doi.org/10.36721/PJPS.2020.33.5.SP.2385-2392.1

Azithromycin induced contractile responses of intestinal smooth muscles: A mechanistic approach

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Abstract: The current work is documented to investigate the actions of azithromycin on intestinal smooth muscles as there are reports of gastrointestinal upsets with use of azithromycin. Azithromycin was tested on rabbit's jejunal and rat's ileal preparations in test concentrations (μM) of 0.01, 0.03, 0.1, 0.3, 1, 3, 5, 10 and 15μM. After mounting the tissues in organ bath containing Tyrode's solution, spasmogenic activity of azithromycin was observed. To explore its possible mechanisms, response of azithromycin was noted in the presence of 0.3μM atropine, 3μM loratadine, 0.3μM ondansetron, 10μM metoclopramide, 0.3μM verapamil, 1μM propranolol, 3μM amiodarone and combination of 0.3μM each atropine, ondansetron, verapamil and propranolol (AOVP). Mean % Emax for azithromycin was 67.6±1.6 and 54.0±2.1 (% of ACh max) for rabbit's jejunal and rat's ileal preparations, respectively. The Mean % Emax for azithromycin in the presence of various antagonists for rabbit's jejunal and rat's ileal preparations was as: 2.4±0.1 and 11.4±1.3 with atropine; 67.9±2.0 and 50.7±1.9 with loratadine; 27.5±0.5 and 34.0±2.9 with ondansetron; 88.4±1.2 and 79.1±3.8 with metoclopramide; 13.6±1.2 and 22.3±2.5 with verapamil; 10.2±2.1 and 15.6±1.4 with propranolol; 68.4±1.3 and 58.0±3.4 with amiodarone. Results reveal that the spasmogenic response of azithromycin is mainly mediated through muscarinic receptors. However, we found involvement of mixed pathway including serotonergic receptors, voltage gated calcium channels and voltage gated sodium channels.

Keywords: Azithromycin, cholinergic receptors, acetylcholine, atropine, voltage gated sodium channels, potassium channels, voltage gated calcium channels.

INTRODUCTION

Muscarinic agonists are used to treat intestinal hypomotility disorders (Ehlert *et al*., 2012). Primarily M_2 and M_3 receptors are found in intestinal smooth muscles (Gao *et al*., 2016). The main excitatory neurotransmitter is acetylcholine (ACh) at muscarinic receptors (Sanders *et al*., 2012). Histamine and serotonin are also responsible for increase in intestinal motility (Mittal *et al*., 2017, Kendig and Grider, 2015, Fabisiak *et al*., 2017). Histamine (H_1 & H_4) and serotonin (5-HT3 & 5-HT4) are other neurotransmitters which remained a focus of clinical studies for intestinal motility disorders. On the other hand, dopamine decreases the intestinal motility (Li *et al*., 2006). There are 5 sub types of dopamine (D1-5) receptors but D-2 receptors are mainly found in the intestinal tract (Ayano, 2016).

Following receptor-neurotransmitter interactions, it is evident that voltage gated ion channels (VGICs) play a key role in the regulation of intestinal motility, therefore, they have remained the main targets for the development of new drugs and interpretation of some of adverse drug reactions of the molecule under consideration. Certain receptor agonists and antagonists interact directly to transform excitatory or inhibitory signals of intestinal tract. In view of this complexity it is not surprising that our understanding regarding the mechanism of actions of different neurotransmitters and VGICs drugs which affect the gut motility is still inadequate. However, recently, considerable advancements have been attained and drug therapy for gut dysmotility is emerging (Mittal *et al*., 2017) VGICs (calcium, sodium & potassium channels) are the chief proteins that express cell excitability; muscles, neurons and secretory cells use VGICs to produce action potentials, that initiate main cellular actions including synaptic vesicle exocytosis and muscular contraction (Lipscombe *et al*., 2013).

