Synergism Among Flavonoids in Inhibiting Platelet Aggregation and H$_2$O$_2$ Production

To the Editor:

In vitro and in vivo studies carried out by Freedman et al. demonstrated that purple grape juice inhibits platelet aggregation and production of superoxide anion and increases the platelet formation of nitric oxide. These findings are of potential relevance for explaining the cardioprotective effect of grape juice and red wine. The authors sought also to investigate the mechanism by which grape juice inhibits platelet function and observed a consistent difference in terms of platelet inhibition among the 5 fractions of grape juice containing flavonoids. We agree with the authors that flavonoids, which are constituent of both red wine and grape juice, contribute to inhibiting platelet activity, but there are some issues that merit consideration.

The first point is to determine whether one or more flavonoids contribute to the antiplatelet effect of red wine or grape juice. Assuming that only one flavonoid inhibits platelet function is not realistic because the concentration of flavonoids in human circulation is low. Surprisingly, there are no data of flavonoids concentration in human circulation after assumption of red wine, but taking into account other sources, such as onions or tea, the plasma concentration would range from 0.6 to 13 μmol/L. Assuming a similar range of concentration after ingestion of red wine or grape juice, it is difficult to imagine that one flavonoid is responsible for the antiplatelet inhibition. Indeed, Freedman’s study and others demonstrated in vitro that much higher concentrations, for instance of quercetin or resveratrol, are necessary for inhibiting platelet function.

On the basis of these considerations, we combined in vitro 2 flavonoids, namely quercetin and catechin, and demonstrated that they are synergistic in reducing platelet formation of H$_2$O$_2$ and inhibiting platelet function by interfering with the activation of phospholipase C pathway. As this effect was observed with concentrations of quercetin and catechin (5 μmol/L and 25 μmol/L, respectively) close to those potentially achievable in blood after wine assumption, we believe that the concept of synergism among the flavonoids could help explain the antiplatelet effect of red wine or grape juice. Under these conditions, the concept of a single flavonoid acting on human platelets is not realistic because the concentration of flavonoids in human circulation is low. Surprisingly, there are no data of flavonoids concentration in human circulation after assumption of red wine, but taking into account other sources, such as onions or tea, the plasma concentration would range from 0.6 to 13 μmol/L. Assuming a similar range of concentration after ingestion of red wine or grape juice, it is difficult to imagine that one flavonoid is responsible for the antiplatelet inhibition. Indeed, Freedman’s study and others demonstrated in vitro that much higher concentrations, for instance of quercetin or resveratrol, are necessary for inhibiting platelet function.

F. Violi
Institute of Clinical Medicine
University La Sapienza
Rome, Italy

P. Pignatelli
F.M. Pulcinelli
Department of Experimental Medicine and Pathology
University La Sapienza
Rome, Italy

Response

The findings from our study are in agreement with the main comments of Violi and colleagues. A central message of this study is that one isolated flavonoid is not responsible for the antioxidant and platelet inhibitory effects that we reported. This is clear from the failure of any single flavonoid group to cause the same effects as the purple grape juice either in vitro or ex vivo. As we were not sure what the relevant flavonoids were, it was difficult to measure specific flavonoids from the subjects who drank the juice. However, we do not believe that the quercetin or resveratrol are the main substances responsible for the platelet inhibitory or nitric oxide–releasing effects. Although previous studies have shown that, in vitro, flavonoids including quercetin, resveratrol, and catechin inhibit platelet aggregation, the physiological relevance of these findings has been questioned in humans because oral supplementation with quercetin causes markedly increased plasma levels but does not alter total, LDL, or HDL cholesterol levels or changes thrombogenic markers including platelet aggregation and platelet thromboxane B$_2$ production. However, Violi and colleagues are correct that flavonoid levels would have provided useful information especially as a point of comparison with the antioxidant levels measured from the plasma of subjects who consumed purple grape juice.

Jane E. Freedman, MD
Crawford Parker III, MD
Liqing Li, MS
Jacob A. Perlman
Balz Frei, PhD
Vadim Ivanov, PhD
Leslie R. Deak, BS
Mark D. Iafrati, MD
John D. Folts, PhD
Boston University School of Medicine
Boston, Mass


Synergism Among Flavonoids in Inhibiting Platelet Aggregation and $H_2O_2$ Production
F. Violi, P. Pignatelli and F.M. Pulcinelli

*Circulation*. 2002;105:e53
*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/105/8/e53

**Permissions:** Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

**Reprints:** Information about reprints can be found online at:
http://www.lww.com/reprints

**Subscriptions:** Information about subscribing to *Circulation* is online at:
http://circ.ahajournals.org//subscriptions/