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

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ABSTRACT

The pharmacodynamic effects of tilmicosin on smooth muscles were investigated in isolated organs. As the effect of graded increased concentrations of tilmicosin on isolated rat's uterine muscles was examined during various stages of sex cycles. Tilmicosin in the tested concentrations produced a dose-dependent negative inotropic effect on isolated rabbit's heart and guinea pig's auricles. Tilmicosin 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 was investigated on isolated frog's gastocnemius muscles and rectus abdominis muscle preparations. It was concluded that, tilmicosin directly stimulate smooth muscles of gastrointestinal tract and depresses those of uterus in various stages of sex cycle as well as cardiac muscles. Tilmicosin might act directly to induce neuromuscular blockade. It was concluded that tilmicosin scarcely any pharmacological properties which might be leading to severe adverse reaction in clinical use.

Key words: Cardiac muscles, neuromuscular junctions, smooth muscles, tilmicosin**.**

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1. INTRODUCTION

Tilmicosin is a broad-spectrum semi-synthetic bactericidal macrolide antibiotic stored at or below room temperature. Tilmicosin apparently concentrates in lung tissues as one dose administered in cattle be durable for at least 72 hours so it was indicated for treatment of respiratory infections associated with tilmicosin-susceptible micro-organisms such as Mycoplasma spp., Pasteurella multocida, Haemophilus somnus and Mannheimia haemolytica in cattle. Tilmicosin produces its antimicrobial and antimycoplasmal effect by binding with the 50 S ribosome subunit of the organism resulting in inhibition of microbial protein synthesis [13]. Therefore, the purpose of this study was to investigate the pharmacodynamic effects of tilmicosin on smooth, cardiac and skeletal muscles.

2. MATERIAL AND METHODS

2.1 Materials:

2.1.1 Drug:

Tilmicosin is a member of macrolide antibiotics. Its activity is attributed to the presence of a macrolide ring, which is a large macrocyclic lactone ring to which one or more deoxy sugars, usually cladinose and desosamine, may be attached. The lactone rings are usually 14-, 15-, or 16-membered [16].

Tilmicosin phosphate is supplied in strength equivalent to 250 mg. tilmicosin. (Advotil AC®, Chemvet for animal health products, Jordon).

2.2.1 Laboratory animals :

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

2.2 Methods:

The method described by Valeri et al. [20] was used for studying the effect of tilmicosin on the isolated ileum of guinea pigs. The method described by Staff members of Pharmacology Department, University of Edinburg [17] was used for studying the effect of tilmicosin on isolated rabbit duodenum, rat's colon and uterine muscle of rats in various stages of sex cycles. The method described by Milenov and Kalfin [5] was used for studying the effect of tilmicosin on rat's fundic strip. The method described by Schlemper and Calixto [15] was used for studying the effect of tilmicosin on isolated guinea pig's tracheal smooth muscle using the glass jar bath apparatus. The glass jar bath was used as described by Vasconcelos et al. [21] for studying the effect of tilmicosin on isolated guinea pig's auricles. The method explained by Hondeghem and Hoffman [11] using Gunn's apparatus (heart infusion assembly) was used for studying the effect of tilmicosin on rabbit's heart. The method explained by Furchgott [3] was used for studying the effect of tilmicosin on rabbit's aortic strip. The method described by Barlow et al. [1] was used for investigating the effect of tilmicosin on the frog's gastrocnemius muscle-sciatic nerve preparation. The effect of tilmicosin on the isolated frog's rectus abdominis muscle was investigated by using the method described by Staff members of Pharmacology Department, University of Edinburg [4].

