Elevated Potassium Intake Inhibits Neointimal Proliferation in the Swine Coronary Artery


Background: Previously, we reported that elevated extracellular potassium concentration in vitro inhibited proliferation and migration of vascular smooth muscle cells, formation of free radical compounds by macrophages, and reduced platelet sensitivity to agonists. More recently, we described a reduction in neointimal proliferation after balloon angioplasty injury in the carotid arteries of rats associated with an elevation of dietary potassium intake during a 4-week experiment. In the present study we conducted a similar investigation in the swine coronary artery balloon angioplasty model.

Procedures: Two groups of seven castrated male swine were studied; for 28 days the normal potassium group consumed a diet containing 0.25% potassium and the high potassium group ate diet containing 2.0% potassium. After 14 days on the diet, balloon angioplasty was performed. After an additional 14 days on the same diets the hearts were removed, and normal and lesioned sections of the artery were analyzed histologically.

Results: The neointimal area was markedly less in the high potassium group than in the normal potassium group, \(0.33 \pm 0.04 \text{ mm}^2\) vs. \(0.74 \pm 0.10 \text{ mm}^2\) \((P < .004)\). Neointimal area-to-total wall area ratio in the normal potassium group averaged 0.199 \(\pm\) 0.018, significantly greater than the ratio computed for the elevated potassium group, 0.120 \(\pm\) 0.015 \((P < .006)\).

Conclusion: These results support the hypothesis that a high level of dietary potassium intake inhibits neointimal proliferation after balloon angioplasty in the swine coronary artery.

Key Words: Angioplasty, diet, restenosis, thrombosis, vascular smooth muscle.

Percutaneous transluminal coronary angioplasty has been used extensively since the technique was introduced 20 years ago by Gruntzig et al. Treatment of stable and unstable angina, and acute myocardial infarction are the most common applications of the procedure, accounting for more than 350,000 cases each year. Currently, in addition to the original procedure involving inflation of a balloon at the site of the lesion, other approaches are also in use, including atherectomy, laser ablation, and endovascular stenting. However, with all of these interventions, the benefits are short lived in many patients due to redevelopment of stenoses at the site of the initial lesion.

In humans after angioplasty restenosis results principally from development of a neointimal proliferative lesion with growth of the wall into the lumen. The underlying basis for restenosis is a response of the artery to vascular trauma with injury to endothelial cells and vascular smooth muscle cells. Four interrelated processes are believed to be involved: 1) thrombosis, due to laceration of the original plaque and exposure of its interior to blood; 2) smooth muscle cell migration from the media to intima; 3) smooth muscle cell proliferation; and 4) fibrosis due to the accumulation of extracellular matrix proteins. Although attempts to design preventive measures have focused on several of these processes, the only notable success in human studies has been with inhibitors of the platelet glycoprotein IIb/IIIa receptor.

Observations by other researchers that population groups that consume diets rich in potassium enjoy significant protection from stroke and other common forms of vascular disease, and the series of reports from Tobian’s laboratory describing vascular protective actions associated with elevated dietary potassium intake in spontaneously hypertensive rats, led us to undertake a series of investigations designed to elucidate possible mechanisms...
that could explain potassium’s beneficial effects. Some of our results demonstrate the following: 1) elevation of extracellular potassium concentration in vitro inhibits proliferation and migration of vascular smooth muscle cells;\textsuperscript{10–12} 2) platelet sensitivity to agonists in vitro is reduced by elevation of potassium concentration;\textsuperscript{13} and 3) canine coronary artery thrombosis in vivo (Folts model) is strongly inhibited by acute elevation of potassium concentration in the plasma.\textsuperscript{13}

Because these processes are believed to contribute to formation of the restenosis lesion as well as other vascular lesions, we tested potassium’s ability to affect development of neointimal proliferation after balloon angioplasty. In our initial study we found an inverse relationship between dietary potassium intake and neointimal proliferation after balloon injury in the rat carotid artery.\textsuperscript{14} This report describes the results of a similar investigation of neointima formation after balloon angioplasty in the coronary arteries of swine. The lesion that develops in this model is similar in many respects to that found after angioplasty in the human coronary artery.\textsuperscript{2,3,15}

**Methods**

Two groups of seven castrated male swine were included in the study. The University of Mississippi Medical Center Laboratory Animal Facilities obtained the animals from a local commercial breeder when they were 6 to 8 weeks of age and weighed approximately 17 kg. They were housed in individual cages, were fed once daily, and had free access to water.

The diets were prepared to our specifications by Purina Test Diets (Richmond, IN). The two preparations were identical except for the potassium content. The normal diet (formula number 5841M-7) contained 0.25% potassium (from native ingredients) and the high potassium diet (5841M-9) had a content of 2.0% (additional potassium was added as KCl). Both contained 0.25% sodium. The animals consumed 3% of their body weight per day of the diet.

