

# IN VITRO EFFECTS OF ANTI MALARIAL DRUG PYRIMETHAMINE - SULPHADOXINE AND ACTIVATED CHARCOAL ON RAT ILEAL SMOOTH MUSCLE

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## ABSTRACT

The mechanical responses of the gastrointestinal tract to anti malarial drug primethamine-sulphadoxine (fansidar) and /or activated charcoal (A/C) were investigated using isolated rat ileal smooth muscle. Fansidar ( $5.0 \times 10^{-2}$  to  $4.5 \times 10^{-1}$  M) elicited concentration-dependent relaxation of the gut smooth muscle. A/C inhibited fansidar induced relaxation, while atropine potentiated action of fansidar. Although the mechanism of action of fansidar is not clear, it is plausible that the effect of fansidar might be direct on the smooth muscle or indirect via neurotransmitter release. The gut vascular smooth muscle exhibited two distinct modes of signaling in the presence of A/C and fansidar: incubation with drug followed by A/C (5 minutes later) significantly ( $p < 0.05$ ) reduced the relaxation responsiveness of the tissue as well as the concentration of the drug compared with the situation when activated charcoal was followed by drug. Fansidar concentration ( $EC_{50}$ ) before and after the application of activated charcoal was  $2.0 \times 10^{-6}$  M and  $1.5 \times 10^{-6}$  M; these elicited respectively, 59% and 41% relaxation of the smooth muscle. These studies show that fansidar relaxes gut smooth muscle, and immediate administration of activated charcoal after drug ingestion reduced the mechanical responsiveness of the smooth muscle to drug. These features may render activated charcoal a unique therapeutic agent in ameliorating the toxicological effects of drugs/poisons in the gastrointestinal tract. There was no detectable difference in the inherent rhythmic spontaneous activity of the ileal smooth muscle in the presence or absence of activated charcoal ( $2.5 \times 10^{-4}$  to  $1.25 \times 10^{-2}$  M).

**Key words:** Anti-malarial drug fansidar, activated charcoal- dual signaling, relaxation, smooth muscle, ileum.

## INTRODUCTION

Anti malarial drugs like quinine and chloroquine in addition to their antimalarial properties hitherto, elicit vascular smooth muscle disturbances (Ebeigbe et al. 1986; Obianime et al. 1994). However, the mechanical responsiveness of the gut smooth muscle to some of the new anti malarial drugs has not been fully understood. Primethamine - sulphadoxine (fansidar) is a drug of choice in the treatment and chemo prophylaxis of malaria in endemic populations, and in particular, is one of the alternate antimalarial drugs used in the treatment of chloroquine - resistant *Plasmodium falciparum* malaria (Anabwani et al. 1996; Nwanyanwu et al. 1996; Wolday et al. 1996; Basco and Ringwald, 1998; Duraisingh et al. 1998; Philipps et al. 1998; Caraballo and Rodriguez-Acosta, 1999). Fansidar is easily

obtainable across the counter in Nigeria and there is the possibility of accidental or deliberate over dosage, yet literature on vascular smooth muscle responsiveness to the drug is still not clear. In

*vitro* studies have shown that the predominant feature of the vascular smooth muscle is its excitation - contraction-coupling ability transduced by  $Ca^{2+}$ . Accordingly, the release of  $Ca^{2+}$ , more specifically, contraction or relaxation, itself, is believed to involve a rise in cytoplasmic  $Ca^{2+}$ , that can be derived from the outside cell via voltage operated channels (VOCs), receptor operated channels (ROCs), voltage - dependent L - type  $Ca^{2+}$  channels, store-operated channels (SOCs) or by released from the sarcoplasmic/endoplasmic reticulum (SR/ER). SR/ER  $Ca^{2+}$  release involves a combination of inositol 1,4,5 trisphosphate ( $IP_3$ ) which functions as a second messenger in a secondary release of  $Ca^{2+}$  by evoking the process of  $Ca^{2+}$  induced  $Ca^{2+}$

release (CICR) (Isenberg, 1994; Yao and Parker, 1994; Yada et al, 1995; Berridge, 1997; Masters et al. 1998). The possibility that changes in membrane potential that results from the opening of  $Ca^{2+}$  sensitive ion channels also regulate  $Ca^{2+}$  movement across the membrane (Yao and Parker, 1994) has been suggested. The duration at half-maximum, the width of the calcium

transient at the point where its concentration is 50% of maximum for  $\text{Ca}^{2+}$  spark is 35ms, spontaneous transient outward current (STOC) 65ms and for spontaneous transient inward current (STIC) 90ms (Berridge, 1997). Thus the variability that exists between different smooth muscle cells makes it difficult to draw general conclusions concerning their calcium signaling mechanisms (Berridge, 1997; Wray, 1997). However, the relative importance of these different contractile machinery/mechanism(s) is not well known, nor how they integrate *in vivo* to elicit changes in vascular smooth muscle and in general.