Azithromycin (AZM) is a broad spectrum, semi synthetic antibiotic which belongs to a group of drugs known as macrolides (Martinez *et al*., 2015, Lu *et al*., 2015, Kong *et al*., 2017). It has prolong half-life (68 hours) with very good tissue penetration (McMullan and Mostaghim, 2015, Kong *et al*., 2017). It is mainly prescribed for bacterial skin, urogenital and respiratory infections (Soda *et al*., 2018, Romøren *et al*., 2012, Beyda *et al*., 2014). It is also clinically effective against non-gonococcal urethritis (Moi *et al*., 2015). Recently its use in COVID-19 infection is suggested as to combat secondary bacterial infections of the respiratory system (Hinks *et al*., 2020).

Certain studies have been conducted to evaluate the effects of AZM on the contractility of intestinal smooth muscles (Ugwu *et al*., 2013, El-Baki *et al*., 2015). Some of these have proved that AZM significantly increases the contractility of intestinal smooth muscles (Broad and Sanger, 2013). Other studies showed controversy

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regarding its effect and role in gastric motility (Nguyen 2014).

So far there are controversial reports about the effects of AZM on the intestinal smooth muscular contractions and the mechanisms involved are still unrevealed. Therefore, the current study is aimed to explore the possible effect(s) along with mechanism of action of AZM on intestinal smooth muscles.

MATERIALS AND METHODS

Drugs and other chemicals

Drugs used in the experiments were E Merck Grade unless specified: Acetylcholine (Poole, England), Azithromycin dihydrate, Atropine, Loratadine, Ondansetron, Metoclopramide, Verapamil, Propranolol and Amiodarone. The drugs were dissolved in distilled water. All solutions were freshly prepared on the same day of experiments.

Animals, experiments and data recording

This laboratory experimental study was performed at Institute of Basic Medical Sciences, Khyber Medical University (KMU), Peshawar after the ethical approval (Dir/KMU-EB/MU/000745) from the Khyber Medical University (KMU) Ethical Board. Rabbits were purchased from local market while rats were brought from National Institute of Health (NIH), Islamabad. The animals were kept for 1 week of acclimatization before the start of experiments at the "Animal house of Institute of Basic Medical sciences, Khyber Medical University" under standard protocols outlined in the "Animals Bye-Laws 2008 of the University of Malakand (Scientific Procedures Issue- 1) (Niaz *et al*., 2009). They were given standard diet and tap water. The animals were fasted 24 hours before the experiments with free access to water. Male and female New Zealand rabbits having 2-2.5kg body weight and Wistar rats having weight 180-220 grams were included in the study. Those animals which were found to be pregnant or in diseased state, were excluded from the study.

In-vitro Experiments

For all the *in-vitro* experiments the concentration response was recorded on rabbits' jejunal and rats' ileal preparations.

Rabbits' jejunal and Rats' ileal Preparations

The abdomen of each rabbit and rat after scarifying was opened through a midline incision. 1-1.5cm pieces of jejunum and ileum were removed from each rabbit and rat respectively (Nath *et al*., 2016, Ali *et al*., 2011). Pieces of isolated jejunum and ileum were mounted individually in an organ bath containing Tyrode's' solution, at 37±1°C, aerated with carbogen gas (a mixture of 95% O_2 and 5% CO_2) (Ghayur *et al*., 2012). The composition of the Tyrode's solution was as: NaCl 8, KCl 0.2, $MgCl_2$ 0.1, $CaCl_2$ 0.2, $NaHCO_3$ 1, NaH_2PO_4 0.05, Glucose 1 gm/liter (Chiragh *et al*., 2006). Each jejunal tissue after mounting was allowed to equilibrate for 30 min (Faisal *et al*., 2018). Those jejunal tissues in which spontaneous contractions were observed were included in the study.