Both groups were fed their prescribed diets for 14 days before the angioplasty procedure. On the day preceding the procedure they were transported from the University of Mississippi Medical Center to the University of Alabama at Birmingham Medical Center where they were housed over night without access to food. The following day they were taken to the Interventional Cardiology Research Laboratory where angioplasty was performed.

Preoperative sedation was achieved by a combination of ketamine (20.0 mg/kg), xylazine (2.0 mg/kg), and atropine (0.05 mg/kg), all given intramuscularly. The animals were intubated and a surgical level of anesthesia was maintained with halothane and oxygen. Under aseptic conditions the right femoral artery was exposed and cannulated with an 8 or 9F sheath. The swine received 200 U/kg of heparin given intravenously. Coronary balloon angioplasty was then performed on an unbranched section of the proximal left circumflex artery. The balloon was inflated six times, each for 30 sec, to give a diameter approximately 25% to 30% greater than the diameter of the vessel. Throughout the procedure the animals’ blood pressure (BP), heart rate, and electrocardiogram were monitored continuously. After the procedure, the femoral artery was ligated and the wound was closed.

The day after the procedure the animals returned to University of Mississippi Medical Center where they were maintained for an additional 14 days on the specially formulated diets. On the 14th day the animals were again sedated and anesthetized. The heart was removed, perfused from the thoracic aorta at 100 mm Hg, first with 500 mL of saline solution, then with 500 mL of buffered 10% formalin. Hearts were shipped to University of Alabama at Birmingham Medical Center, Department of Pathology, for examination of the injured vessels.

**Tissue Preparation and Analysis**

The left circumflex coronary artery containing approximately 1 to 2 cm of normal coronary just proximal and distal to the injured segment was dissected from the heart. The vessel was serial sectioned at 2- to 3-mm intervals, 5-μm sections, starting 1 to 2 cm proximal to the injured segment, continuing through the entire injured segment, including 1 to 2 cm of normal vessel distal to the injured segment. Sections were embedded in paraffin and fixed to glass microscope slides.

**Morphometric Measurement**

Histologic sections were coded and examined by one of us (PGA) blinded to the treatment of the animal. The presence of medial dissection, any disruption of internal or external elastic lamina, and the amount of thrombotic material were quantitated using a modification of the scoring system described by Karas et al.\textsuperscript{16} Briefly, the degrees of medial laceration and external elastic lamina stretch were given a grade of 0 for no injury, 1 for partial medial laceration, 2 for complete medial laceration, and 3 for complete medial laceration and stretching of the external elastic lamina. The amount of thrombotic material at the injury site was evaluated using a scoring system of 0 to 5, with 0 indicating no thrombotic material discernible within the neointima and 5 indicating a well-formed thrombus within the injured vessel segment.

Digital images of two to three histologic vessel cross sections from uninjured segments proximal and distal to the injured vessel segment were used to obtain the mean values for vessel outer diameter, vessel wall area, lumen area and vessel wall thickness. The two to three sections for the injured artery segment that demonstrated the greatest degree of vessel injury were similarly evaluated to obtain the same vessel measurements; however, in the injured artery segments the total vessel wall area included both the media and neointima. The maximal neointimal
The animals consumed their entire ration each day, and as a result gained weight at a normal rate during the 28-day feeding period; the normal potassium group gained an average of 6.7 ± 0.5 kg and the high potassium group gained 6.2 ± 0.3 kg.

The fluoroscopically estimated vessel diameter before angioplasty averaged 2.4 ± 0.2 mm in the normal potassium group, and 2.4 ± 0.1 mm in the group eating the high potassium diet. The inflated diameter of the balloons used in the normal potassium group average 3.1 ± 0.2 mm, and in the high potassium group the mean value was 3.0 ± 0.1 mm. Vessel dimensions at the lesion site 14 days after angioplasty are given in Table 1. Although the outer diameters of the two groups were similar, the lumen area of the high intake group averaged 3.36 ± 0.07 mm², significantly larger than the area of the group on normal potassium intake, 3.05 ± 0.12 mm². The wall area-to-lumen area ratio of the high intake group was significantly less than that of the normal intake group, 1.25 ± 0.03 v 1.96 ± 0.25.

At the time of the angioplasty procedure the normal potassium group gained weight at a normal rate during the 28-day feeding period; the normal potassium group gained an average of 6.7 kg, whereas the mean from the high potassium group was 3.95 ± 0.12 mmol/L, significantly greater than the normal potassium group mean (P < .003) (Table 2). At the time of sacrifice a second sample was collected by direct puncture of the aorta. The mean values were not significantly different for the two groups; the normal potassium mean was 3.64 ± 0.14 mmol/L and the high potassium group mean was 4.09 ± 0.26 mmol/L (P < .191). Analyses for plasma renin activity (PRA) and plasma aldosterone concentration were performed on the blood samples obtained at the time of sacrifice. The PRA mean values were 1.01 ± 0.22 and 0.41 ± 0.16 ngAI/mL/h for the normal and high potassium groups, respectively (P < .081); the plasma aldosterone group means were 109.7 ± 9.8 ng/dL and 220.9 ± 67.0 ng/dL for the normal and high groups (P < .124).