Recently, in clinical practice as well as in experimental animal and human studies, literature search revealed that activated charcoal constitutes a useful method in ameliorating the toxicological effects of drugs, chemicals and poisons in the gastrointestinal tract (Ali *et al.* 1996; Iddid and Lee, 1996; Tomimaru *et al.* 1996; Manoguerra, 1997; Palatnick *et al.* 1997; Larsen and Cummings, 1998). However, there is little or no information on the mechanical responses of the vascular smooth muscle in the presence or absence of fansidar and /or activated charcoal (A/C). The purpose of this study therefore was to characterize the effects of fansidar and/or activated charcoal using rat ileal smooth muscle preparation, which have not been previously reported. It is recognized that the responsiveness of the different types of smooth muscle to drugs and ionic variations differs (Aziba and Okunola, 1999).

## MATERIALS AND METHODS

Albino rats (*Ratus ratus*) of the Wistar strains of either sex (200 to 250g) were stunned, decapitated and rat ileum dissected into strips of (2 – 3-cm) long and mounted in a bath for isometric tension recording and super perfused

with Tyrode solution (mM: 120 NaCl, 4.0 KCl, 24  $\text{NaHCO}_3$ , 1.0  $\text{mgCl}_2$ , 0.4 $\text{NaH}_2\text{PO}_4$ , 1.8  $\text{CaCl}_2$ , 6.1 glucose and 5.0 sodium pyruvate, 5%  $\text{CO}_2$ , pH 7.34, temperature  $37^\circ\text{C}$ ). After equilibration, strips were subjected to fansidar stimulation in the presence or absence of activated charcoal

(Micromedex Thomson Health care) administered as 50% slurry to produce a dose – response curve. The concentration- dependent responsiveness was then repeated in the presence of receptor agonist acetyl choline (ACh) and receptor antagonist (atropine). Drug dose were expressed as final bath concentrations.

Data are quoted as mean  $\pm$  S.E.M; the number of experiments is given as n, paired or unpaired students 't' test as appropriate, were used to verify statistical significance at  $p < 0.05$ .

## RESULTS

Fansidar between final bath concentrations  $5.0 \times 10^{-2}$  M and  $4.5 \times 10^{-1}$  M evoked concentration- dependent relaxation of the isolated rat ileal smooth muscle (n=8). Strips incubated with fansidar followed by activated charcoal (final bath concentration  $1.25 \times 10^2$  M) elicited comparatively and significantly ( $p < 0.05$ ) smaller relaxation responses compared with those exposed to activated charcoal followed by fansidar (n=8) (fig. 1). Fansidar concentration ( $\text{EC}_{50}$ ) at the point where its concentration is 50% of maximum before and after application of activated charcoal was  $2.0 \times 10^0$  M and  $1.5 \times 10^0$  M (fig.1); these elicited 2.0/3.5 (or 57%) and 1.5/3.5 (or 43%) relaxation of the smooth muscle, respectively. Fig.2 depicted also that, at constant fansidar concentration ( $10^2$  mg / ml) followed by activated charcoal ( $5.0 \times 10^{-2}$  g/L) elicited comparatively smaller (41%) relaxation responses while perfusion with activated charcoal ( $5.0 \times 10^{-2}$  g/ml) followed by fansidar ( $10^2$  mg/ml) evoked

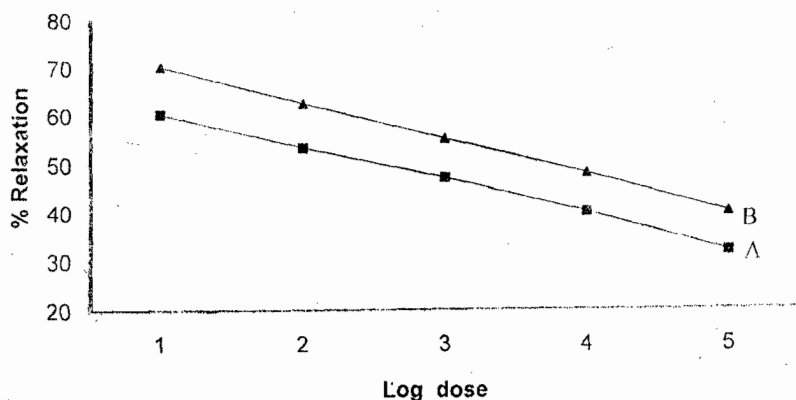


Fig. 1: Responsiveness of smooth muscle to fansidar before and after application of activated charcoal. A: fansidar followed by activated charcoal, B: activated charcoal followed by fansidar.