Effect of azithromycin on intestinal preparations of test animals

The spasmogenic effect of AZM was evaluated on rabbits' jejunal and rats' ileal preparations. AZM was added cumulatively in doses of 0.01, 0.03, 0.1, 0.3, 1, 3, 5, 10, 15µM (final bath concentrations). Response for each dose was recorded for 2 minutes. AZM was tested in similar concentrations for the next series of experiments for exploring its mode of action. As intestinal tissues are predominant with muscarinic receptors, hence we quantified the spasmogenic response as % of acetylcholine response. The contraction of isolated tissue preparation was expressed as percent of the control response produced by ACh (3µM). Briefly describing, the tissues were pre-treated with specific agonist and antagonist of target receptors or its ion channel blockers. The concentrations at which the receptor and channel blockers showed maximum decrease in the amplitude of contractions was considered as standard for pre-treating the relevant tissue, after which the above-mentioned concentrations of AZM were added. Intestinal contractions were recorded using Force Transducer (Model No: MLT 0210/A Pan Lab S.I.) attached with Power Lab. (Model No: 4/25 T) AD Instruments, Australia. Data were recorded at range 20 mv, Low pass 5Hz X 10 gain using input 1, rate 40/S, as per our previous reported procedure (Ali *et al*., 2013).

Effect of AZM in the presence of Atropine (muscarinic receptor blocker)

Since intestine is predominant with cholinergic muscarinic receptors, hence for possible mechanism of spasmogenic effect of AZM, each jejunal and ileal tissue was pre-treated with atropine (0.3µM), a standard anticholinergic muscarinic drug. Each tissue was stabilized for 15 min. Then different cumulative concentrations of AZM (0.01, 0.03, 0.1, 0.3, 1, 3, 5, 10, 15 µM) were added and response to each dose was noted for 2 min (Ali *et al*., 2017).

Effect of AZM in the presence of Loratadine (histamine receptor blocker)

Tissues were pre-treated with (3µM) Loratadine. After 15 min, different concentrations (0.01, 0.03, 0.1, 0.3, 1, 3, 5, 10, 15 µM) of AZM were then added without washing the tissue and response to each dose was recorded for 2 min (Faisal *et al*., 2018).

Effect of AZM in the presence of Ondansetron (serotonin receptor blocker)

Ondansetron (0.3µM) was used to pre-treat the tissues for the blockade of serotonergic receptors. After waiting for

15 min, AZM was added in different concentrations (0.01, 0.03, 0.1, 0.3, 1, 3, 5, 10, 15 µM)) to check the response. Response produced by each dose was recorded for 2 min (Nabi *et al*., 2020, Ali *et al*., 2016).

Effect of AZM in the presence of Metoclopramide (dopamine receptor agonist)

Each tissue was pre-treated with (10µM) Metoclopramide. After 15 min, AZM in different cumulative concentrations (0.01, 0.03, 0.1, 0.3, 1, 3, 5, 10, 15 µM) was added without washing the tissue. Response shown by each dose was recorded for 2 min (Kamel, 2015).

Effect of AZM in the presence of Verapamil (voltage gated calcium channel blocker)

Tissues were pretreated with (0.3µM) Verapamil. AZM was added in different concentrations (0.01, 0.03, 0.1, 0.3, 1, 3, 5, 10, 15 µM) to check the response. Response produced by each dose was recorded for 2 min (Faisal *et al.,* 2018).

Effect of AZM in the presence of Propranolol (sodium channel blocker)

Propranolol (1µM) was added to block the sodium channels in the tissues. After 15 min, AZM in different cumulative concentrations (0.01, 0.03, 0.1, 0.3, 1, 3, 5, 10, 15 µM) was added without washing the tissue. Response shown by each dose was recorded for 2 min (Ahounou *et al*., 2012).

Effect of AZM in the presence of Amiodarone (potassium channel blocker)

Each tissue was pre-treated with (3µM) Amiodarone. After 15 min, AZM in different increasing concentrations (0.01, 0.03, 0.1, 0.3, 1, 3, 5, 10, 15 µM) was added without washing the tissue. Response shown by each dose was recorded for 2 min (Roden, 2016).

