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Pentoxifylline increases cochlear blood flow while decreasing blood pressure in guinea pigs

J.K.M. Coleman¹, W.S. Quirk², H.A. Dengerink¹ and J.W. Wright¹

¹ Department of Psychology, Washington State University, Pullman, Washington, U.S.A. and ² Kresge Hearing Research Institute, University of Michigan, Ann Arbor, Michigan, U.S.A.

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The effects of pentoxifylline on cochlear blood flow (CoBF) were investigated in anesthetized guinea pigs by laser Doppler flowmetry and intravital microscopy red blood cell velocity measurement. Intra-arterial infusion of pentoxifylline (3, 4, and 5 mg/kg/min) produced dose-dependent reductions in blood pressure, accompanied by significant elevations in CoBF that were not dose-dependent. These results are in general agreement with previous findings from our laboratory utilizing normotensive and spontaneously hypertensive rats, however, in contrast with rats, guinea pigs revealed an initial decrease in CoBF followed by an increase. Also, pentoxifylline produced relatively smaller elevations in CoBF in guinea pigs as compared with those previously reported in rats. Taken together these results support the hypothesis that pentoxifylline increases vascular perfusion by decreasing blood viscosity and increasing the plasticity of red blood cells.

Cochlear blood flow; Blood pressure; Laser Doppler flowmeter; Intravital microscopy; Pentoxifylline; Guinea pig

Introduction

Decreases in cochlear blood flow (CoBF) reduce oxygen and nutrient availability and removal of waste products from the inner ear, and have been suggested to contribute to functional pathologies of the inner ear (Axelsson et al., 1983; Dengerink and Wright, 1988; Hawkins, 1971). These otopathologies include noise-induced hearing loss and Meniere's disease (Borg, 1982; Dulan et al., 1987). Since the cochlear vasculature represents the principal route by which medical treatments can be delivered to the cochlea, increased CoBF may facilitate treatment of these otopathologies (Dengerink and Wright, 1988). However, there are problems associated with increasing CoBF via: 1) vaso-constrictive agents such as angiotensin II (Wright et al., 1985) and vasopressin (Osborn et al., 1987) and blood volume expan-

sion agents such as glycerol (Larson et al., 1982a), dextran (Hultcrantz and Nuttall, 1987), and mannitol (Larson et al., 1982b; Quirk et al., in press) given the concomitant increases in blood pressure; 2) vasodilation using compounds such as vinca-mine (Coleman, unpublished observations) and carbogen (Goodwin et al., 1984; Melnick, 1984) which can promote a hypotensive response; and 3) osmotic agents such as dextran which may produce rebound effects and kidney failure (Hultcrantz and Nuttall, 1987). There is the further problem that increases in CoBF can prompt local autoregulatory responses that may preclude increased blood flow (Lawrence et al., 1977; Miller and Dengerink, 1988; Quirk et al., 1988a, 1989).

Our laboratory has been investigating various strategies to elevate CoBF while attempting to avoid, or diminish the above problems. It has been previously noted that increased blood viscosity and red blood cell (RBC) rigidity are closely related to sensorineural hearing impairment (Browning et al., 1986). We reasoned that if an agent could be found that decreased blood viscosity while having minimal effects on vasodilation,

Correspondence to: John W. Wright, Department of Psychology, Washington State University, Pullman, WA 99164-4820, U.S.A.

blood osmolarity and pressure, its use for increasing CoBF may be superior to the presently available compounds. Pentoxifylline appears to be such a compound. This rheologic agent increases CoBF without elevating blood pressure, presumably by altering RBC membrane flexibility, thus promoting erythrocyte penetration into microcapillaries less than one-half their diameter (Antignani et al., 1987; Ehrly, 1976; Manrique and Manrique, 1987). This improved circulation has been used to inhibit ischemic attacks and increase blood flow in patients suffering from peripheral artery disease (Smith et al., 1986).

A recent report from our laboratory utilizing rats (Quirk et al., 1988b) indicated that pentoxifylline decreased blood pressure in a dose-related fashion while facilitating CoBF. However, the guinea pig's low baselevel blood pressure prompts the question as to whether pentoxifylline will be as effective in this species. We have previously reported that larger doses of angiotensin II were required to produce equivalent elevations in CoBF in the guinea pig as compared with the laboratory rat, while CoBF was more closely correlated with blood pressure in the guinea pig than the rat (Flynn et al., 1989). A second question concerns the validity of CoBF measurements using the laser Doppler flowmeter technique (Nuttall, 1988). Therefore, in the present investigation we compared the influence of pentoxifylline upon CoBF in the guinea pig utilizing the laser Doppler flowmetry and intravital microscopy techniques.

