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# Evaluation of the Vasomodulatory Activity of some Cardiotoxic Drugs

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**ABSTRACT**

The inadequacy of approaches for control of drug-induced cardiotoxicity, suggests the possibility that yet to be explored mechanisms are involved. This study was performed to assess arthemether-lumefanthrine combination (A/Lcomb), 5-fluorouracil (5-Fu) and cisplatin for their vasomodulatory potential as an attempt to identify the possible contribution of such modulation to their adverse effect on the cardiovascular system. This was done using isolated rat aortic ring preparations securely positioned in tissue baths aerated with carbogen (95% carbon dioxide and 5% oxygen). Following tissue equilibration, the direct effect of A/Lcomb, 5-Fu and cisplatin on aortic rings were determined followed by an evaluation of their effect on noradrenaline ($3.33 \times 10^{-4}$ mg/ml) and potassium chloride (4.48 mg/ml) pre-contracted aortic rings. Noradrenaline and potassium chloride pre-contracted aortic rings were significantly relaxed by A/Lcomb. The anti-neoplastic, 5-Fu, significantly increased contractility of aortic rings with or without pre-contraction by noradrenaline or KCl. Cisplatin also significantly increased contractility of noradrenaline pre-contracted tissues. The findings of this study revealed the vasomodulatory action of these drugs, an effect that may contribute to their cardiotoxic potential. A more detailed study to further elucidate the mechanisms by which these vasomodulatory responses are mediated is ongoing.

**Keywords**: vasoconstriction, vasodilation, aortic ring, arthemeter-lumefantrine, 5-fluorouracil

## INTRODUCTION

Cardiovascular disorders are a major cause of death globally. In some patients, a malfunction of the cardiovascular system can be attributed to the cardiotoxic effect of a drug the patient is on. Various drugs have multiple effects on the cardiovascular system. Drug-induced cardiac failure, arrhythmia, diastolic dysfunction, ischaemic events and myocardial infarction have been reported (Feenstra *et al.*, 1999; Pai and Nahata, 2000). Major culprits include anticancer agents such as 5-fluorouracil and cisplatin and antimalarials such as halofantrine and chloroquinie. Arthemeter-lumefantrine, the much studied and widely used antimalarial combination therapy recommended for resistant malaria, has also been implicated in cardiac dysrythmias (WHO, 2017) and oxidative stress-induced cardiovascular and renal toxicity (Asiedu-Gyekye *et al*., 2016; Angus, 2014; Efferth and Kaina, 2010). The vascular system is functionally linked to the heart and their functions are interconnected. Disruption in the structure and function of endothelial cell of the vascular system and subsequent hypertension could result in a damage to the heart, and vice versa. An imbalance in the regulation of vasodilation and vasoconstriction of blood vessels may result in cardiotoxic responses (Mladˇenkaˇ *et al*., 2018). These sort of changes could arise with the use of drugs. Some approaches for prevention and control of these effects have been adopted. One such approach is the simultaneous treatment of patients with protective substances thought to interact beneficially with the possible mechanisms of cardiac damage induced by the cardiotoxic drug (Zhang *et al*., 2016). This indicates the need to first understand the cardiotoxic mechanisms involved. The inadequacy of current approaches in the control of drug-induced cardiotoxicity (evidenced by continuous reports of drug-induced crdiotoxicity), suggest the possibility that there are yet to be explored mechanisms implicated here. Drug induced cardiac diseases including heart failure and cardiomyopathy have been proposed as due to mechanisms such as production of reactive oxygen species, disturbance of the mitochondrial energy metabolism, intracellular calcium overloading, toxic endothelial damage followed by extravasation of toxic metabolites with resultant myocyte damage and interstitial haemorrhage and oedema (Mladˇenkaˇ *et al*., 2018). Vasomodulation due to interaction of implicated drugs with receptors on the smooth muscles of blood vessels have also been reported (Feenstra *et al*., 1999). Sager *et al*. (2013) indicated that vasomodulatory studies are veritable preclinical study approaches that may be conducted to elucidate mechanisms involved in drug induced alterations of blood pressure.

