In vitro pharmacologic effect of two endothelin-1 antagonists on equine colonic arteries and veins

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Objective—To evaluate the effectiveness of 2 potential endothelin (ET)-1 antagonists in blocking the contractile responses of equine colonic vessels to increasing concentrations of ET-1.

Sample Population—Mesenteric vessels from 6 clinically healthy horses.

Procedure—Colonic vessels (arterial and venous rings) were placed in organ baths with oxygenated Tyrode solution at 37 C. Each was attached to a force transducer interfaced with a polygraph, and 2 g of tension was applied and equilibrated for 45 minutes. Then, B-1 (PD 142893) and B-2 (PD 145065) ET-1 antagonists were tested. One ring from each vessel type was used as a control for determining concentration-response relationships of ET-1 (10^-10 to 10^-4M). Three rings of each vessel type were incubated with 3 concentrations of each antagonist (10^-8, 10^-6, and 10^-4M for 30 minutes before ET induced contractions were determined. The maximum contractile response and pA2 values were determined.

Results—Vessels contracted in a concentration-dependent manner to ET-1. Arteries responded slowly but reached greater contractions. Veins responded immediately with sustained contractions. Both antagonists inhibited contractions in a concentration-dependent manner with significant differences at 10^-6 and 10^-4M for arteries and 10^-6M for veins. Complete blockade of contractions was observed with B-2 (10^-4M). The pA2 values for B-1 were 8.26 and 6.82 for arteries and veins, respectively, whereas they were 8.25 and 7.21 for B-2.

Conclusion and Clinical Relevance—Both antagonists effectively blocked ET-1-induced contractions of equine colonic vessels. Because B-2 is water soluble and caused complete blockade at 10^-4M, it appears to be the preferred antagonist. (Am J Vet Res 2001; 62:154–159)

Endothelium, a metabolically active layer of cells lining blood vessels, has specialized functions in the regulation of blood pressure, vascular permeability, vascular tone, inflammatory cell adhesion, coagulation, and platelet aggregation. 

Endothelium synthesizes and releases several chemical mediators involved in relaxation and contraction functions that regulate vasomotor tone. The important endothelium-derived vasorelaxants are nitric oxide (NO) and prostacyclin; endothelin-1 antagonists include endothelins (ET) and prostaglandins that induce contractile activity. Endothelins are a family of peptides containing 21 amino acid residues with 2 disulfide bridges linking Cys1-Cys15 and Cys3-Cys11. Three ET (ie, ET-1, ET-2, and ET-3) have been identified, and each is encoded by a separate gene.

Endothelin-1 is the most potent vasoconstrictor known. It contracts vascular and nonvascular smooth muscle. Physiologic functions of ET-1 include aiding closure of the ductus arteriosus immediately after birth and maintaining basal vascular tone via interaction with NO. Also, it has been suggested that ET-1 has a role in mobilizing pooled blood from veins. Endothelin also is involved in various pathologic conditions. It has been suggested that ET-1 is involved in arterial hypertension. Plasma ET-1 concentrations are high in humans with hypertrophic cardiomyopathy. High concentrations of ET-1 are found in bronchoalveolar lavage fluid obtained from asthmatic patients. Excessive production or inadequate degradation of ET-1 is associated with pulmonary hypertension. The production of ET-1 is stimulated by several inflammatory mediators such as vasopressin, angiotensin, bradykinin, thrombin, lipopolysaccharide, tumor necrosis factor-α, and interleukins. Production of ET-1 is increased by low shear stress and decreased by high shear pressure. Enhanced release of catecholamines and formation of angiotensin II that is evident during heart failure stimulates ET-1 synthesis.

Endothelin antagonists have been suggested as a treatment to improve hemodynamic performance and long-term survival in patients with heart failure. Endothelin-1 also may be involved in the systemic inflammatory response to sepsis, endotoxemia, hemorrhagic shock, multiple organ failure, and anaphylaxis. In human medicine, clinical studies conducted with ET antagonists have indicated a potential therapeutic benefit with these agents by lowering vascular resistance.

Many horses with strangulating large-colon volvulus die despite surgical correction and intensive medical care. Sustained reduction in colonic blood flow after correction could account for progression of disruption of the mucosal barrier subsequent deterioration of the condition of affected horses. Blood flow during reperfusion of the large colon after experimental occlusion of venous or arteriovenous vessels may be decreased as a result of increased resistance of the colonic vasculature secondary to venoconstriction.
urally acquired strangulating large-colon volvulus have substantial venous or arteriovenous occlusion with moderate to severe endothelial damage. A substantial body of evidence exists that suggests the vasoconstrictor action of ET-1 could be involved in the pathobiologic aspects of various diseases; similarly, it is quite possible that ET-1 plays a role in colonic vasoconstriction leading to further intestinal injury. Therefore, we hypothesized that administration of a pharmacologic antagonist of ET-1 during the immediate perioperative period after correction of volvulus could be beneficial by reducing resistance of the colonic vasculature. Thus, the purpose of the study reported here was to determine the effectiveness of 2 ET-1 antagonists for inhibiting the in vitro contractile response of equine colonic vasculature to ET-1.