3. RESULTS

The effect of graded increased concentrations of tilmicosin 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 Table 1. The effect of tilmicosin 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 tilmicosin on the gastrointestinal motility and the results showed that tilmicosin had a direct intestinal smooth muscles stimulant effect and had an antihistaminic like effect on rat fundic strip. Tilmicosin exerts its depressent effect on uterine muscles in non estrous, estrous, early and late pregnancy stages of sex cycle which revealed to a direct effect of tilmicosin on uterine motility as shown in figure (1 A,B). Tilmicosin depressed the isolated guinea pig auricles, rabbit's heart figure (2) and this a negative inotropic effect of tilmicosin was not referred to $ß_1$ adrenergic blocking effect as adrenaline (1µg/ml bath) was able to produce its cardiac stimulatory effect in presence of tilmicosin (160 µg/ml bath). Tilmicosin (160 µg/ml bath) was able to produce its inhibitory

Table 1 the effect of tilmicosin 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	rat's fundic strip	Guinea pig's tracheal chain
0.25	No effect	No effect	No effect	No effect	No effect
10	Slight stimulation in the force.	Slight stimulation in the force	Slight stimulation in the force	Slight inhibition in the force.	No effect
20	Moderate stimulation in the force	Slight stimulation in the force	Marked stimulation in the force and rate of contractions	Moderate inhibition in the force.	No effect
40	Marked stimulation in the force and rate of contractions	Moderate stimulation the force.	Marked stimulation in the force and rate of contractions	Moderate inhibition in the force.	No effect
80	Marked stimulation in the force and rate of contractions	Moderate stimulation the force.	Marked stimulation in the force and rate of contractions	Moderate inhibition in the force.	No effect
160	Maximum stimulation	Marked stimulation in the force of contractions	Maximum stimulation	Marked inhibition in the force and rate of contractions	No effect
320	----------	Marked stimulation in the force of contractions	----------	Marked inhibition in the force and rate of contractions	No effect
640	----------	Maximum stimulation	----------	Maximum inhibition	No effect

(----------) Not done

Table 2 Effect of tilmicosin 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
0.0625 → 1	No effect	No effect	No effect	No effect
5	No effect	No effect	No effect	Slight inhibition in the force
10	No effect	No effect	Slight inhibition in the force	Slight inhibition in the force
20	Slight inhibition in the force	Slight inhibition in the force and frequency	Slight inhibition in the force and frequency	Slight inhibition in the force and frequency
40	Slight inhibition in the force	Slight inhibition in the force and frequency	Slight inhibition in the force and frequency	Moderate inhibition in the force and frequency
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	Marked inhibition in force and frequency	Marked inhibition in force and frequency	Marked inhibition in force and frequency	Marked inhibition in force and frequency
320	Complete relaxation	Complete relaxation	Complete relaxation	Complete relaxation

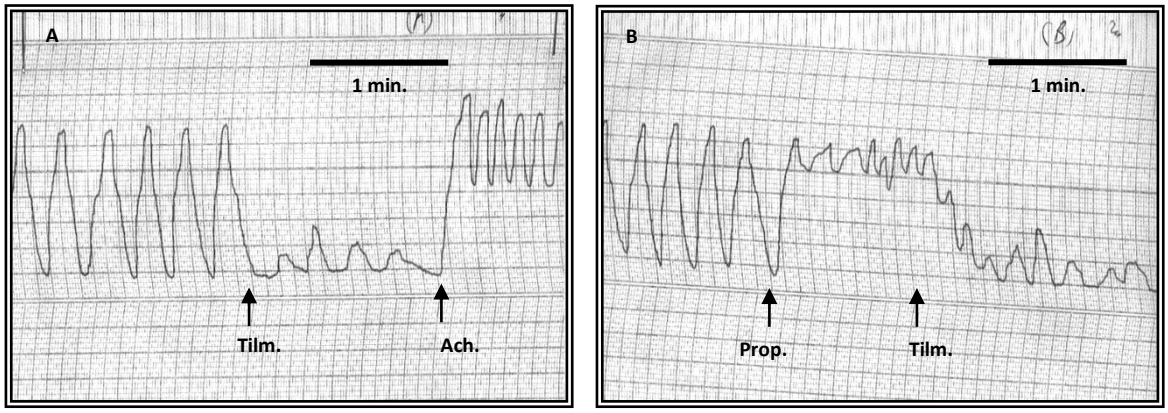

Figure 1 (A): Site of action of tilmicosin (Tilm.) on isolated rat's uterus during late pregnant stage.* 160 µg/ml bath tilmicosin (Tilm.) followed by 0.25 µg/ml bath acetylcholine (Ach.). Figure 1 (B): Site of action of tilmicosin (Tilm.) on isolated rat's uterus during late pregnant stages. * 1 µg/ml bath propranolol (Prop.) followed by 160 µg/ml bath tilmicosin (Tilm.).

