



## NITRIC OXIDE PATHWAY-MEDIATED RELAXANT EFFECT OF PPAR (PERACISOME PROLIFERATOR ACTIVATED RECEPTOR)-GAMMA AGONIST IN THE GUINEA-PIG ISOLATED TRACHEA.

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**ABSTRACT... Objectives:** This study focuses on the role of the mediator of smooth muscles i.e. nitric oxide in the effect of rosiglitazone on the relaxation of contractions in guinea pig trachea induced by histamine. **Study Design:** Experimental Study. **Setting:** Department of Pharmacology, Army Medical College, Rawalpindi. **Period:** 1<sup>st</sup> December 2018 till 14<sup>th</sup> December 2018. **Material & Methods:** The activity of the trachealis muscle of the guinea pigs was noted by the use of oxygenated solution of Krebs-Henseleit. The contractions were noted with rosiglitazone alone as well as in presence of an ester of L-nitro-arginine-methyl, which can antagonize the activity of NO. The activity was measured by the use of an isometric force displacement transducer and was then recorded on an oscillograph with four channels. **Results:** The ester L-nitro-arginine-methyl was able to reduce the relaxant effect of rosiglitazone on the contractions induced by histamine. **Conclusion:** It can be suggested that the relaxation induced by rosiglitazone is mediated in part through NO.

**Key words:** Nitric Oxide (NO), PPAR Agonist, Tracheal Relaxation.

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## INTRODUCTION

The enormous increase in the prevalence of chronic obstructive pulmonary disease and asthma<sup>1</sup>, diseases characterized by hyperreactivity of bronchi and bronchospasm, has attracted considerable attention to the development of unconventional therapeutic options against representative diseases. Thiazolidinediones are valuable agents used as a treatment option in patients suffering from type II diabetes mellitus (T2DM) as well as metabolic syndrome.<sup>2,3</sup> The therapeutic benefits of TZDs expand beyond their use in diabetes. Their importance in various other ailments including Alzheimer's disease, pancreatitis and inflammatory airway diseases has also been studied.<sup>4,5,6,7,8</sup> Several studies show that thiazolidinediones and PPAR agonists have beneficial effects in models of asthmatics as well as chronic obstructive pulmonary disease (COPD) leading to the reduction in the responsiveness and hyper reactivity of airways.<sup>9,10</sup> They activate various members of the steroid aka nuclear receptor superfamily, importantly PPAR-  $\gamma$ . The

activation of a number of genes that lead to inflammation is suppressed by the PPAR- $\gamma$  ligands and rosiglitazone, hence exhibiting themselves as potent anti-inflammatory agents.<sup>11,12</sup> It has been acknowledged that PPAR- $\gamma$  ligands such as TZDs have a role in cell proliferation and malignancy.<sup>13,14</sup> PPAR-  $\gamma$  is found in the airway passages as well as lungs. The epithelium, airway smooth muscles, as well as the cells of inflammation are responsible for the expression of PPAR- $\gamma$ .<sup>15,16</sup> As a result of the identification of PPAR-  $\gamma$  in lung tissue and cells associated with inflammation in the lungs and airways, the PPAR-  $\gamma$  has been studied as a target for interventional study.

PPAR ligands are capable of modulation and remodeling of the airway by decreasing the migration of the airway smooth muscles and production of the matrix. A number of current studies have demonstrated that the PPAR- $\gamma$  agonists have beneficial effects in models of asthma.<sup>17,18,19</sup> Airway submucosal and structural cells, show an increased expression of PPAR $\gamma$

during the inflammatory and remodeling response in asthma.<sup>20,21</sup> In a pharmacological model, rosiglitazone reversed the pro-asthmatic changes induced by persistent beta 2 receptor activation, thereby, improving the therapeutic index of the agonists of  $\beta_2$  receptors.<sup>22</sup> Synergistic interaction between PPAR- $\gamma$  agonists and agonists of the  $\beta_2$  receptor on bronchial smooth muscle cell proliferation has been observed.<sup>23</sup> The inflammation as well as the remodeling of the airways induced by ovalbumin (OVA) in a mouse model, were decreased significantly by the intranasal administration of rosiglitazone. The number of the inflammatory cells including the eosinophils and neutrophils were decreased so were the levels of IL-5 and IL-13. In addition there was a decrease in the thickness of the airway smooth muscle layer and reduced deposition of the collagen in the airway.<sup>24</sup> It has been postulated that rosiglitazone has activity on the airways sparing the parenchyma of the lung since rosiglitazone did not affect the compliance of the lung. This inhibition by rosiglitazone occurred without significant effect on the OVA-induced increase in the inflammatory cells in bronchoalveolar lavage. Therefore, it can be a possibility that rosiglitazone modulates airway hyperreactivity (AHR) by a mechanism that is independent of inflammatory cell recruitment to the airway.