Materials and Methods

Sample collection—The study was approved by the Institutional Animal Care and Use Committee of Louisiana State University. Six adult horses euthanatized for reasons unrelated to the cardiovascular or gastrointestinal systems were used. Horses were euthanatized by injection of sodium pentobarbital (7.5 mg/kg of body weight, IV). Ventral mediastinal celiotomy was performed, and mesenteric vessels (arteries and veins) from the left ventral colon were collected and placed in oxygenated (95% O2-5% CO2) Tyrode solution. Vessel segments were gently cleansed of excess connective and adipose tissue, and cut perpendicular to the long axis into 4-mm-wide rings.

Experimental procedure—One side of each ring was fixed to the floor of an organ bath containing oxygenated Tyrode solution at 37 C, and the other side was attached to a force-displacement transducer interfaced with a polygraph. On the basis of previous studies in our laboratory, an initial tension of 2 g was applied to each ring. The rings were allowed to equilibrate for 45 minutes. During that period, the bath solution was gently replenished with fresh Tyrode solution at 15-minute intervals, and the tension was readjusted to maintain 2g.

After equilibration, 2 ET-1 antagonists, PD 142893 and PD 145063 (ie, B-1 and B-2, respectively) were tested for their effectiveness in inhibiting the contractile response of the vasculature to various concentrations of ET-1. For each antagonist, 4 arterial and 4 venous samples from each horse were used. One ring from each artery and vein was used as a control specimen to determine the concentration-response relationship to ET-1. The other 3 rings of each vessel type were incubated with a specific concentration of ET-1 to determine the concentration-response relationship to ET-1. Each vessel was used for only 1 ET-1 concentration-response curve. Dry weight of each ring was determined on an analytical scale after allowing it to dry at room temperature (20 to 22 C) until additional weight loss was not detected.

Determination of drug antagonism values—A method for the measurement of drug antagonism (pA2) is defined as the negative common logarithm of the molar concentration of an antagonist that will reduce the effect of twice the dose of an agonist to that of a single dose. The pA2 values were determined. Using the concentration-response curves for ET-1, a mean concentration-response curve of ET-1 was constructed for the control samples (without antagonist) and for the treatment groups (3 concentrations of each blocker). Then, the concentration required to produce 50% of the maximal response to ET-1 (EC50), before and after addition of the ET antagonists, was determined for arterial and venous samples. At higher antagonist concentrations, the EC50 values for the ET-1 concentration-response relationship required extrapolation, using a computer software program.

The next step was to determine concentration ratios for each antagonist. The concentration ratio is the ratio of the EC50 of the control curve (without the antagonist) to the EC50 of ET-1 after addition of an antagonist. Because 3 concentrations of each antagonist were used, 3 concentration ratios were determined for each antagonist. Finally, pA2 values were determined by plotting the log (concentration ratio - 1) on the y-axis against the molar concentration of the antagonist on the x-axis. The slope was extended to intersect the point on the x-axis that corresponds to the molar concentration of the antagonist and to zero on the y-axis. Because the y-axis is plotted as a value of log (concentration ratio - 1), the value of zero of the y-axis represents an actual concentration ratio of 2. The negative log (1/molar concentration at which the line intersects the x-axis) is the pA2 value.

Statistical analysis—Responses of the vessels to various concentrations of ET-1 were standardized by representing the contractile responses (ie, milligrams of tension) for each concentration per milligram of dry weight of the vessel samples. Effectiveness of each concentration of the 2 antagonists on the maximal contractile response of the same vessel type at each concentration-response relationship to ET-1 was compared with that for control samples not receiving an antagonist, using an ANOVA. The response elicited by the highest concentration of ET-1 was defined as the maximal response. Comparisons between the response of each antagonist on arteries and veins also were performed, using an ANOVA. Post-hoc comparisons were made, using the Tukey test. A value of P < 0.05 was considered significant for all tests.