STATISTICAL ANALYSIS

Intestinal responses (%) of both rabbits' jejunal and rats' ileal preparations were plotted as percent of ACh maximum concentration response using Graph Pad Prism version 6. Similarly, in the presence and absence of each antagonists for possible involvement in intestinal tissues, intestinal responses were expressed in tables. Their Emax in presence of antagonist was compared with Emax of the tissues without respective antagonists by using t-test. P-value ≤ 0.05 was considered significant. All experiments were run in triplicate.

RESULTS

Effect of AZM on rabbits' jejunal and rats' ileal preparations

AZM produced significant spasmogenic response both in rabbits' jejunal and rats' ileal preparations. The mean Emax for AZM was 67.6±1.6% and 54.0±2.1% for rabbits' jejunal and rats' ileal preparations respectively (table 1).

Effect of AZM on rabbits' jejunal and rats' ileal preparations in the presence of Atropine

The spasmogenic response produced by AZM in the presence of Atropine was significantly reduced both in rabbits' jejunal and rats' ileal preparations. The mean Emax for AZM in the absence and presence of atropine for rabbits' jejunal preparations was 68.3±1.3% and 2.4±0.1% respectively, while for rats' ileal preparations it was 57.8±1.8% and 11.4±1.3% respectively. When the mean of Emax was compared in the absence and presence of atropine, it was found significant with $p<0.0001$ for rabbits' jejunal and $p<0.0001$ for rats' ileal preparations (table 1).

Effect of AZM on rabbits' jejunal and rats' ileal preparations in the presence of Loratadine

The response of AZM in rats' ileal preparations in the presence of Loratadine was insignificant $p>0.05$ (table 1).

Effect of AZM on rabbits' jejunal and rats' ileal preparations in the presence of Ondansetron

AZM was unable to produce its full spasmogenic response in the presence of Ondansetron. The mean Emax for AZM in the absence and presence of Ondansetron was 68.3±1.0 and 27.5±0.5 for rabbits' jejunal preparations, and 55.6±1.0% and 34.0±2.9% for rats' ileal preparations, respectively. When the Emax for AZM in the absence and presence of Ondansetron was compared, a significant difference of $p<0.0001$ and $p=0.0003$ was found for rabbits' jejunal and rats' ileal preparations, respectively (table 2).

Effect of AZM on rabbits' jejunal and rats' ileal preparations in the presence of Metoclopramide

In the presence of metoclopramide, AZM slightly increased the amplitude of rabbits' jejunal and rats' ileal contractions. The differences between the readings were statistically insignificant $p>0.05$ (table 2).

Effect of AZM on rabbits' jejunal and rats' ileal preparations in the presence of Verapamil

The spasmogenic response produced by AZM in the presence of verapamil was significantly reduced both in rabbits' jejunal and rats' ileal preparations. The mean Emax for AZM in the presence of verapamil for rabbits' jejunal preparations was 13.6±1.2% and for rats' ileal preparations was 22.3±2.5%. When the Emax for AZM in the absence and presence of Verapamil was compared, a significant difference of $p<0.0001$ and $p=0.0003$ was found for rabbit's jejunal and rat's ileal preparations, respectively (table 3).

Table 1: Effect of Azithromycin (uM) alone and in the presence of Atropine, Loratadine on rabbit's jejunal preparations and rat's ileal preparations (Mean ± SD, n=3)