Nitric oxide (NO) is an established regulator of diverse physiologic processes. Its involvement in host defense, neurotransmission, platelet aggregation, gastrointestinal and vascular smooth muscle regulation is all well recognized. NO has an established role in the tone of the airway smooth muscles.<sup>26,27</sup> NO stimulates the mechanisms leading to the relaxation of smooth muscles in the airways. In addition the enzyme, Nitric oxide synthase is found in the airways pointing towards the possible involvement of NO in the pathophysiology of asthma.<sup>28</sup> Nitric oxide possesses broncho-dilatory property that in part is mediated by the synthesis of s-nitrosothiols (SNOs).<sup>29</sup> This compound leads to the activation of guanylyl Cyclase which eventually induces bronchial relaxation.<sup>30</sup> Studies have shown of NO and related signaling pathways in various

effects of PPAR- $\gamma$  including the brain and endothelial system.<sup>31,32</sup> Pioglitazone treatment also significantly increased total enzyme activity of NOS in hypertensive rats.<sup>33</sup>

It is evident that the exact mechanism of the airway relaxant effects of the PPAR- $\gamma$  agonists is not well established. In consideration of the role of No in airways and its association with effects of PPAR-  $\gamma$  elsewhere in the body, we studied the modulation of the smooth muscle relaxation by inhibitors of No in an isolated intact guinea pig trachea that had been treated with rosiglitazone.

## MATERIAL & METHODS

### Guinea pig trachea isolation

The study was conducted from 1<sup>st</sup> December 2018 till 14<sup>th</sup> December 2018. The guidelines of the institutional care of animals were followed. Dunken Hartley guinea pigs (500-600g) were killed by cervical dislocation. The chest was opened through an incision in the midline. Through surgery a segment of the cervical trachea measuring 8-10mm was taken out intact. The tracheal segment was not manipulated any further and was directly placed in a pre-oxygenated Kreb's Henseleit solution. The solution had the following composition per 100ml (mM): NaCl 118.2, KCl 4.7,  $\text{CaCl}_2$  2.5,  $\text{Mg}_2\text{SO}_4$  1.2,  $\text{NaHCO}_3$  25,  $\text{KH}_2\text{PO}_4$  1.3 and glucose 11.7. A gas mixture of 95%  $\text{O}_2$  and 5%  $\text{CO}_2$  was bubbled into the solution.

### Studies on Isometric Tension

In order to look for the changes in the muscle tension, the intact segment of the trachea was placed in an organ bath with 50ml water and Kreb's Henseleit solution maintained at 37°C, and supplied with oxygen. The strip of the trachea was attached at one end to the the oxygen tube while the other end of it was attached to an isometric force displacement transducer of Harvard model number & 72-4494. The recordings of the transducer were then recorded through an oscillograph with four channels. Equilibration of the trachea was performed against a passive load weighing 2 grams kept at a temperature of 37°C for time duration of 45 min.

**Stimulation by Drugs**

**Effect of rosiglitazone on the contraction induced by histamine**

Following the equilibration period performed initially, the baseline tension was adjusted by addition of  $10^{-3}$  M histamine. Histamine was washed out from the organ bath once the effect of histamine was recorded to reach a plateau. Then the tension at the baseline was restored by allowing the tissue to relax. Then forth the same experiment was repeated by increasing the histamine concentrations to  $10^{-4}$  M,  $10^{-5}$  M,  $10^{-6}$  M and  $10^{-7}$  M randomly. The tissue was allowed to relax for a period of ten minutes between all the concentrations. A concentration response curve was then constructed after calculating the mean values at different concentrations.

Next a fixed dose of  $100 \mu\text{M}$  of rosiglitazone was used against which the effect of histamine was noted. Rosiglitazone  $100 \mu\text{M}$  was added and was left to be in contact with the tracheal tissue for a period of 15 minutes. Then the contractions produced by histamine were recorded on this tissue. A mean was calculated for the six experiments and a concentration response curve constructed.

