Folic acid (from Latin folium, meaning leaf; pteroylglutamic acid) is a B vitamin that facilitates the transfer of 1-carbon units in numerous biosynthetic reactions for 2 classes of important cellular functions: biological methylation and the contribution of formyl units to the synthesis of nucleotides (Figure). The interest in folic acid for the treatment of cardiovascular disease stems from its critical role in converting homocysteine to methionine. Hyperhomocysteinemia was found to be associated with a higher risk of cardiovascular disease in epidemiological studies, and dietary folic acid fortification lowers plasma homocysteine levels. Although recent trials have failed to demonstrate a benefit of lowering homocysteine for cardiovascular disease, it is not time to close the book on folic acid and cardiovascular health. Folic acid has been found to improve endothelial function independent of its homocysteine-lowering effect in several clinical studies. A dramatic cardioprotective effect of folic acid reported by Moens and colleagues in this issue of Circulation could potentially bring folic acid to the center stage in the management of ischemic heart disease.

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In this study, Moens et al9 treated rats with a very high dose of folic acid for 1 week (10 mg/d, roughly 400 times over the high recommended clinical dose of 5 mg/d when normalized to body weight) and then subjected them to myocardial ischemia (ligation of anterior coronary artery for 30 minutes). The hearts of the treated rats maintained cardiac function during ischemia and developed virtually no infarct after reperfusion, whereas the hearts of untreated rats suffered severe injury and developed an infarct in 60% of the area at risk. Perhaps most remarkably, a similar protection could also be achieved by acute intravenous delivery of folic acid 10 minutes after the onset of ischemia, suggesting that the protection is attributable to the nongenomic effects of folic acid. Thus, the protective effects of a super high dose of folic acid are comparable if not superior to ischemic preconditioning, the most powerful protection against ischemic injury demonstrated so far; yet, it can be achieved by treatment initiated after the onset of ischemia.

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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How Does Folic Acid Cure Heart Attacks?

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What are the underlying mechanisms of such a dramatic effect? Several possibilities can be considered based on the metabolic role of folic acid (Figure). Folic acid or its metabolite 5-methyl tetrahydrofolate has been shown to reduce superoxide production. In addition, 5-methyl tetrahydrofolate improves nitric oxide production and prevents superoxide generation via uncoupling of nitric oxide synthase by stabilizing tetrahydrobiopterin, a critical cofactor of endothelial nitric oxide synthase, or by regenerating tetrahydrobiopterin from its inactive form (dihydrobiopterin). These mechanisms likely account for the benefit of folic acid on endothelial function observed in previous studies. Consistent with these studies, Moens et al9 also observed antioxidant effects of folic acid in their model and presented evidence that endothelial nitric oxide synthase uncoupling was markedly reduced in the ischemic hearts treated with folic acid. As the authors noted, however, considering the relatively low potency of folic acid or 5-methyl tetrahydrofolate in free radical scavenging compared with other powerful antioxidants such as vitamin C or tempol, it seems unlikely that the cardioprotective effect of folic acid could be solely attributed to its antioxidant capacity. It has not yet been determined, however, whether the very high dose of folic acid used in the study yields a substantially greater antioxidant effect than was previously attainable in vivo. Nevertheless, the authors showed that a similar effect could be achieved with 10% of the dose, that, 1 mg/d. To determine whether the dose is a critical factor for cardioprotection, the threshold effective dose needs to be identified, and the tissue level of folic acid and its metabolites should be determined in subjects treated with the threshold dose.

An interesting and novel hypothesis proposed by Moens et al9 is that supplying high concentrations of folate would increase purine synthesis and thus sustain ATP levels in the heart during ischemia. Folate participates in the transfer of 1-carbon formyl units from donor molecules to form purine and pyrimidines (thymine). Folic acid itself does not have coenzyme activity and must be converted to tetrahydrofolate acid (THF). The THF coenzymes that are active in purine biosynthesis are N9,N10-methenyl THF and N10-formyl THF, both of which donate formyl groups to the construction of the purine ring (Figure). Also important for purine biosynthesis, folate mediates the interconversion of serine and glycine, the latter of which is also used to build the purine ring (Figure). Thus, the biochemistry for the key role of folate coenzymes for the synthesis of the purine ring is well understood.

The loss of purines from the ischemic myocardium has been appreciated for decades and is a hallmark of ischemia. Identifying ways to prevent the loss of purines or to increase de novo purine synthesis is the holy grail of cardiac biochemists. Many have tried, as the stakes are high. During ischemia, when ATP synthesis by oxidative phosphorylation is severely impaired after the onset of ischemia, the most powerful protection against ischemic injury demonstrated so far; yet, it can be achieved by treatment initiated after the onset of ischemia.