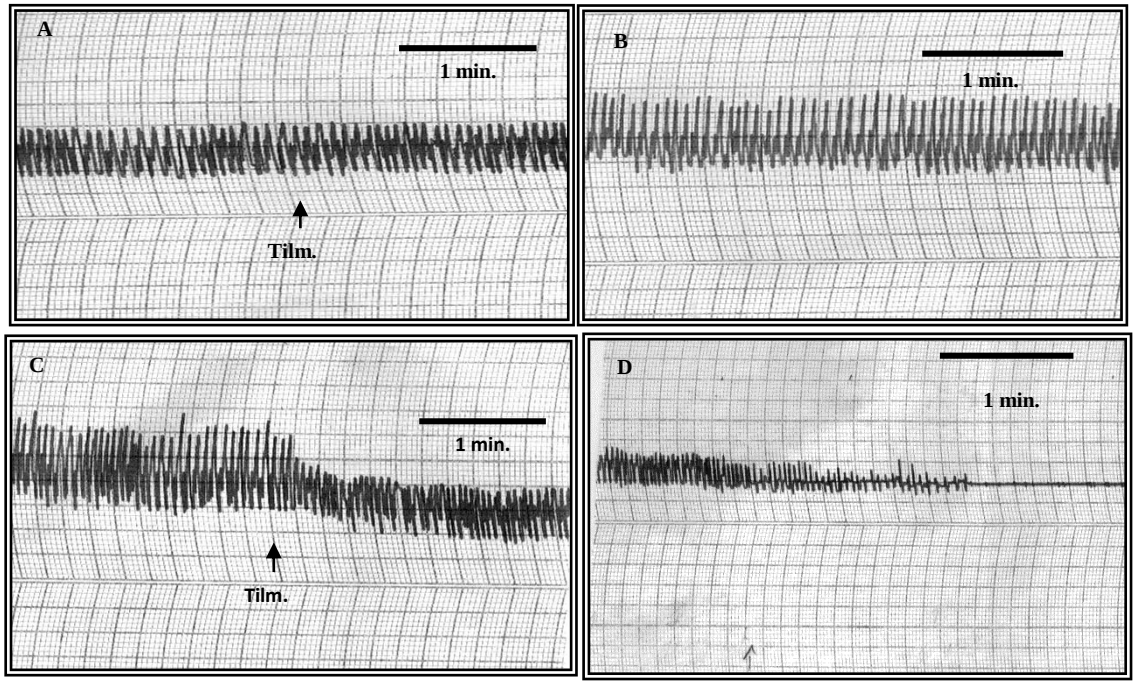

Figure (2): Effect of tilmicosin (Tilm.) on isolated guinea pig's auricles. (A) 0.5 µg/ml bath tilmicosin (Tilm.). (B) 40 µg/ml bath tilmicosin
(Tilm.). (C) 160 µg/ml bath tilmicosin (Tilm.).
(D) 640 µg/ml bath tilmicosin (Tilm.).

muscle which was nearly similar to the action of procaine. The effect of graded increased concentrations of tilmicosin on isolated frog's rectus abdominis muscle had a neuromuscular blockade in presence of acetyl choline.