Conc (µM)	Azithromycin (3uM) (alone)		In the absence & presence of Atropine (0.3uM)				In the absence & presence of Loratadine (3uM)			
	Effect of Azithromycin on rabbit's jejunum in % of Ach	Effect of Azithromycin on rat's ileum in % of ACh	Effect of Azithromycin on rabbit's jejunum in the absence of Atropine	Effect of Azithromycin on rabbit's jejunum in the presence of Atropine	Effect of Azithromycin on rat's ileum in the absence of Atropine	Effect of Azithromycin on rat's ileum in the presence of Atropine	Effect of Azithromycin on rabbit's jejunum in the absence of Loratadine	Effect of Azithromycin on rabbit's jejunum in the presence of Loratadine	Effect of Azithromycin on rat's ileum in the absence of Loratadine	Effect of Azithromycin on rat's ileum in the presence of Loratadine
0.01	33.9±2.8	4.0±2.8	25.8±0.9	3.1±1.5***	2.4±0	0±0**	31.3±3.6	22.9±1.0**	3.2±1.4	4.8±4.2
0.03	38.6±1.9	9.7±4.2	29.5±1.1	3.1±1.5***	5.6±2.8	2.4±4.2	37.6±2.6	26.1±1.2***	7.3±0	8.1±3.7
0.1	45.3±1.6	11.4±3.7	40.5±0.8	2.3±0.1***	9.7±2.4	4±3.7	43.2±5.3	36.0±1.4	10.6±1.3	12.2±2.4
0.3	47.7±3.8	15.5±3.7	48.1±1.4	2.3±0.1***	13.8±1.4	4±3.7**	50.7±2.7	44.1±3.9	14.7±2.2	11.9±0.4
1	50.5±3.7	26.9±0.1	57.0±1.2	2.4±0***	22.1±4.9	6.5±3.7**	56.5±1.6	65.6±3.3**	26.4±2.1	19.5±4.2
3	52.5±4.9	35.1±1.4	63.3±1.1	2.4±0.1***	35.9±1.4	10.6±1.3***	59±1.7	66.1±3.0**	36.9±0.1	31.9±4.2
5	65.9±1.3	44.9±2.8	67.8±1.0	2.4±0.1***	45.8±1.3	11.4±1.3***	65.5±1.3	67.5±1.9	45.7±3.1	44.3±2.1
10	67.7±1.6	54±2.1	68.3±1.3	2.4±0.1***	57.8±1.8	11.4±1.3***	68.8±0.6	67.9±2	52.4±2.8	48.1±1.3
15	67.7±1.6	54±2.1	68.3±1.3	2.4±0.1***	57.8±1.8	11.4±1.3***	68.8±0.6	67.9±2	52.4±2.8	48.1±1.3

Table 2: Effect of Azithromycin (uM) in the presence of ondansetron & metoclopramide on rabbit's jejunal preparations and rat's ileal preparations (Mean ± SD, n=3)

Conc (µM)	In the absence & presence of Ondansetron (0.3uM)				In the absence & presence of Metoclopramide (10uM)			
	Effect of Azithromycin on rabbit's jejunum in the absence of ondansetron	Effect of Azithromycin on rabbit's jejunum in the presence of ondansetron	Effect of Azithromycin on rat's ileum in the absence of ondansetron	Effect of Azithromycin on rat's ileum in the presence of ondansetron	Effect of Azithromycin on rabbit's jejunum in the absence of metoclopramide	Effect of Azithromycin on rabbit's jejunum in the presence of metoclopramide	Effect of Azithromycin on rat's ileum in the absence of metoclopramide	Effect of Azithromycin on rat's ileum in the presence of metoclopramide
0.01	27.8±1.7	12.4±0***	3.2±1.4	5.6±2.8	37±1.1	38.4±1.9	29.2±0.7	28.6±1.3
0.03	35.8±1.5	14±1.4***	5.6±2.8	6.5±1.3	42.5±2	43.7±1.2	33.7±1.1	35.2±1.5
0.1	43.2±1.3	14±1.4***	9.7±2.4	7.3±2.4	43.7±1.1	47.6±0.8	35.5±1	36.8±0.2
0.3	49.4±0.6	15.7±1.3***	13.8±1.4	9.4±2.4*	50.5±1.9	51.3±3.7	41.3±0.9	44.2±3.8
1	53.8±1.3	17.3±0***	27.2±0.4	12.1±4.1*	54.4±2.1	56.2±2.9	45.1±0.8	48.9±0.4
3	58.7±0.4	17.9±1.1***	35.2±1.3	20.6±4.2**	61.7±1.2	64.1±2.1	52.4±1.8	54.2±0.2
5	64.3±1.5	21.6±1.1***	45.9±1.0	31.2±1.4***	62.1±1.7	65.1±1.4	60.4±2.2	67.8±5.1
10	68.3±1.0	27.5±0.5***	55.6±1.0	34±2.9***	68.7±2.2	70.3±1.9	64.4±1.9	68.8±2.9
15	68.3±1.0	27.5±0.5***	55.6±1.0	34±2.9***	68.7±2.2	70.3±1.9	64.4±1.9	68.8±2.9