Oversize balloon injury in the normal swine coronary artery produced damage to the media, which in most cases resulted in laceration. In many of the vessel segments evaluated the media was completely transected in one location and the external elastic lamina had been stretched. The injury scores for the two groups were similar, 2.53 ± 0.06 for the normal group, and 2.59 ± 0.16 in the high potassium group. This level of injury indicates that in the majority of the specimens, the media were partially or completely lacerated. At 14 days after angioplasty, the discontinuity of the media was filled by neointima. The neointima consisted of primarily smooth muscle cells that had a fusiform to somewhat stellate morphology with pale eosinophilic cytoplasm, consistent with the secretory smooth muscle phenotype. An abundant extracellular ma-

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<th>Table 1. Vessel dimensions at the lesion site</th>
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W/L ratio = wall area-to-lumen area ratio; NS = not significant.

Results

The animals consumed their entire ration each day, and as a result gained weight at a normal rate during the 28-day feeding period; the normal potassium group gained an average of 6.7 ± 0.5 kg and the high potassium group gained 6.2 ± 0.3 kg.

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At the time of the angioplasty procedure the normal potassium group mean value for potassium concentration was 3.07 ± 0.19 mmol/L, whereas the mean from the high potassium group was 3.95 ± 0.12 mmol/L, significantly greater than the normal potassium group mean (P < .003) (Table 2). At the time of sacrifice a second sample was collected by direct puncture of the aorta. The mean values were not significantly different for the two groups; the normal potassium mean was 3.64 ± 0.14 mmol/L and the high potassium group mean was 4.09 ± 0.26 mmol/L (P < .191). Analyses for plasma renin activity (PRA) and plasma aldosterone concentration were performed on the blood samples obtained at the time of sacrifice. The PRA mean values were 1.01 ± 0.22 and 0.41 ± 0.16 ngAI/mL/h for the normal and high potassium groups, respectively (P < .081); the plasma aldosterone group means were 109.7 ± 9.8 ng/dL and 220.9 ± 67.0 ng/dL for the normal and high groups (P < .124).

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Other abbreviation as in Table 1.
trix material surrounded the smooth muscle cells. There were occasional small blood vessels deep within the neo-intima and occasional inflammatory cells, primarily macrophages and a few lymphocytes. Deep within the neo-intima and along the edge of the external elastic membrane there was often an accumulation of thrombotic material. The presence of thrombotic material was more frequent and more severe in specimens from the normal potassium group than in the high potassium group.

The neointimal thickness (Fig. 1) in the hyperkalemic group averaged 58% less than that of the normal potassium group (267 ± 18 μm v 708 ± 111 μm, P < .002); the neointimal area (Fig. 2) was also markedly less in the high potassium group than in the normal potassium group, 0.33 ± 0.04 mm² v 0.74 ± 0.10 mm² (P < .004). Neointimal area-to-total wall area ratios were computed (Fig. 3); in the normal potassium group the ratio averaged 0.199 ± 0.018, significantly greater than the ratio computed for the elevated potassium group, 0.120 ± 0.015 (P < .006). Similarly, the neointimal-to-medial area ratio was significantly less in the high potassium group than in the low intake group, 0.138 ± 0.19 v 0.252 ± 0.28, respectively.

The thrombus score data are presented in Fig. 4. The high potassium group’s score averaged 72% less than the score from the normal potassium group, 0.59 ± 0.20 v 2.14 ± 0.20 (P < .006).

**Discussion**

The results of this study extend those of our previously reported work in the rat model of neointimal proliferation in the carotid artery after balloon angioplasty. As in the previous study, elevated potassium intake was associated with a strongly significant reduction in neointimal formation. Although these results are confirmatory of our previous studies, and results of both studies are consistent with the hypothesis that potassium has a vascular protective effect, additional investigations using longer time courses, more moderate potassium intake levels, and more mature animals must be undertaken to provide the comprehensive evidence necessary to extend the implications of the hypothesis toward a clinical setting.