Materials and Methods

Animals and maintenance

Male adult guinea pigs (Hartley strain) 120 to 150 days of age, weighing from 400–600 g, were derived from stock obtained from Simonsen Laboratories (Gilroy, CA) and were housed in an AAALAC approved vivarium on a 12:12 h light : dark cycle initiated at 0700 h, with food and water available ad libitum. All animals in this study were utilized in strict accordance with NIH guidelines for animal research.

Surgical and treatment protocol

Twenty-four animals were randomly assigned to 1 of 3 groups (8 animals each), and anesthetized

with Ketamine Hydrochloride (100 mg/kg, Bristol-Myers, Inc.) and Rompun (2 mg/kg, Haver Inc.). Each animal was prepared with a flow-through catheter of the right carotid artery (PE 60, Clay Adams) designed to allow infusion and continuous recording of blood pressure. Details of this surgical procedure have been described elsewhere (Quirk et al., 1989). The left bulla was exposed and opened for access to the cochlea using a ventral approach. The needle probe (0.9 mm o.d.) of the laser Doppler (Advanced Optokinetics Corporation, Model 2100) was placed on the basal turn of the cochlea via a micromanipulator as described by Goodwin et al. (1984). Blood pressure was monitored using a Statham transducer (Model P23AC) and a Grass Instruments polygraph (Model-5D). Baselevel CoBF and blood pressure were monitored for at least 10 min or until stable. Once baselevel measures of CoBF and systemic blood pressure were achieved, an intra-arterial infusion of 0.15 M NaCl (50 μ l/min) was initiated, followed by one dose of pentoxifylline (Sigma, P-1784: 3, 4, or 5 mg/kg/min in a volume of 50 μ l/min of 0.15 M NaCl, Travenol 2F7124) titrated to a pH of 7.0–7.2. Thus, the animals of each group received a control infusion (0.15 M NaCl) and one of the doses of pentoxifylline with 10 min between the control infusion and the pentoxifylline dose or until baselevel measures were regained. The infusions were counter-balanced with 4 guinea pigs of each group receiving pentoxifylline first and 4 guinea pigs receiving saline first. Blood pressure and CoBF were recorded continuously throughout the baselevel period and subsequent 10 min infusion periods. For statistical purposes only 8 treatments with 0.15 M NaCl were randomly selected from the 24 total runs, and the systemic blood pressure and CoBF changes induced by this control treatment were compared against the effects of the three doses of pentoxifylline.

Three additional guinea pigs were anesthetized as described above and were prepared for measurement of RBC velocity using the intravital microscopy technique as developed by Nuttall (1987a). Again the bulla was exposed for access to the cochlea. A rectangular portion of bone (approximately 0.2 \times 0.3 mm) was scored by knife blade and removed to expose the stria vascularis

vessels in the second turn of the cochlea. These capillaries were monitored by a fluorescence video monitor with a magnification of approximately $1000\times$.

To provide fluorescence of the plasma and thereby the exposed capillary network, 0.05 ml of FITC-dextran (MW = 150 kDa; 5% solution by weight in 0.15 M NaCl) was intra-arterially infused (Nuttall, 1987a,b). A tracking velocity correlator (RBC Velocity Tracking Correlator, Model 102, Instrumentation for Physiology and Medicine, Inc., San Diego, CA) which utilizes photometric signals derived from detectors sensitive to light scintillation or modulation due to moving particles, was used for determining RBC velocity. These RBC velocities were measured before, during, and after the intra-arterial infusion of 0.15 M NaCl (50 μ l/min for 10 min) and pentoxifylline (7.5 mg/kg/min for 10 min).

Statistical analyses

For those animals utilized in the laser Doppler flowmetry experiment, baselevel blood pressures prior to treatment were compared by a one-way analysis of variance (ANOVA). Maximum changes in blood pressure from baselevel were scored at 30 s intervals throughout each 10 min infusion period for each animal. This blood pressure data set was submitted to a 4 (groups) \times 2 (doses) ANOVA with repeated measures on the second factor. The CoBF measures were scored as a percent of baselevel, and because they did not meet the assumptions of the ANOVA they were submitted to Chi-square non-parametric analysis. Significant effects were further evaluated by Newman-Keuls post-hoc tests with the level of significance set at $P < 0.01$.

The blood pressure and CoBF changes measured prior to, and during, the infusion of pentoxifylline using the intravital microscopy technique were evaluated using *t*-tests for related measures.

Results

There were no significant differences in pre-infusion anesthetized baselevel blood pressure among the treatment groups utilized for determination of CoBF by laser Doppler flowmetry.