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reduced and the energy reserve compounds such as glycogen and phosphocreatine have been consumed, the mismatch in ATP demand and ATP synthesis leads to a loss of purines. As shown by experiments measuring the rate of repletion of the ATP pool after ischemia in the Reimer/Jennings and Braunwald laboratories in the 1980s, among others, de novo purine synthesis is many orders of magnitude slower than ATP synthesis via glycolysis or oxidative phosphorylation. It takes 4 to 5 days to replete 30% of the ATP pool after regional ischemia in the dog heart. De novo purine synthesis takes a great deal of energy, requiring 9 ATPs to make 1 new purine ring. The idea that supplying folate could accomplish this is almost too good to be true. Nevertheless, there are convincing physiological data showing that supplying high concentrations of folate protects the purine pool and promotes better contractile performance. A recent article by Lamberts et al. reported preserved diastolic function and preserved total adenine nucleotides in a rat model of right heart hypertrophy when animals were supplied with folate in a dose similar to that used by Moens et al.; folate was supplied along with D-ribose for 6 weeks. The strategy of Lamberts et al. was to supply precursors of both the purine base and the sugar moiety of ATP. Their results are impressive; preserved total adenine nucleotides were found in treated hypertrophied rat hearts, whereas total adenine nucleotide levels fell by as much as 50% in untreated rats. Thus, there is precedent for preservation of the total adenine nucleotide pool with a long-term supply of high doses of folate, at least in combination with ribose.

Is there evidence in the report by Moens et al. of increased de novo purine synthesis? Several important omissions in the presentation of the purine data make it difficult to draw firm conclusions about whether folate prevented the development of infarct by preserving purine pools. The ATP values for control hearts (Figure 3 of Moens et al.) are about half of what has been reported for rat hearts, and the ratio of AMP to IMP is also too low. Another is the omission is in the reporting of the major nucleosides adenosine and inosine in the ATP degradation pathway (ATP → ADP → AMP → adenosine → inosine → hypoxanthine → xanthine). Inosine and hypoxanthine are the major unphosphorylated purines that accumulate in ischemic tissue. A significant amount of uric acid produced from xanthine could come from nonmyocytes in the heart, and how much should be added to the myocyte purine pool is unclear. Finally, it is unfortunate that they ignored the guanines, as the fate of the purine ring from IMP requires knowing both the adenine and the guanine pools (Figure).

The most important question to ask is whether the idea that high-dose folate preserves ATP by increasing de novo purine synthesis has any support from the data presented. The observation that pretreatment was not necessary to prevent infarction is key here, for it defines a time period during which the proposed mechanism could operate. Looking along the ATP synthesis/degradation pathway, comparisons of hypoxanthine, xanthine, uric acid, IMP, and AMP in folate-supplied and untreated ischemic rat hearts show essentially no differences due to treatment. Note that adenosine and inosine were not included in this analysis, and that biochemical measurements were not made during reperfusion. The major difference is in the levels of ATP and ADP in the ischemic heart: 1047 versus 1682 nmol/g. Could 600 nmol adenine nucleotide/g tissue be made in 20 minutes in the rat heart via de novo ATP synthesis? Mauser et al. measured de novo synthesis in control Wistar rats as 1.5 nmol/g wet weight per h, in good agreement with the original measurements made by Zimmer et al. Ischemia increased the rate ≈ 2-fold, ribose 5-fold, 5-aminoimidazole-4-carboxamide ribonucleoside 9-fold, and adenosine 90-fold, to ≈ 135 nmol/g wet weight per h. Making 600 nmol/g wet weight in only 20 minutes or making 1800 nmol/g per h is another factor of 15 over supplying adenosine, which supplies ribose as well as purine ring. As de novo purine synthesis requires 9 ATPs for every 1 new ATP made, this amount would have to be multiplied by another factor of 10. Finally, one cannot help
but wonder where the ribose came from for the folate effect. If increased de novo synthesis rather than preservation of nucleotides secondary to reduced ATP utilization were the mechanism explaining folate-protection from infarction, it would be an extraordinary stimulation of a very slow metabolic pathway.

Thus, the remarkable, almost magical, effect of folate on the ischemic myocardium has no clear mechanism. The work by Moens et al9 raises more questions than it provides answers to the role of folic acid in the management of ischemic heart disease. As with all new ideas, their study points the way to many future experiments, including biochemical analysis of the reperfused myocardium, direct measurement of de novo purine synthesis, determination of the effects of folate on severe ischemia, identification of the minimally effective dose, determination of the requirement for ribose, etc. As the effects demonstrated here are so powerful and as folic acid is highly affordable, further investigations are certainly warranted for both scientific and social causes.

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References

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