4. DISCUSSION

The present investigation showed that, tilmicosin *in vitro* stimulated the contractility of guinea pig's ileum, rat's colon and rabbit's duodenum. The stimulatory effect of tilmicosin was proportional to the graded tested concentrations. Presence of atropine sulphate as muscarinic cholinergic receptor blocker and large dose of nicotine sulphate as ganglionic (Nicotinic receptor) blocker did not inhibit the stimulatory effect of tilmicosin. In addition, the adrenaline as adrenoceptor agonist produced its inhibitory effect in presence of tilmicosin. These results proved that, tilmicosin might directly stimulate the intestinal smooth muscles of rabbit's duodenum, guinea pig's ileum and rat's colon. These obtained results were similar to those obtained by [6] who investigated that the gastrointestinal motor stimulating activity of erythromycin was associated with the release of endogenous motilin in the dog. In addition [7] clarified the nature of the side effects of macrolide antibiotics on the gastrointestinal tract, the motor-stimulating activity of these agents in unanesthetized dogs. The results showed that erythromycin and oleandomycin, strongly stimulate gastrointestinal motor activity, an action accompanied by vomiting at large doses. Also [14] reported that erythromycin was a potent stimulator of gastrointestinal motor activity. *In vitro* studies suggested that it mimics motilin, a peptide that stimulated motor activity in human and in rabbit via smooth muscle receptors concluding that erythromycin is a motilin receptor agonist. Tilmicosin in vitro inhibited the uterine motility during non-pregnant stages (estrus and non-estrus) and also inhibited the uterine motility during early and late pregnant stages. The effect was dose dependant. These effects might be attributed to the direct action of the tilmicosin on the isolated uterus and these obtained results were consistent with those recorded by [4], who concluded that erythromycin produced a decrease in the pregnant rat myometrial activity in vitro, independent of the stimulant. Also [2] reported that clarithromycin inhibited myometrial contractions in isolated human myometrium independent of stimulus. The aim of this study was to investigate the effects of clarithromycin on oxytocin, prostaglandin F2α and KCl-induced contractions of human myometrium *in vitro*. In addition [12] studied the effect of gentamicin sulfate on the contractility of myometrium isolated from non-pregnant cows and noted that gentamicin sulfate cause depression of neuromuscular function, is a drug of choice in intrauterine antibiotic treatment of bovine chronical or subclinical uterine infections.

The guinea pig's tracheal smooth muscles seemed to be not affected by the action of tested concentraions of tilmicosin. In presence of tilmicosin, histamine was not able to produce its stimulatory effect, thus tilmicosin blocked the action of histamine on the tracheal smooth muscles. The obtained results in this study was opposite to those obtained by [19] who suggested that erythromycin, roxithromycin and clarithromycin may inhibit cholinergic neuroeffector transmission in the human airway smooth muscle, probably by reducing exocytotic release of acetylcholine from the nerve terminals. [18] reported that azithromycin has an antiproliferative and autophagic effect on rabbit tracheal smooth muscle cells. They concluded that azithromycin reduced the viability of human bronchial smooth muscle cells possibly by leading to apoptotic cell death.

The obtained results in this study on the cardiovascular muscles proved that, tilmicosin had a negative inotropic effect on the isolated guinea pig's auricles and rabbit's heart. Tilmicosin produced a direct and dose dependant depression of the myocardial contractility. This negative inotropic effect of tilmicosin was not referred to either $ß_1$ adrenergic blocking effect or cholinergic stimulant effect, as adrenaline (2 µg/ml