Table 3: Effect of Azithromycin (uM) in the presence of verapamil & propranolol on rabbit's jejunal preparations and rat's ileal preparations (Mean ± SD, n=3)

Conc (µM)	In the absence & presence of Verapamil (0.3uM)				In the absence & presence of Propranolol (1uM)			
	Effect of Azithromycin on rabbit's Jejunum in the absence of verapamil	Effect of Azithromycin on rabbit's Jejunum in the presence of verapamil	Effect of Azithromycin on rat's ileum in the absence of verapamil	Effect of Azithromycin on rat's ileum in the presence of verapamil	Effect of Azithromycin on rabbit's Jejunum in the absence of propranolol	Effect of Azithromycin on rabbit's Jejunum in the presence of propranolol	Effect of Azithromycin on rat's ileum in the absence of propranolol	Effect of Azithromycin on rat's ileum in the presence of propranolol
0.01	43.4±1	2.4±0***	5.5±2.7	2.4±0	32.8±0.5	9.0±2.8***	3.2±1.4	3.2±1.4
0.03	45.7±1.2	2.4±0***	6.5±3.7	2.4±0	37.9±1.5	9.0±2.8***	4.8±2.4	3.2±1.4
0.1	48.9±0.5	2.4±0***	9.9±2.7	2.4±0**	47±1.6	9.8±2.5***	9.7±2.4	5.7±2.8
0.3	52.2±0.8	4.3±0.5***	17.2±4.3	3.2±1.4**	51.6±1.3	9.8±2.5***	12.2±2.4	6.5±1.4**
1	56±0.7	8.1±0.5***	20.7±5.3	5.7±1.4***	53.9±1.3	9.9±2.5***	24±2.4	5.7±1.4***
3	59±0.4	8.4±0.2***	34.4±0.2	12.3±2.5***	57.7±1.4	9.9±2.5***	35.2±1.3	8.2±1.3***
5	63.3±0.8	9.7±0.7***	43.3±2.4	18.1±2.8***	66.6±2.1	10.2±2.1***	45.1±2.5	9.8±2.4***
10	68.9±0.4	13.6±1.2***	53.2±3.7	22.3±2.5***	68.2±1.1	10.2±2.1***	54.8±1.1	15.6±1.4***
15	68.9±0.4	13.6±1.2***	53.2±3.7	22.3±2.5***	68.2±1.1	10.2±2.1***	54.8±1.1	15.6±1.4***

$p \leq 0.05$ = *, $p \leq 0.01$ = **, $p \leq 0.001$ = ***

Table 4: Effect of Azithromycin (uM) in the presence of amiodarone & atropine, ondansetron, verapamil, propranolol (AOVP) on rabbit's jejunal preparations and rat's ileal preparations (Mean ± SD, n=3)