Results

Addition of ET-1 produced concentration-dependent contractile responses of equine colonic arterial and venous rings (Fig 1 to 4). Control veins began to contract at a lower concentration of ET-1 (10^-5 M) compared with control arteries (10^-4 M). Veins responded with an immediate but sustained contraction, whereas the responses of arteries were slower to develop. However, the maximal contractile responses of
arteries to ET-1 (255 to 265 mg of tension/mg of dry weight) were greater than the contractile responses of veins (95 to 110 mg of tension per mg of dry weight). Both ET antagonists caused a concentration-dependent inhibition of ET-1-induced contraction of arteries and veins. At a concentration of 10^{-7} M for either antagonist, the ET-1-induced maximal response of venous rings was greater, but not significantly so (P = 0.2), than the values for control rings. On the other hand, arteries responded less, but not significantly so (P = 0.08), to ET-1 after addition of ET antagonists at 10^{-6} M, compared with the response of control arteries. This was most apparent with B-2.

Both antagonists inhibited the contractile responses of arteries and veins to ET-1 at 10^{-6} and 10^{-5} M (Fig 1 to 4). Colonic arteries were significantly (P < 0.001) inhibited at 10^{-6} and 10^{-5} M. However, colonic veins were inhibited significantly (P = 0.006) for both antagonists only at 10^{-5} M. Although not significantly different, B-2 caused more substantial blockade of contraction of colonic arteries and veins, compared with B-1.

Calculated pA2 values for colonic arteries and veins treated with B-1 were 8.26 and 6.82, respectively (Fig 5). For B-2, calculated pA2 values were 8.25 and 7.21 for colonic arteries and veins, respectively.

Discussion
The study reported here revealed important information regarding the response of colonic vessels of horses to ET-1 and the effectiveness of 2 ET antagonists. Colonic veins were more sensitive to ET-1 than arteries, as indicated by their response to a lower concentration of ET-1. Once the tissues begin to respond, arteries contracted to a greater magnitude than veins,
as determined on a force per unit-weight basis, leading to greater maximal contractions for arteries than veins at the highest concentration of ET-1 used in the study (10^{-7}M). The study also revealed that the 2 ET-1 antagonists (B-1 and B-2) were effective blockers of contractile responses of ET-1 on equine colonic arteries and veins. Endothelin-1, a potent contractile agent derived from the endothelium of vessels, contracted colonic arteries and veins in a concentration-dependent manner. The antagonists caused a concentration-dependent blockade of contractions, which indicated the existence of a true antagonism between ET-1 and the antagonists on colonic vessels.

Endothelin-1, an important member of the 3-member peptide family of ET, is the most potent vasoconstrictor currently known in mammals.26 There are 2 types of ET receptors identified for ET-1 (ie, ET_A and ET_B). Stimulation of ET_A receptors, which are located on smooth muscle cells, causes contraction of smooth muscles (vascular and nonvascular), whereas stimulation of ET_B receptors, which are located on endothelial cells, causes relaxation through release of NO.30 Contractions attributable to stimulation of ET_B receptors have been reported in various regional vessels in several species.31,32 Comparison of responses to ET-1 in the study reported here and to norepinephrine in another study conducted by our laboratory group28 indicates that ET-1 produces contraction in equine colonic arteries that is at least an order of magnitude greater than that produced by norepinephrine. The intense vasoconstriction produced by ET-1 likely results in decreased blood flow to organs supplied by those vessels. This property of ET-1 is used physiologically to initiate closure of the ductus arteriosus, maintain vascular tone, and enhance responses of various inflammatory agents.33 Endothelin-1 reportedly is involved in arterial hypertension, cardiomyopathy, bronchoconstriction, pulmonary hypertension, shock, and heart failure.29

The ability of ET-1 to cause intense vasoconstriction and its ability to release NO through stimulation of ET_B receptors leads us to speculate that ET-1 may be involved in ischemia-reperfusion injury. In experimental occlusion of arteries, the failure of reestablished vessels of the large colon, colonic blood flow remains decreased below baseline values for at least 4 hours after cessation of occlusion and restoration of flow.34,35 Similar conditions occur in naturally acquired gastrointestinal tract diseases in horses in which blood flow is reduced or eliminated as a result of intestinal volvulus. Even after surgical correction of large-colon volvulus and restoration of blood flow, 60 to 80% of horses died despite intensive medical and supportive care.37 It is possible that reduced blood flow to the affected region after surgical correction could contribute appreciably to colonic mucosal injury; disruption of the mucosal barrier, and subsequent hypovolemic and endotoxic shock.38 This reduced blood flow could be caused by release of inflammatory and vasoactive mediators including ET-1. In seeking an effective antagonist to prevent or counteract the constriction of blood vessels caused by ET-1, we evaluated the effectiveness of 2 ET antagonists for inhibiting ET-1-induced contraction of colonic vessels. Because it could lead to adverse effects affecting cardiovascular and pulmonary function, we urge caution in the therapeutic use of ET-1 antagonists on a long-term basis, even in horses with diseases that may cause increased concentrations of ET-1.3 However, short-term administration of ET-1 antagonists following surgical correction of naturally acquired colic could potentially be beneficial in preventing a continuation of diminished blood flow in the immediate postoperative period. Transient inhibition of the effects of ET-1 could be useful in restoring blood flow at these critical times without the deleterious effects of chronic ET-1 blockade.