**Effect of L-NAME on the contractions induced by histamine and rosiglitazone induced relaxation**

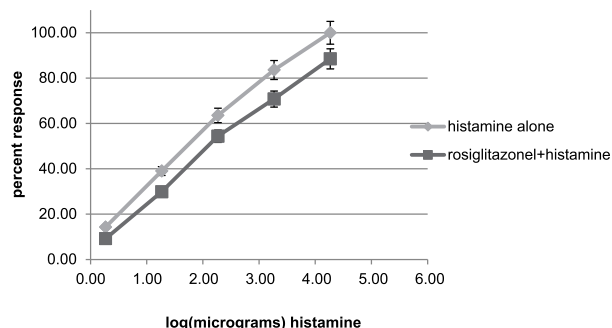
In an attempt to study the role of endogenous NO in the contractility of trachea, six experiments were performed. Trachea was first incubated with the NO synthetase inhibitor known as L-NAME in a concentration of  $120 \mu\text{M}$ . It was then followed by the addition of rosiglitazone and increasing histamine concentrations randomly. A mean was calculated for the six experiments and a concentration response curve constructed.

**Statistical Analysis**

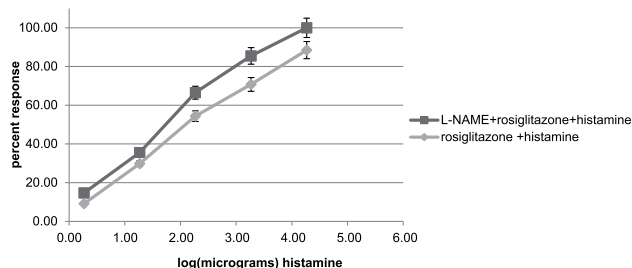
The data obtained from the experiments was noted as mean  $\pm$  SEM. All the calculations were done using SPSS version 20. The comparative data were presented through the one way ANNOVA along with the Tukey's post hoc test. The percentage of the response as well as the deviation was calculated. A value of  $p < 0.05$  was taken as significant statistically.

**RESULTS**

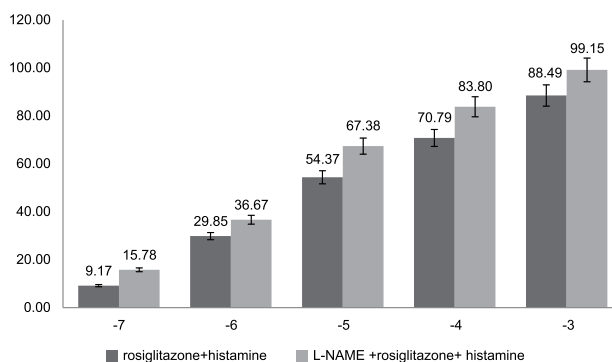
Action of rosiglitazone on the tissue that had the histamine pre-treatment both in the presence as well as the absence of L-NAME were noted. Statistically significant ( $P < 0.05$ ) difference was found when the mean values shown with the differing concentrations of histamine ranging from  $10^{-7}$  to  $10^{-3}$ M were compared first to the group that had the pretreatment with rosiglitazone and then to the group that had the pretreatment with both L-NAME as well as rosiglitazone. The mean deviation was calculated to be 25.34 percent.



**Figure-1. Shows the curve demonstrating the relaxation of the histamine induced contractile response by pretreatment with Rosiglitazone.**



**Figure-2a: Shows the comparison between percent response in only Rosiglitazone pretreated group (in blue) and pretreatment with both L-NAME and rosiglitazone (in red).**



**Figure-2b: Bar diagram showing histamine induced contractions after pretreatment with Rosiglitazone and response to histamine after pretreatment with both L-NAME and Rosiglitazone.**

Serial no.	Gp 3	Gp 6	Gp 3	Gp 6	Gp 3	Gp 6	Gp 3		Gp 6	Gp 3	Gp 6
	Concentration ( $\mu\text{M}$ )										
	$10^{-7}$	$10^{-7}$	$10^{-6}$	$10^{-6}$	$10^{-5}$	$10^{-5}$	$10^{-4}$	$10^{-4}$	$10^{-3}$	$10^{-3}$	
1	7	11	20	31	41	50	57	63	68	78	
2	8	10	25	30	42	49	55	66	70	75	
3	6	12	23	29	39	53	55	65	66	77	
4	7	14	25	28	43	52	56	68	71	78	
5	7	15	20	28	45	55	53	65	68	76	
6	8	12	27	26	45	57	56	66	72	81	
Mean	7.17	12.33	23.33	28.67	42.5	52.67	55.33	65.5	69.17	77.5	
SD	0.75	1.86	2.87	1.75	2.34	3.01	1.37	1.64	2.23	2.07	
SEM	0.31	0.76	1.17	0.71	0.96	1.23	0.56	0.67	0.91	0.85	
P value	0.000*		0.117*		0.000*		0.000*		0.000*		

Table-I. Comparison among groups of the responses of the pig tracheal muscle.