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Vascular smooth muscle contraction is important for maintaining vasomotor tone. However, sustained vascular smooth muscle contraction may be deleterious in pathologic vasospasm. Vasospasm contributes to mesenteric ischaemia, myocardial infarction, and stroke associated with subarachnoid haemorrhage and often complicates peripheral arterial reconstructions. On the other hand, vasodilatation induced by drugs have been attributed to flushing, hypotension, chest pain, among others. Closely examining the potential of drugs to induce constrictive and dilatory changes in vascular smooth muscle is thus important in efforts to manage drug-induced cardiovascular dysfunction. The drugs, 5-fluorouracil (5-Fu), cisplatin and arthemether-lumefanthrine combination (ALcomb) were selected for this study based on reports of their cardiotoxicity from literature and communication with physicians. The aim of this study was to determine their role in vasomotor tone regulation using rat aortic rings.

## MATERIALS AND METHODS

### Experimental animals

Male and female albino rats of about 8-9 weeks old with an average weight of 200 g ± 40 g were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos. They were maintained under standard environmental conditions and allowed free access to water and food (Animal Care Service Consult, Ogere-Remo, Ogun state, Nigeria). The experimental procedures were carried out in accordance with the 2011 United States National Institute of Health's guideline for laboratory animal care and use.

### Tissue Preparation and Isometric Tension Measurement

Following acclimatization and fasting overnight, rats were humanely sacrificed by stunning and cervical dislocation. Their thoracic aorta were then excised and immediately transferred to a petri dish filled with ice cold Krebs- Henseleit physiological solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM $KH_2PO_4$, 25 mM $NaHCO_3$, and 11.1 mM glucose) maintained at pH 7.4. The aortae were cleaned of adhering fat and connective tissue and then cut into 3 – 4 mm long rings or segments. Care was taken to avoid abrading the intimal surface in order to maintain the integrity of the endothelial layer. For the experiment, the aortic rings were placed hanging from two triangular tissue holders, one of which was held in place in the isolated tissue bath (Radnoti organ bath, ADInstruments, New Zealand) filled with Krebs-Henseleit solution, aerated with 5% $CO_2$ and 95% $O_2$ and maintained at 35-37 $^oC$. The other tissue holder was connected to a force transducer (ADInstrument, New Zealand) connected via a response recording system (Powerlab, ADInstrument, New Zealand) to personal computer installed with Labchart 8 (ADInstrument, New Zealand) for data acquisition. The rings were stretched to an initial tension of 2 g (determined in preliminary experiments) and allowed to equilibrate for 60 min while washing at 15 minutes interval. The absence of vasodilatory responses to ACh was taken as evidence that aortic rings were functionally denuded of endothelium, while its presence was evidence of intact endothelium (Owolabi et al., 2005). The results presented in this report were obtained from endothelium denuded aortic rings.

### Evaluation of the direct effect of test drugs on vascular tone

In this series of experiments, varying concentrations of the test drugs 5-Fu (0.0167, 0.0500, 0.1667 or 0.5000 mg/ml); cisplatin (0.001, 0.003, 0.010 or 0.033 mg/ml) and A/Lcomb (0.13/0.80, 0.40/2.38 or 0.78/4.75 mg/ml respectively obtained from 0.1, 0.3 and 0.6 ml of 40/240 mg dose combination of artemeter/lumefantrine) were evaluated. The different concentrations were added respectively to the tissue baths containing endothelium intact or denuded tissues prepared as described above at 4 minutes interval. Each addition was preceded by washing of the tissue and attainment of baseline tracings. (Yang *et al*., 2015).

### Evaluations of mechanisms underlying the effects of test drugs

In order to verify the mechanisms involved in the effect of the test drugs, the following studies were performed using slight modifications of the method described by Lee *et al*., (2013). In the first set of experiments, aortic rings were pre-contracted with noradrenaline ($3.33 \times 10^{-4}$ mg/ml); maximum contractions were noted within 15 minutes. The tissue was then washed by draining the tissue bath of the physiological solution containing the drug (noradrenaline) and replacing with fresh physiological solution. After obtaining steady baseline tracings, the tissue was subjected to the same concentration of noradrenaline following pre-treatment with 5-Fu (0.500 mg/ml)) for 5 minutes. The tissue's response was then observed for 15 minutes. The results for noradrenaline alone and noradrenaline in the presence of 5-Fu pre-treatment of the arotic rings were compared. This procedure with noradrenaline was repeated with KCl (4.48 mg/ml) to test the influence of 5-Fu on $K^+$ channel pathway. The effect of ALcomb at 0.78/4.75 mg/ml and cisplatin at 0.033 mg/ml (data not shown) on

noradrenaline- and KCl-induced contractions were also evaluated using similar procedures.