The mechanisms involved in the response to balloon injury that may have been affected by elevated dietary...
potassium intake cannot be determined from the data obtained in this study. However, we demonstrated previously that elevation of extracellular potassium concentration markedly reduced the rate of three processes believed to be causally related to neointimal proliferation and restenosis lesion development: thrombus formation, vascular smooth muscle cell migration, and vascular smooth muscle cell proliferation. Potassium’s potential effectiveness in inhibiting thrombosis was analyzed using an in vivo model in the dog circumflex artery as initially described by Folts. An acute increase in the potassium concentration from 3.53 ± 0.05 to 6.10 ± 0.09 mmol/L reduced by 56% the rate thrombus formation. The importance of thrombus formation in neointimal development was emphasized by the experiment by Willerson et al who observed a strong correlation between frequency of cyclic blood flow reductions in damaged coronary arteries of dogs and the severity of neointimal thickening during a 21-day period. When inhibitors of thrombus formation were given to the animals, well-correlated reductions in cyclic blood flow and neointimal thickening were recorded. In the present study the finding of a thrombus score in the high potassium group 72% lower than in the normal intake group is consistent with an antithrombogenic effect of potassium being causally related to the reduction in neointimal proliferation observed in the high potassium intake group. Vascular smooth muscle cell migration was studied in vitro, and was found to be inhibited by elevation of potassium concentration in the medium within the physiologic range, from 3 to 6 mmol/L. Potassium’s effect on proliferation of vascular smooth muscle cells was also studied in vitro. Increasing potassium concentration from 3 to 7 mmol/L reduced cell numbers by 61%, and increasing the concentration from 3 to 4 mmol/L reduced the count by 18%. An effect of elevated extracellular potassium concentration associated with greater than normal intake on either vascular smooth muscle cell migration, proliferation, or thrombus formation could provide the mechanism for the observed inverse relationship between potassium intake and neointimal lesion formation.

The effects of increases in potassium concentration on these vascular cell function may be mediated by stimulation of the activity or expression of sodium, potassium-ATPase in the cell membrane, which would decrease intracellular sodium concentration and increase the concentration gradient driving the sodium–calcium exchange mechanism. This is the hypothesis proposed by Canadry et

** FIG. 2. Neointima area data from the normal and high potassium (K) intake groups. The area of neointima averaged 50% less in the high potassium intake group than in the normal intake group. Double asterisk denotes statistically significant difference between the mean values of the two groups, P < .01.**
al\textsuperscript{18} to account for the inhibition by potassium of cell proliferation and DNA synthesis in glial cells in culture. In studies in vascular smooth muscle, the reduction in tension associated with elevation of potassium concentration in the physiologic range has been attributed by Haddy\textsuperscript{19} and Haddy and Scott\textsuperscript{20} to an increase in sodium, potassium-ATPase activity. Also, Songu-Mize et al\textsuperscript{21} demonstrated that physiologic increases in dietary potassium intake or in plasma potassium concentration act in vivo to increase vascular smooth muscle sodium, potassium-ATPase activity. Intracellular sodium concentration is known to increase markedly before initiation of vascular smooth muscle proliferation\textsuperscript{22}; increases in extracellular potassium concentration from 3 to 7 mmol/L were shown by McCabe and Young\textsuperscript{10} to reduce by 74\% the increase in intracellular sodium concentration in vascular smooth muscle just before serum-stimulated proliferation. In those studies, the proliferation rate was also markedly reduced by increases in potassium concentration. Increases in the same range in extracellular potassium concentration were shown by Jones\textsuperscript{23} to increase sodium efflux from vascular smooth muscle cells in vitro. Similarly, in other cell types increased expression of sodium, potassium-ATPase induced by hyperkalemia has been associated with reduction in intracellular sodium concentration.\textsuperscript{24}

Any reduction in intracellular sodium concentration associated with elevation of extracellular potassium concentration could be expected to stimulate calcium extrusion from the cell by the sodium–calcium exchange mechanism, and subsequently, lead to a reduction in intracellular calcium concentration. There is increasing evidence that intracellular calcium concentration plays critically important roles in regulating vascular smooth muscle cell migration,\textsuperscript{25,26} proliferation,\textsuperscript{27,28} and platelet activation.\textsuperscript{29} This series of interacting hypotheses, each of which is well substantiated individually, relating changes in extracellular potassium concentration to regulation of intracellular calcium activity may explain the cellular mechanisms responsible for potassium’s vascular protective effects.

The plasma renin activity data from the high potassium intake group averaged 0.41 ± 0.16 ng AI/mL/h, less than half the average of the normal intake group, 1.01 ± 0.22 ng AI/mL/h. Although the group means were not significantly different in this study, other investigators\textsuperscript{30,31} have consistently reported an inverse relationship between po-
The observation of potassium’s effectiveness to markedly reduce neointimal development in the pig circumflex artery after angioplasty is consistent with our previous findings of potential vascular protective effects in in vitro experiments, during short-term in vivo experiments in dogs, and in long-term studies in rats and rabbits.

The mechanisms of potassium’s effects may include inhibition of thrombus formation and a direct effect on proliferation or migration of vascular smooth muscle cells. This series of positive results in a wide range of models and time courses encourages us to pursue the potential of potassium’s beneficial clinical effects.

References