P-value &gt; 0.05 = Not significant (Ns)

Concentration of Histamine (M)	Percent Contraction (%)		Percent Deviation (%)
	Group 3	Group 6	
$10^{-7}$	9.17	15.78	53
$10^{-6}$	29.85	36.67	20.51
$10^{-5}$	54.37	67.38	21.36
$10^{-4}$	70.79	83.80	20.48
$10^{-3}$	88.48	99.15	11.36

Table-II. Comparison among groups of the percent responses &amp; the percent deviations.

Mean (%) Deviation: 25.34

## DISCUSSION

Rosiglitazone, when added before eliciting tracheal contraction with histamine, leads to decrease in the percent contraction. Adding the inhibitor of NO synthesis, L-NAME to the incubation medium for a period of 30 minutes significantly reduced the relaxant effect of rosiglitazone on the histamine induced contractions (Figure-1). Rosiglitazone induced relaxation has been previously observed in isolated tracheal muscle of rats pre-contracted with carbachol.<sup>34</sup> However, the mechanism of the tracheal relaxation caused by the agonist of PPAR- $\gamma$  is not completely understood. It has been suggested that PPAR- $\gamma$  stimulated NO release activates signaling in the renal cortex of pre-hypertensive rats after pioglitazone treatment.<sup>33</sup> The relaxation in contractile response of tracheal muscle in the presence of L-NAME can be explained on the basis of involvement of NO in the relaxing effect of rosiglitazone on airway smooth muscle. NO is produced through a variety of isoforms of the enzyme NO synthase (NOS). These enzymes use amino acid i.e L-arginine,

oxygen as well as NADPH as substrates for the production of NO and L-citrulline. The various forms of NO show an inhibitory non adrenergic as well as non cholinergic action within the neurons, the endothelium and epithelium. On the other hand the pro inflammatory cytokines secrete inducible NOS (iNOS) that is found in macrophages as well as epithelial cells.<sup>35</sup> The cholinergic neurotransmission may be interfered with because of the released nitric oxide either by the functional antagonism on smooth muscle of the airway or through inhibiting the acetylcholine release via the cholinergic nerve terminals. Through the phosphorylation of eNOS, NO takes part in the cardio-protective activity of the agonists of PPAR- $\gamma$ , rosiglitazone.<sup>36,37</sup> The restoration of the vascular dysfunction by rosiglitazone takes place through the up-regulation of the NO system in the endothelium, as shown in the fructose-fed rat model.<sup>38</sup> A study illustrated the role of NO in the activity of pioglitazone on the spatial recognition memory.<sup>39</sup> Similar role of NO maybe present in the airways. Our study hypothesizes that NO is

involved in the effect of the thiazolidinediones on the airways. In accordance to our findings, a study conducted in pioglitazone improved endothelial function through an increase in nitric oxide bioavailability and partly a decrease in oxidative stress after administration of pioglitazone.<sup>33</sup> Studies suggest that activation of the PPAR- $\gamma$  receptor can influence and change the expression of signaling molecules of the insulin signaling cascade (IRS, PI3K, Akt, eNOS, 5-AMP kinase, GLUT4, etc.) connected with improvement of insulin sensitivity and/or endothelial dysfunction.<sup>40</sup> Similar mechanism may be involved in the airways. Additional studies are required in order to establish the role of NO in the relaxant activity of the rosiglitazone. This study basically focuses on the larger airways and so the action of these drugs on the smaller airway muscles is to be elucidated. In addition it has been demonstrated through various studies that most of the peripheral smaller airways have a much less contribution the total airflow resistance as compared the large airways<sup>41,42,43</sup> thus making large airways an important target of agonists.

## CONCLUSION

The results of this study are suggestive that the rosiglitazone induced relaxation on the trachea contracted via histamine is in part through the synthesis and release of NO, thus indicating the possible influence of cGMP pathway mediating the action of NO in the regulation of airway smooth muscle tone.

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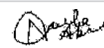



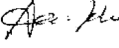
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