**Statistical analysis**

Results are expressed as mean ± SEM. One-way analysis of variance followed by Dunnett's multiple comparison post-hoc test as well as two way analysis of variance followed by Bonferoni's multiple comparison post hoc tests were used to compare data among groups. Differences among groups were considered significant at $p < 0.05$. These statistical analyses were performed using Graphpad prism 6 statistical analysis software.

## RESULTS

**The direct effect of test drugs on vascular tone**

The fixed dose A/Lcomb (0.13/0.80, 0.40/2.38 or 0.78/4.75 mg/ml) induced concentration-dependent negative contractile response (relaxation) of endothelium denuded rat aorta. This effect was significant at 0.40/2.38 mg/ml and 0.78/4.75 mg/ml with p values less than 0.01 and 0.001 respectively (Figure 1). The antineoplastic, 5-Fu on the other hand concentration-dependently contracted thoracic aorta in a manner that was however only significant ($p < 0.05$) at 0.50 mg/ml of 5-Fu (Figure 2). Finally, cisplatin, another antineoplastic agent concentration-dependently relaxed endothelium-denuded aorta at 0.003 mg/ml ($p < 0.05$), 0.010 mg/ml ($p < 0.0001$) and 0.033 mg/ml ($p < 0.0001$) as shown in Figure 3.

**Mechanism of vasomodulatory action of test drugs**

Consistent with the vasodilatatory effect of A/Lcomb shown above, at 0.078/4.75 mg/ml, A/Lcomb significantly ($p < 0.0001$) reversed the vaso-constrictive action of noradrenaline ($3.33 \times 10^{-4}$ mg/ml) in a time-dependent manner (Figure 4). On the other hand, 5-Fu apparently enhanced the vaso-constrictive action of the same concentration of noradrenaline; this effect was however not statistically significant (Figure 5). Regarding potassium channel pathway, A/Lcomb significantly ($p < 0.001$ at 3 min and $p < 0.0001$ at 6 – 15 min of observation period) reversed KCl-induced contraction of rat aortic ring tissues (Figure 6). On the other hand, time-dependent increase in KCl-induced contraction of these tissues was observed with 5-Fu, significant ($p < 0.05$) at 9 and 12 minutes of observation (Figure 7).

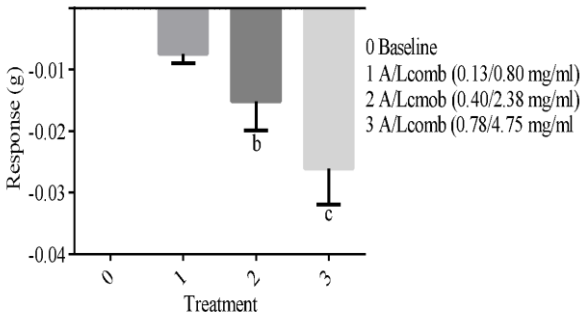


Figure 1: Effect of A/Lcomb on rats endothelium denuded aortic rings. Bars represent response mean ± S.E.M (n = 4 - 6). $^{b}p < 0.01$, $^{c}p < 0.001$ vs baseline (One way analysis of variance followed by Dunnett's multiple comparison test) . A/Lcomb - artemeter/lumefantrine combination

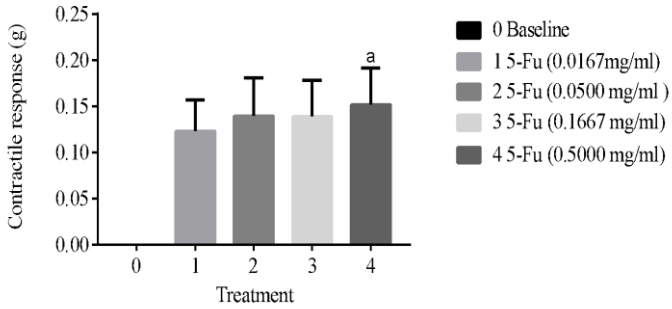


Figure 2: Effect of 5-Fu on rats endothelium denuded aortic rings. Bars represent response mean ± S.E.M (n = 6). $^{a}p < 0.01$ vs baseline (One way analysis of variance followed by Dunnett's multiple comparison test). 5-Fu -5-fluorouracil

