PRELIMINARY INVESTIGATIONS ON THE EFFECTS OF A STRONGYLUS VULGARIS LARVAL EXTRACT, MONONUCLEAR FACTORS AND PLATELET FACTORS ON EQUINE SMOOTH MUSCLE CELLS IN VITRO

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ABSTRACT

Factors involved in the proliferation of equine vascular smooth muscle cells were studied in vitro. The most prominent proliferative responses in cultured vascular smooth muscle cells were induced by Strongylus vulgaris larval antigen extract (LAE) and platelet-derived factors. Less significant proliferative responses were obtained with conditioned media from S. vulgaris LAE stimulated and from unstimulated equine mononuclear leukocytes. Additionally, vascular smooth muscle cells exposed to S. vulgaris LAE developed numerous perinuclear vacuoles and were more spindle-shaped than control or smooth muscle cells exposed to other factors. Equine mononuclear leukocytes exposed to LAE developed prominent morphological changes, including enlargement, clumping and increased numbers of mitotic figures.

Keywords: horse, in vitro proliferation, leukocytes, platelet, smooth muscle, Strongylus vulgaris.

INTRODUCTION
Specific factors responsible for vascular smooth muscle cell alterations in the cranial mesenteric artery have not been investigated. In human atherosclerosis, the smooth muscle cell is important in the pathogenesis of the lesion (Ross, 1985). Most atherosclerosis studies centre around the potential role various factors may have on proliferation or synthetic capabilities of vascular smooth muscle cells. One of the most potent proliferative stimuli investigated to date is platelet-derived growth factor (PDGF) (Bowen-Pope and Ross, 1982; Ross, 1985; Walker et al., 1986). Although platelets are a source of this factor, PDGF-like proteins are also produced by endothelial cells (Gajdusek et al., 1980; DiCorleto and Bowen-Pope, 1983), smooth muscle cells (Nilsson et al., 1985; Walker et al., 1986) and monocytes (Ziats and Robertson, 1981).

Histological examination of the naturally occurring cranial mesenteric arterial lesion has identified several potential stimulants of smooth muscle cell proliferation (Morgan, 1988). Larval antigen was considered to be a potential stimulant since 45%
of the arteries with evidence of arteritis contained larvae, while larvae were never present in normal arteries (Morgan, 1988). Also, factors involved in the initiation and progression of thrombosis may have stimulated smooth muscle cell proliferation, since thrombi were seen in 44% of the arteries with evidence of arteritis but were absent in normal arteries (Morgan, 1988). Additional possible stimulants include factors produced by the inflammatory cells, such as macrophages, lymphocytes and eosinophils, which were commonly found in cranial mesenteric arteries with evidence of arteritis (Morgan, 1988).

The presence of larvae, thrombi or inflammatory cells in affected arteries, as compared to their absence in normal arteries, suggests that factors derived from these may have a role in the pathogenesis of vascular smooth muscle cell changes in the affected arteries by stimulating vascular smooth muscle proliferation. The purpose of this study was to examine the effect of these factors on vascular smooth muscle cells in vitro so as to characterize more adequately their role in the pathogenesis of cranial mesenteric arteries.

MATERIALS AND METHODS

Smooth muscle cell cultures

The vascular tissue was obtained from a 10 cm long segment of the thoracic aorta of a recently euthanized horse by carefully removing the endothelium and adventitia to leave the medial layer. Multiple 2 mm³ sections of medial layer were subjected to limited enzymatic digestion with collagenase (Casey et al., 1984). Smooth muscle cells were washed and resuspended in Dulbecco Modified Eagle's Media containing 10% fetal bovine serum (DME/10% FBS) with 10 units/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml Fungizone. The cells were then aliquoted into 25 cm³ flasks (Falcon, Lincoln Park, New Jersey), allowed to grow to confluency, trypsinized and passaged at a 1:5 ratio. Upon reaching confluent growth, the cells were trypsinized and resuspended in DME/10% FBS containing 10% dimethylsulphoxide and frozen at −70°C for future use. The purity of the cultured smooth muscle cells was confirmed by electron microscopy (Charnley-Campbell et al., 1979). Additionally, cultures of cells were examined with an inverted microscope to ensure that they exhibited the typical morphology and growth pattern of smooth muscle cells (Charnley-Campbell et al., 1979).

Collection and preparation of platelet-containing plasma

Whole blood was collected in acid citrate dextrose (ACD) solution from healthy horses and platelet-poor and platelet-rich plasma were subsequently prepared by differential centrifugation (Busch et al., 1976). In addition, a preparation designated as super platelet-rich plasma was prepared by resuspending platelets which were removed from the platelet-poor plasma into an aliquot of platelet-rich plasma. The platelet release reaction was accomplished in all three plasma preparations by the addition of 0.2 ml of 1.0 mol/L CaCl₂ and 5 units/ml thrombin (Thrombostat, Parke-Davis, Morrow, Georgia).