Conc (µM)	In the absence & presence of Amiodarone (3uM)			In the absence & presence of Atropine, Ondansetron, Verapamil, Propranolol (0.3uM)		
	Effect of Azithromycin on rabbit's Jejunum in the absence of amiodarone	Effect of Azithromycin on rabbit's Jejunum in the presence of amiodarone	Effect of Azithromycin on rat's ileum in the absence of amiodarone	Effect of Azithromycin on rabbit's Jejunum in the absence of AOVP	Effect of Azithromycin on rabbit's Jejunum in the presence of AOVP	Effect of Azithromycin on rat's ileum in the absence of AOVP	Effect of Azithromycin on rat's ileum in the presence of AOVP
0.01	35.3±2.8	30.9±1.3	4±2.8	27.8±1.7	0.0±0.0***	7.5±0.4	0.0±0.0***
0.03	44.5±4.9	37.5±1.4	5.6±2.8	4.1±1.3	35.8±1.5	0.0±0.0***	11.5±3.8	0.0±0.0***
0.1	55.6±7.6	48.8±1.7	11.3±3.8	6.5±1.3	43.2±1.3	0.0±0.0***	12.4±2.4	0.0±0.0***
0.3	60.9±5.5	58.5±2.2	15.5±3.7	10.5±1.3	49.4±0.6	0.0±0.0***	16.8±2.4	0.0±0.0***
1	64.6±2.3	60.8±1.2	23.6±5.6	22.2±2.2	53.8±1.3	0.0±0.0***	22.4±3.9	0.0±0.0***
3	67±2.5	62.5±3.9	35.1±1.4	38.7±7.4	58.7±0.4	0.0±0.0***	30±3.7	0.0±0.0***
5	68.4±1	65.9±1.8	44.9±2.8	49.3±2.0	64.3±1.5	0.0±0.0***	38.8±3.1	0.0±0.0***
10	69.8±0.5	68.4±1.3	62.9±3	58.0±3.4	68.3±1	0.0±0.0***	45±3.2	0.0±0.0***
15	69.8±0.5	68.4±1.3	62.9±3	58.0±3.4	68.3±1	0.0±0.0***	45±3.2	0.0±0.0***

p≤0.05 = *, p≤0.01 = **, p≤0.001 = ***

Effect of AZM on rabbits' jejunal and rats' ileal preparations in the presence of Propranolol

The amplitude of contraction in rabbits' jejunal and rats' ileal was markedly reduced when AZM was given in the presence of propranolol. The Emax in the presence of propranolol was found to be 10.2±2.1% for rabbits' jejunal preparations and 15.6±1.4% for rats' ileal preparations. A significant difference of $p<0.0001$ for rabbits' jejunal preparations and $p<0.0001$ for rats' ileal preparations was found when the Emax of AZM in the absence and presence of propranolol was compared (table 3).

Effect of AZM on rabbits' jejunal and rats' ileal preparations in the presence of Amiodarone

The response of AZM in both rabbits' jejunal and rats' ileal preparations in the presence of amiodarone was not significant $p>0.05$ (table 4).

Effect of AZM on rabbits' jejunal and rats' ileal preparations in the presence of Atropine, Ondansetron, Verapamil & Propranolol (AOVP)

The spasmogenic response of AZM was completely lost in the presence of atropine, ondansetron, verapamil and propranolol (AOVP), both in rabbit's jejunal and rat's ileal preparations. When the Emax for AZM in the absence and presence of AOVP was compared, a significant difference of $p<0.0001$ and $p<0.0001$ was found for rabbits' jejunal and rats' ileal preparations, respectively (table 4).

DISCUSSION

The beauty of the study is that it explored the mechanisms of the effects of AZM on the small intestinal contractility in detail. Results of the present study confirmed spasmogenic response by AZM as it increased intestinal contractility by acting on muscarinic and serotonergic receptors, and voltage gated calcium and sodium channels. The effects of AZM in the presence of loratadine, metoclopramide and amiodarone were found to be insignificant.

We found similar results for possible effects of AZM on intestinal preparations as reported by El-Baki *et al.* He performed a study to determine the pharmacological effects of AZM on different isolated smooth muscle preparations of experimental animals. After recording the normal contractions of rabbit's jejunum, AZM was given in different concentrations ranging from 1-16µg/ml. It was found that AZM increased the amplitude of jejunal contractions in all concentrations in a dose-dependent manner (El-Baki *et al*., 2015). Similar results were reported by Chini *et al*., (Chini *et al*., 2012) and Sifrim *et al*., (Sifrim *et al*., 1994). In contrast to our results Chiragh *et al*., stated that the prokinetic effect of AZM is not well-sustained in comparison with other macrolides. Thus,

showing AZM not to be a beneficial therapeutic agent to produce prokinetic actions (Chiragh *et al*., 2006).