canula) was able to produce its cardiac stimulatory effect in presence of tilmicosin (640 µg/ml canula) and after addition of atropine sulphate (25 µg/ml canula), tilmicosin (640 µg/ml canula) was able to produce its inhibitory effect. Contraction of the cardiac cells is believed to be dependent upon the intracellular concentration of available calcium ions in the vicinity of the contractile apparatus. Forty male Balb/C mice were used as materials. Ten mice were used as a control group, and thirty mice were injected with tilmicosin (25 mg/kg body weight, SC, single injection) and monitored for 3 days. The results obtained in this study showed that use of tilmicosin caused temporary increases in cardiac muscle creatine kinase activity and serum total protein level in male. Also [21] studied that tilmicosin administration at doses of 50 and 70 mg/kg appears to alter selected cardiac enzymes and total sialic acid concentration indicating its cardiotoxic and strong prooxidant effect in mice. And it is opposite to what reported by [10] as they reported that cardiotoxicity has been demonstrated after both intravenous and oral administration of erythromycin but has never been reported with the newer macrolides. It was reported that a case of ventricular dysrhythmias that occurred after six therapeutic doses of clarithromycin. The dysrhythmias resolved after discontinuation of the drug. In addition, [9] concluded that erythromycin therapy was associated with prolongation of myocardial repolarization that manifests after the first few doses in a majority of patients. It was observed that, tilmicosin had no effect on the smooth muscle of aorta. In the presence of tilmicosin, nor adrenaline was not able to produce its stimulatory effect, thus tilmicosin appeared to cause an alpha adrenergic blocking effect on isolated rabbit's aortic strip.

The effect of tilmicosin on skeletal muscle preparations (frog's gastrocnemius muscle sciatic nerve and frog's rectus abdominis muscle) was investigated. The tilmicosin elicited a marked neuromuscular blocking activity in response to indirect muscle twitches, also tilmicosin exhibited a local anaesthetic activity on frog's gastrocnemius sciatic nerve preparation.

Trials were performed to detect the site of action of tilmicosin on the skeletal muscle preparations. The results showed that, tilmicosin did not impaire the stimulatory effect of neostigmine and acetylcholine on rat's phrenic nerve hemidiaphragm preparation. Therefore the neuromuscular blocking effect of tilmicosin seemed to be attributed to two mechanisms; the first might be due to local anaesthetic effect of tilmicosin which is responsible for blocking of conduction through sciatic and phrenic nerve. The second mechanism might be attributed to calcium ions antagonistic effect of the tilmicosin [8]. Calcium ions influx is necessary for acetylcholine release as well as other neurotranmittors and hormones found that, the twitch tension of gastrocnemius muscle evoked by electrical stimulation of sciatic nerve was slightly reduced following administration of clarithromycin and azithromycin respectively [22].

The neuromuscular blocking activity of tilmicosin on skeletal muscle preparations in the present work determined that, tilmicosin reduced spontaneous locomotor activity in mice [26].

Conclusion:

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

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تأثير التيلميكوزين على العضلات الملساء والعضلات القلبية وتأثيره على التوصيل العضلي العصبي.

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الملخص:

تمت دراسة التأثير الفارماكوديناميكى لعقار التيلميكوزين على حركة العضلات الملساء موضحا على العضـــلات المعزولة. حيث تمت دراســـة التأثير التزايدى التدريجي لعقارالتيلميكوزين فى عضـــلة الرحم فى الجرزان فى المراحل المختلفة من الدورة الجنســـية. حيث يعطى عقار التيلميكوزين تأثيرا ســـلبيا يعتمد على الجرعـة فى العينـات المعزولـة من قلب الأرانب وأذينين الأرانب الغينية. ووجد أن عقار التيلميكوزين ليس له أى تأثير على العينات المعزولة من عضـلة القصـبة الهوائية للأرانب الغينية وشـريان الأورطى فى الأرانب. بينما يظهر تأثيره على التوصـيل العضـلي العصـبي للعينات المعزولة من عضـلة السـاق البطنية بالعصـب الوركي وعضلة البطن المستقيمة المنزوعتين من الضفادع المصرية. وهذا يتلخص في أن عقار التيلميكوزين له تأثير تنشيطي مباشر على العضـلات الملسـاء للقناة المعد معوية وتأثير تثبيطي لعضـلة الرحم في المراحل المختلفة من الدورة الجنســية والعضـــلات القلبية. وهذه النتائج تدل على أن عقار التيلميكوزين له خصـــائص فارماكولوجية من الممكن أن تؤدى الى تأثيرات مضادة فى الاستخدام الاكلينيكى.

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