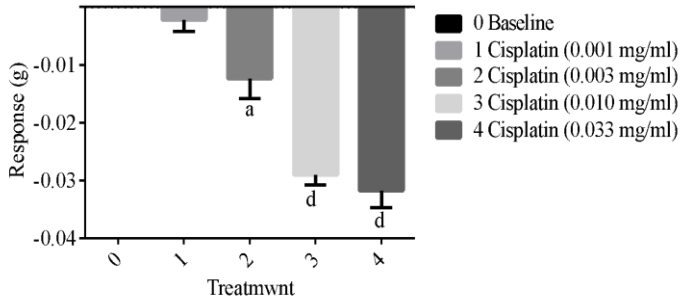


Figure 3: Effect of cisplatin on rats endothelium denuded aortic rings. Bars represent response mean ± S.E.M (n = 8 - 10). $^{a}p < 0.01$, $^{d}p < 0.0001$ vs baseline (One way analysis of variance followed by Dunnett's multiple comparison test).

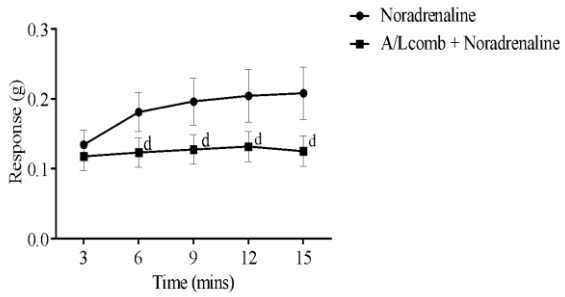


Figure 4: Effect of A/Lcomb (0.078/4.75 mg/ml) on noradrenaline (3.33 x $10^{-4}$ mg/ml) induced contraction of rats endothelium denuded aortic ring. Points on the line graph represent mean ± S.E.M (n=8). $^{d}p< 0.0001$ for A/Lcomb + noradrenaline vs noradrenaline (Two way ANOVA followed by Bonferoni's test).

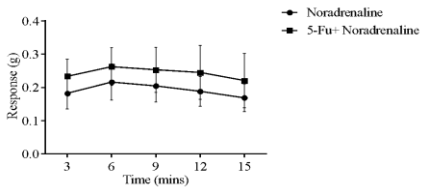


Figure 5: Effect of 5-Fu (0.50 mg/ml) on noradrenaline (3.33 x $10^{-4}$ mg/ml) induced contraction of rats endothelium denuded aortic ring. Points on the line graph represent mean ± S.E.M (n=7).

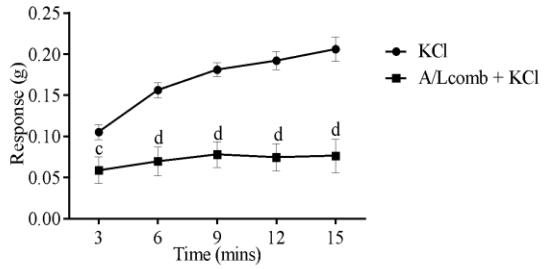


Figure 6: Effect of A/Lcomb (0.078/4.75 mg/ml) on KCl (4.48 mg/ml) induced contraction of rats endothelium denuded aortic ring. Points on the line graph represent mean ± S.E.M (n=8). $^{c}p < 0.001$, $^{d}p< 0.0001$ for A/Lcomb + KCl vs KCl (Two way ANOVA followed by Bonferoni's test).

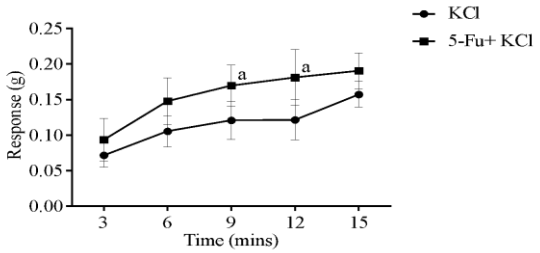


Figure 7: Effect of 5-Fu (0.50 mg/ml) on KCl (4.48 mg/ml) induced contraction of rats endothelium denuded aortic ring. Points on the line graph represent mean ± S.E.M (n=5). $^{a}p < 0.05$ for 5-Fu + KCl vs KCl (Two way ANOVA followed by Bonferoni's test).