ACh has been shown to increase the amplitude of small intestinal contractility and its frequency in the circular muscle layers (Grasa *et al*., 2004). In a study effects of AZM were evaluated on isolated jejunum of rabbits and ileum of guinea pigs. In relation to our findings, this study also confirmed that AZM exerts its effect on the jejunal motility via muscarinic receptors but in contrast to our results this study did not show involvement of muscarinic receptors by AZM in the motility of ileum (El-Baki *et al*., 2015).

In the same study the role of serotonin and calcium channels in the colonic contractions induced by AZM was further investigated. Isolated colon was first given serotonin antagonist, then without washing the tissue different concentrations of AZM were given. Same procedure was repeated for calcium channel blocker. It was found that colonic contractions induced by AZM were not affected in the presence of serotonin receptor blockers, denoting that these receptors are not involved in the mechanism of AZM affecting the colon motility. However, the colonic contractions were weakened in the presence of calcium channel blocker, showing the involvement of calcium channels in the AZM induced colonic contractions (El-Baki *et al*., 2015). Our results are dissimilar to the findings of this study as our study also confirmed the involvement of serotonergic receptors. However, our results like the results of this study are also suggestive of voltage gated calcium channels mediated effects of AZM because in the presence of voltage gated calcium channels blocker, the increase in the intestinal contractility was significantly minimal. This suggests that the intestinal contractions have been facilitated by promoting the influx voltage gated calcium channels as in the presence of verapamil, the contractile response is decreased. Hence, we can inference that as IP_3 Ca^{2+} release is facilitated by Ca^{2+} influx through voltage operated channels. Thus, it is possible to speculate that the AZM might cause the spasmogenic effect through increase in extra cellular influx of Ca^{2+}.

AZM is not found to act through dopamine receptors as in the presence of metoclopramide the AZM increases the intestinal contractility though not statistically significant. It might be because metoclopramide also slightly possesses serotonin agonistic effect, so there is a possibility that a small rise in the intestinal contraction of metoclopramide treated group may be because of the effect of metoclopramide on serotonin receptors (Isola and Adams, 2019).

AZM failed to increase the intestinal contractions in the presence of receptor operated Na_V channel blocker, confirming to affect the process of depolarization. During depolarization, the membrane potential quickly moves from negative to positive. The Na_V channels open in reaction to an early change in voltage. As the Na^+ rush back into the cell, the interior of the cell become positive, ultimately changing the membrane potential from negative to positive. When the interior of the cell becomes more positively charged, depolarization of the cell is complete, and the channels close again (Vincent, 2015).

AZM effect was completely blocked in the presence of all these antagonists suggesting the involvement of mixed pathways in the spasmogenic response produced by AZM. Though it is evident that the maximum spasmogenic response is mediated through muscarinic receptors of the intestine as atropine is a standard antimuscarinic cholinergic receptor blocker.

The other prospective aspect of this study is that azithromycin may significantly affect the absorption of drugs which requires more time for absorption like digoxin. Hence, our studies open new window of research to basic scientists and clinicians to study further the possible drug drug interactions of AZM and other primary drug therapies that requires AZM in combination with other drugs.

CONCLUSIONS

Our results suggest that azithromycin increases the contractility of rabbits' jejunal and rats' ileal preparations by involving multiple receptors and voltage gated ion channels. Decreased contractility of rabbits' jejunal and rats' ileal preparations in the presence of atropine, ondansetron, verapamil and propranolol confirmed the core involvement of muscarinic receptors following mixed pathway involving serotonergic receptors, voltage gated calcium and sodium channels in the production of spasmogenic response by azithromycin.

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