool for studying the process involved in calcium movement across membrane in a variety of systems [23]. Also, the obtained results were similar to those recorded that tobramycin, amikacin and ribostamycin produced dose-dependent neuromuscular blockade on the rat isolated phrenic nerve-diaphragm preparation and on the sciatic nerve-gastrocnemius muscle and the neuromuscular blockade produced by

these antibiotics was reversed by calcium chloride, whereas it was not influenced by neostigmine methylsulfate.

From the present study, it could be concluded that, spectinomycin directly inhibit the smooth muscles of gastrointestinal tract, those of uterus as well as cardiac muscles [24]. Spectinomycin in all tested concentrations did not induce any effect on the resting tonus of guinea pig's tracheal chain and rabbit's aortic strip. Spectinomycin had a neuromuscular blocking activity on the skeletal muscle preparations.

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تاثير الأسبكتينومايسين على العضلات الملساء والعضلات القلبية

وتاثيره على التوصيل العضلى العصبى

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الملخص العربي

تمت دراسة التأثير الفارماكوديناميكى لعقار الأسبكتينومايسين على حركة العضلات الملساء موضحا على العضلات المعزولة حيث تمت دراسة التأثير التثبيطين التدريجي لعقار الأسبكتينومايسين فى عضلة الرحم فى الجرزان فى المراحل المختلفة من الدورة الجنسية حيث يعطى عقار الأسبكتينومايسين تأثيرا إيجابيا يعتمد على الجرعة فى العينات المعزولة من قلب الارانب واذينين الارانب الغينية. ووجد ان عقار الأسبكتينومايسين ليس له أي تأثير على العينات المعزولة من عضلة القصبة الهوائية للأرانب الغينية وشريان الأورطي فى الارانب، بينما يظهر تأثيره على التوصيل العضلي العصبي للعينات المعزولة من عضلة الساق البطنية بالعصب الوركي وعضلة البطن المستقيمة المنزوعتين من الضفادع المصرية. وخلص البحث الى ان عقار الأسبكتينومايسين له تأثير تثبيطي مباشر على العضلات الملساء للقناة المعد معوية وتأثير تثبيطي لعضلة الرحم فى مراحل الشبق وما قبل الشبق وتأثير تثبيطي لعضلة الرحم ف مراحل الحمل والعضلات القلبية وهذه النتائج تدل على ان عقار الأسبكتينومايسين له خصائص فارماكولوجية من الممكن ان تؤد الى تأثيرات مضادة فى الاستخدام الإكلينيكي.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(1)256–264 سبتمبر 2013)