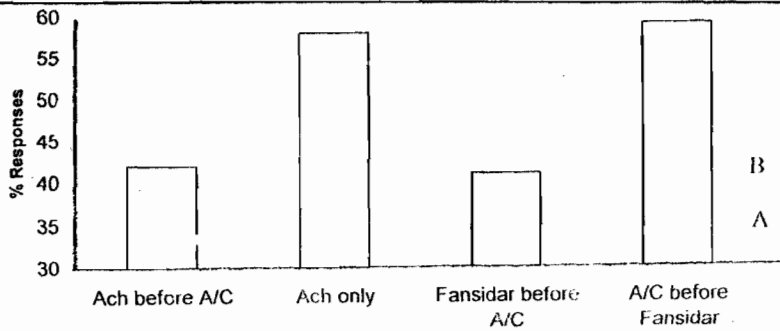


Fig. 2: Responsiveness of smooth muscle to ACh ( $10^{-5}$  mg/ml) and fansidar ( $10^{-7}$  mg/ml) in the presence / absence of activated charcoal (A/C)

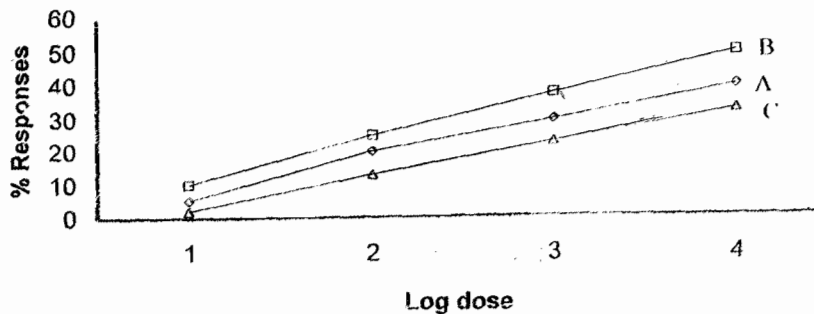


Fig. 3: Responsiveness of smooth muscle to fansidar (A), fansidar and atropine (B), fansidar and acetylcholine (C)

significantly higher (59%) relaxation responses ( $n=6$ ); this difference was also significant ( $p < 0.05$ ). The difference in smooth muscle relaxation evoked by fansidar alone compared with those elicited with the drug followed by activated charcoal was not statistically significant ( $p > 0.05$ ).

On the other hand, strips incubated with ACh ( $10^{-5}$  mg/ml) followed by activated charcoal ( $5 \times 10^{-2}$  g/ml) ( $n = 8$ ) caused comparatively smaller contraction (42%), but strips stimulated by ACh ( $10^{-5}$  mg/ml) in the absence of activated charcoal potentiated contraction (58%), this difference was also significant ( $P < 0.05$ ) (fig 2).

After obtaining a dose-response relaxation effect of fansidar in rat ileal strips, atropine potentiated action of fansidar ( $n=6$ ) while ACh inhibited relaxation responses of the strips ( $n=8$ ) (fig. 3). Atropine concentration response curve were shifted to the left while that of ACh were shifted to the right (fig. 3).

## DISCUSSION

The present results demonstrate that fansidar unlike other antimalarial drugs like quinine and chloroquine (Ebeigbe et al. 1986; Obianime et al. 1994) evoked relaxation responses of the isolated rat ileal smooth muscle, which was inhibited by ACh but enhanced by

atropine. It is not completely clear from the present investigation which mechanism(s) is involved in fansidar-induced relaxation of the gut smooth muscle. Thus it is reasonable to speculate that the effects of fansidar can be explained either by a direct action on calcium channels or indirect via neurotransmitter released or alternatively by an indirect action due to the deformability of the cell membrane (Burton and Hutter, 1990; Ben-Tabou et al. 1994; Yao and Parker, 1994; Yada et al. 1995; Masters et al. 1998). This study revealed that the vascular smooth muscle preparations in the presence of activated charcoal and drug exhibited two distinct modes of signaling or dual action: perfusion with fansidar followed by activated charcoal resulted in reduction in fansidar gradient which greatly reduced its relaxation effects (figs 1&2). This phenomenon perhaps may highlight the mechanics or potent factor which makes activated charcoal (or any other known absorbent) an important gastrointestinal decontaminant after poisoning. This is because the presence of activated charcoal in the gastrointestinal tract after fansidar appears to present a low potential for hazard (in case of accidental or deliberation ingestion of drug). This is in accord with previous studies (Idid and Lee, 1996; Salgia and Kosnik, 1999), which suggested that administration of an

absorbent (activated charcoal) as early as possible after ingestion of poison would decrease the effect of the poison in the gastrointestinal tract.

On the other hand, perfusion with activated charcoal followed by fansidar perhaps led to activated charcoal absorbing as much as possible of other substances extractable from the gut smooth muscle; the absorbent previously loaded with such extractable substances may have similar molecular structure and size to the latter, the resultant effect being potentiative action by the drug (figs 1&2). In fact, it has been observed from *in-vitro* study of the efficacy of absorbents assayed and their *in vivo* application in a range of animals, that addition of activated charcoal to animal feeds sequestered mycotoxins and reduced their gastrointestinal absorption of toxins (Ramos *et al* 1996). However, the kinetics involved in these two distinct modes of smooth muscle-associated- activated charcoal signaling in the presence of drug has not been described.

In conclusion, this preliminary study demonstrated that fansidar relaxes smooth muscle, the effect is significantly modified in the presence of activated charcoal: the absorbent being present in the gut before fansidar must have absorbed some other particles including micro-organisms, thereby exposing the tissue more to the drug. Incubation of the tissue with fansidar followed by activated charcoal reduced

the effects of the drug on the gut smooth muscle. These results suggest that activated charcoal therapy may be beneficial in reducing the harmful effects of drugs in the gastrointestinal tracts.

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