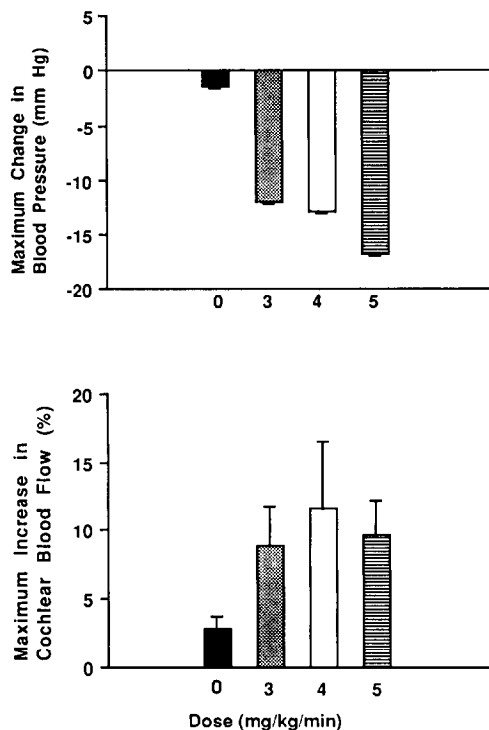


Fig. 1. Maximum changes in blood pressure (upper panel) and cochlear blood flow (lower panel) from baselevel induced by 10 min infusions of 0, 3, 4, and 5 mg/kg/min doses of pentoxifylline. The mean (\pm SEM) blood pressure baselevel before infusion was 52.3 (\pm 1.3) mm Hg. The maximum decreases in blood pressure were 11.9 (\pm 0.3), 12.9 (\pm 0.2) and 16.4 (\pm 0.2) mm Hg for the 3, 4, and 5 mg/kg doses, respectively. The maximum elevations in CoBF were 8.9 (\pm 2.9), 11.6 (\pm 4.9), and 9.7 (\pm 2.5)% for the 3, 4, and 5 mg/kg doses, respectively.

The overall mean (\pm SEM) baselevel blood pressure across groups during the pre-infusion period was 52.3 (\pm 1.3) mm Hg. Fig. 1 (top panel) presents the maximum change in blood pressure induced by each dose of pentoxifylline. Although the 0.15 M NaCl infusion yielded minimal effects on blood pressure, significant decreases were observed following the infusion of the 3, 4, and 5 mg/kg/min doses of pentoxifylline, and these decreases followed a dose-response pattern: 11.9 (\pm 0.3), 12.9 (\pm 0.1), and 16.4 (\pm 0.2) mm Hg, respectively ($F = 18.28$, $df = 3,28$, $P < 0.001$). Post-hoc analyses indicated that each dose was significantly different from the next. These decreases in blood pressure represented percent decrements from baselevel of 22.8, 24.7, and 31.4%, respectively.

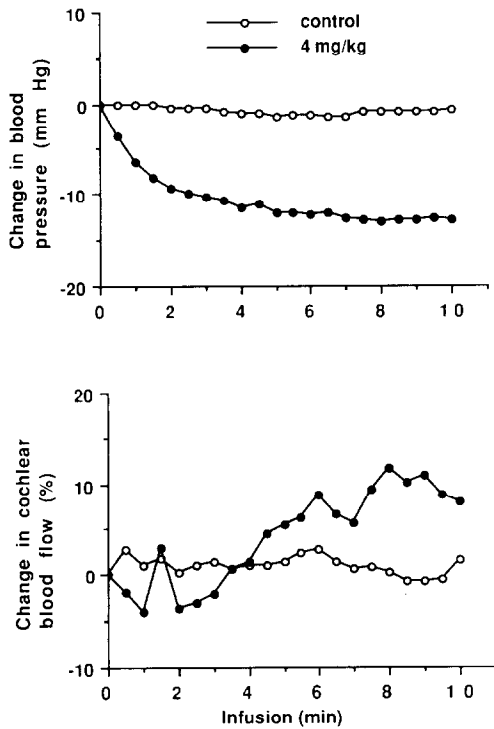


Fig. 2. Mean changes in blood pressure (upper panel) and cochlear blood flow (lower panel) during infusion of 0.15 M NaCl or the 4 mg/kg/min dose of pentoxifylline. The mean decrease in blood pressure during pentoxifylline infusion was 12.9 mm Hg, while the maximum increase in CoBF above baselevel was 12.1%. The 4 mg/kg/min dose of pentoxifylline is representative of the 3 and 5 mg/kg/min doses.