**DISCUSSION**

Our study revealed that A/Lcomb has vasodilatatory activity; an effect that has been linked to reflex tachycardia, fluid retention, flushing, headaches and hypotension. Reflex tachycardia is known to exacerbate myocardial ischaemic events and angina (Kester *et al*., 2012). In addition, papaverine, a coronary vasodilator is known to induce cardiac arythmias in humans (Kim et al., 2007). A report by the World Health Organization in 2017 revealed that arthemeter-lumefantrine has potential to induce cardiac dysrhythmias. This effect could be due to a number of mechanisms, which remain to be explored. So far, our study shows that the vasodilating effect of A/Lcomb can be independent of endothelium derived mediators (such as nitric oxide), given the observations made in endothelium denuded aortae. The fact that in the presence of A/Lcomb, the vasoconstrictive action of noradrenaline is reduced significantly indicates that the effect of A/Lcomb may involve interference with noradrenergic receptor pathways. This could be via direct adrenergic receptor interaction or other less direct sympatholytic pathways. Investigation of its precise activity in this regard will provide useful insight. In a similar manner A/Lcomb inhibited the vasoconstrictive activity of KCl, a vasoconstrictive agent that acts majorly by depolarization, which leads to extracellular $Ca^{2+}$ influx via voltage-dependent calcium channel and then activation of the actin-myosin complex (Karaki *et al*., 1984). Thus, a reversal of vascular contraction by A/Lcomb could be demonstrative of its potential to prevent extracelluar calcium influx and the resulting downstream response cascade. Interestingly, calcium channel blockers such as verapamil and fantofarone have shown promising results against chloroquine resistant *Plasmodium falciparum* (Scheibel *et al*.., 1987; Adovelande *et al*., 1998; Van Schalkwyk *et al*., 2001), a form of malaria A/Lcomb is also used to manage. Further

investigations in this respect will thus reveal more precise mechanism(s) as it relates to this pathway and also possibly provide insight into more therapeutics implications of A/Lcomb in clinical medicine practice. It is also worth noting that vasodilatory activity is associated with reduced blood pressure, which may contribute to some of the side effects of A/Lcomb, namely: dizziness, light-headedness, headaches and fatigue. It has been proposed that vasospasm induced by 5-Fu could be responsible for its myocardial ischaemia effect as coronary angiography appear to indicate (Shah *et al*., 2012). Several pathways have been suggested for this vasoconstriction including up-regulation of the vasoconstrictor, endothelin 1 and activation of protein kinase C activity. Evidence suggests the need for more research to further elucidate pathophysiological mechanisms for 5-Fu-induced coronary artery vasospasm. Our study reveal that contraction of aortic rings by 5-Fu within the tested range of concentrations can occur via mechanism that are endothelium-independent. Its failure to influence noradrenaline-induced vasoconstriction indicates that its action in the absence of endothelium dependent pathways does not significantly involve noradrenergic mechanisms. With respect to its effect on KCl-induced contraction, 5-Fu showed potential to increased aortic tissue contraction induced by this depolarizing agent that enhances $Ca^{2+}$ influx into the smooth muscle cells in an additive manner. Vasoconstriction with 5-Fu explains a possible effect by which the chest pain, signs of ischaemia, ventricular dysfunction, coronary spasm usually seen with 5-Fu usage occurs (Feenstra *et al*., 1999; Chong and Ghosh, 2019). An exploration of this additive interaction between 5-Fu and KCl in endothelium denuded aortic tissue may serve to uncover information relevant to controlling (to some extent at least) the menace of cardiotoxicity induced by 5-Fu. Early stages of study on cisplatin, another anticancer agent featured in this study also reveal its potential to modulate vasomotor tone. Further studies in our laboratory aimed at elucidating more precise mechanisms for these effects which contribute to the limitations of the therapeutic benefits of these drugs in clinical practice are ongoing in our laboratory.

## CONCLUSION

This study reveals that the antimalarial, A/Lcomb and anticancer drugs such as 5-Fu and cisplatin have vasomodulatory actions that may contribute to their undesired effects on the cardiovascular system. A more detailed study to further elucidate the mechanisms by which these vasomodulatory responses are mediated will provide information necessary for improvement of therapeutic out comes with these drugs.

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