The ET-1 antagonists used in our study are non-specific competitive receptor antagonists that affect ET_A and ET_B receptors.34,35 Competitive receptor antagonism of 2 antagonists can be compared, using pA_2 values. The concept of the use of pA_2 values for measuring antagonism is based on a mathematical formulation in which agonists and antagonists compete for the same receptors, and this competition obeys the laws of mass action. In drug antagonism, the equation of laws of mass action refers to events taking place on receptors rather than to observable responses of the tissues. However, considerations are made for events between receptor activation and the tissue response in applying these equations. Thus, an assumption is made in this mathematical model that equal responses before and after addition of an antagonist involve an equal number of receptors. On the basis of this model, pA_2 values for both antagonists were determined for comparative purposes.

The pA_2 value also is a measure of the affinity of a competitive antagonist for its receptor, which, in the study reported here, reflected the effectiveness of B-1 and B-2 as antagonists of ET-1. The pA_2 value is a mathematical constant.35 The pA_2 value of an antagonist indicates the negative common logarithm of the molar concentration required to reduce the response of the agonist to half, which in our study was a concentration that reduced the effect of ET-1 by half. Thus, the lower the molar concentration required to reduce the response by half, the higher the pA_2 value and the more effective the antagonist.

In the study reported here, B-2 had a higher pA_2 value than B-1 on colonic veins (7.21 and 6.82, respectively). Thus, B-2 was more effective in producing blockade of veins than B-1 (Fig 3 and 4). At 10^{-5}M, B-2 completely blocked the effect of ET-1 at all concentrations used in this study. This caused difficulties in determining EC_{50} values. Use of high concentrations of ET-1 (10^{-5} and 10^{-4}M) was prohibitive because of expense. Therefore, extrapolation of concentration-response relationships, using a computer software program, was required for EC_{50} determinations. Because the pA_2 values and findings from the maximal response analysis correlated well, we assumed that the extrapolated data are a reflection of the true pharmacologic antagonism. On arteries, the pA_2 values of B-1 and B-2 were 8.25 and 8.26, respectively. These pA_2 values indicated that both antagonists were equipotent on colonic arteries (Fig 1 and 3).

The greater pA_2 values for colonic arteries, com-
pared with values for colonic veins, indicated the blocking effect of these ET-1 antagonists was better in arteries, which is also evident from examination of their concentration-response curves (Fig 1 vs 2; Fig 3 vs 4). At 10^{-7} M, both antagonists enhanced ET-1-induced vasoconstriction. One explanation for this phenomenon observed for colonic veins is that these antagonists could act as a partial agonist at lower concentrations. However, this is unlikely, because when the tissues were incubated with the antagonists prior to addition of ET-1, basal tone did not increase. Additionally, this type of response was not observed in the arteries. A more likely possibility for enhanced contraction of veins after addition of the antagonists may be explained by the possibility for enhanced contraction of veins after response was not observed in the arteries. A more like-
non observed for colonic veins is that these antagonists could act as a partial agonist at lower concentrations. ETA receptors leads to contraction, whereas stimulation of ETB receptors leads to relaxation through the release of NO and prostaglandins. In other studies conducted by our group, we documented that denuding the endothelium causes greater enhancement of responses to contractile agents in the arteries than in the veins. Those studies indicated NO plays a major role in the control of arterial caliber by inducing relaxation. Both of the ET-1 antagonists used in the study reported here are nonselective antagonists of ETA and ETB receptors. Because the enhanced effect of ET-1 was observed only in colonic veins, using both antagonists at 10^{-7} M, it is quite likely that the distribution of ETA and ETB receptors plays an integral role in this phenomenon. It is possible that veins contain a lower number of ETB receptors as a result of differences in the endothelium; these ETB receptors may have been blocked completely at the lower concentration of the antagonists, thus preventing relaxation through the release of NO. Simultaneous with complete blockade of ETB receptors, the ETA receptors may have been insufficiently blocked because of a large number of ETA receptors and the difficulty of the antagonists to access these smooth muscle receptors (ie, endothelial layer vs muscular layer). Both B-1 and B-2 were effective blockers of ET-1 effects on equine colonic vessels. These agents were better antagonists for arteries than veins. Because B-2 was a superior blocker and has better solubility in water, compared with B-1, B-2 has advantages for additional studies on potential therapeutic uses in modifying colonic vasomotor tone.

References