Fig. 1 (bottom panel) displays the maximum changes in CoBF for each dose of pentoxifylline. Chi-square analysis indicated that these elevations in CoBF were significantly different from baselevel. Specifically, the doses 3, 4, and 5 mg/kg/min of pentoxifylline resulted in maximum elevations of $9.9 (\pm 2.1)$, $12.1 (\pm 4.7)$, and $9.7 (\pm 2.5)\%$, respectively. Chi-square analysis for the 3, 4, and 5 mg/kg doses yielded $\chi^2 = 14.75$, 14.75 , and 24.75 respectively, $P < 0.001$. Fig. 2 presents the patterns of changes in blood pressure (top panel) and CoBF (bottom panel) for the 4 mg/kg dose of pentoxifylline which is representative of the other two doses. During the infusion there was a decrease in blood pressure accompanied by an initial decrease in CoBF followed by a gradual continuous increase during the remainder of the infusion period.

There was also a significant decrease in systemic blood pressure during pentoxifylline infusion (7.5 mg/kg/min) evidenced by those animals used for intravital microscopy analysis ($t = 6.25$, $P < 0.001$). This decrease was from a mean (\pm SD) baselevel of $58 (\pm 2.1)$ to $51 (\pm 3.4)$ mm Hg. And there was a concomitant significant elevation in RBC velocity in the stria vascularis vessels in these animals, from $227.7 (\pm 17.9)$ to $248.3 (\pm 22.7)$ $\mu\text{m/s}$ ($t = 2.49$, $P < 0.05$). During the 10 min post-infusion recovery period, systemic blood pressure recovered to baselevel while RBC velocity remained elevated at $255.5 (\pm 23.6)$ $\mu\text{m/s}$.

Discussion

The results of this investigation indicate that pentoxifylline increases cochlear blood flow in guinea pigs as previously reported for the laboratory rat (Quirk et al., 1988b). These elevations in CoBF occurred despite significant decreases in systemic blood pressure that appear to be attributable to the rheological effects of pentoxifylline rather than any vasodilative effects (Ehrly, 1976; Smith, 1986). Although the present results from guinea pigs generally agree with the previous findings in rats (Quirk et al., 1988a) there were differences. In contrast with rats there was an initial decrease in CoBF during the infusion of the 3 and 4 mg/kg/min doses followed by increases that did not follow a dose response pattern as seen in rats. Also in guinea pigs higher doses of pentoxifylline were required for effect and resulted in smaller reductions in absolute blood pressure than were observed in rats (Quirk et al., 1988a), although the average percent decrements in blood pressure from baselevel were greater in guinea pigs than rats. Specifically, the maximum percent decrements in blood pressure from baselevel for rats were 10.2, 17.2, and 21.6%, at the 1.5, 2, and 3 mg/kg doses, respectively; while in guinea pigs these decrements were 22.8, 24.7, and 31.4% at doses of 3, 4, and 5 mg/kg, respectively. The most effective dose of pentoxifylline in those guinea pigs used for laser Doppler analysis was 4 mg/kg which promoted blood pressure decreases of approximately 13 mm Hg, and CoBF increases of approximately 12% above baselevel. Although the 5 mg/kg dose did not follow a dose-response

pattern it illustrates the effectiveness of pentoxifylline in elevating cochlear blood flow despite a blood pressure decrease of nearly 16.4 mm Hg (31.4% from baselevel). The increase in CoBF persisted beyond the period of infusion.

The intravital microscopy results generally agree with those derived from laser Doppler flowmetry in that systemic blood pressure decreased during the infusion of pentoxifylline, while cochlear red blood cell velocity was elevated. It should be noted that a higher dose of pentoxifylline was employed in the animals used for intravital microscopy measurements, and this dose yielded a 7 mm Hg decrease in systemic blood pressure from baselevel, while the decreases noted in the animals used for laser Doppler analysis ranged from 11.9 to 16.4 mm Hg. At present we have no ready explanation for this discrepancy. It is conceivable that this dampening of effect is due to differences in the amount of anesthetic given to these two groups of animals. Specifically, because intravital microscopy is more sensitive to animal movements than the laser Doppler flowmeter, the intravital microscopy animals may have been inadvertently maintained at a somewhat deeper anesthetic plane. This may have made the animals less responsive to the hypotensive effects of pentoxifylline (Flynn et al., 1988). Histological measures concerned with the impact of noise exposure on the cochlear vasculature generally suggest reduced blood flow with exposure to noise (Axelsson et al., 1981; Axelsson and Dengerink, 1987; Dengerink et al., 1985). These findings have been corroborated with laser Doppler flowmetry (Thorn and Nuttall, 1987) and intravital microscopy (Quirk et al., 1990) studies. Thus, if acoustic trauma results from diminution of blood flow, then pentoxifylline may help to increment blood flow. With respect to basic research, compounds such as pentoxifylline may facilitate our understanding of cochlear blood flow dynamics and thus provide insight into the development of new more effective pharmacological treatments for otopathologies of vascular origin.

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