Oregon Wine Advisory Board
Research Progress Report

1997 - 1998

The Effect of Phenolics Found in Oregon Pinot Noir on Macrophage Gene Expression

Dr. Dennis R. Koop
Department of Physiology and Pharinacology
Oregon Health Sciences University

Degenerative diseases of aging such as cancer, cardiovascular disease, and brain dysfunction are increasingly found to have, in part, an oxidative origin. As a result, dietary antioxidants play a major role in minimizing this damage and preventing or delaying the pathophysiology. Population groups that generally do not smoke (a significant oxidative insult to the body), do not drink heavily, or do not eat much meat, but instead have a diet rich in fruits and vegetables have an overall cancer mortality about half that of the general population and live several years longer (1,2).

There are numerous studies which suggest that moderate consumption of alcoholic beverages confers a beneficial effect on one's well being (3,4). This is due, in part, to the presence of phenolic compounds (such as flavonoids) that possess antioxidant properties in some alcoholic beverages. This is particularly true for red wines where the level of many flavonoids exceeds that found in white wines, beers, and distilled beverages.

Oxidant stress is an activating stimulus for the NF-*k*B/Rel family of transcription factors, which have binding sites in the promoter region of many genes involved in inflammatory, immune, and acute phase responses. The effect of three wine polyphenolics (catechin, quercetin and resveratrol) on the LPS-stimulated activation of NF-*k*B and the subsequent production of TNF-*a*, IL-6 and nitric oxide was determined in RAW 264.7 macrophages. Our initial studies in vitro used concentrations of the phenolics present in Pinot noir samples. Much remains to be learned about the pharmacokinetics of these components when ingested. However, we feel that the concentrations that we selected represent a good starting point for future dose response studies and for the testing of mixtures of more than one component.

We utilized gel mobility shift assays to monitor the activation of NF-*k*B. Unexpectedly, the three polyphenolics did not inhibit LPS-dependent activation of the dimeric NF-*k*B complex, p50/p65. The activation of this complex results in an increase in the transcription of a number of genes. We investigated the effects of the three wine components on mRNA levels and the final levels of TNF-*a*, IL-6, and nitric oxide. As shown in Figure 1, LPS-dependent production of IL-6 and NO was inhibited by greater than 70% by both quercetin (200 uM) and resveratrol (100 uM) while catechin had no effect. In contrast, while the same concentration of quercetin

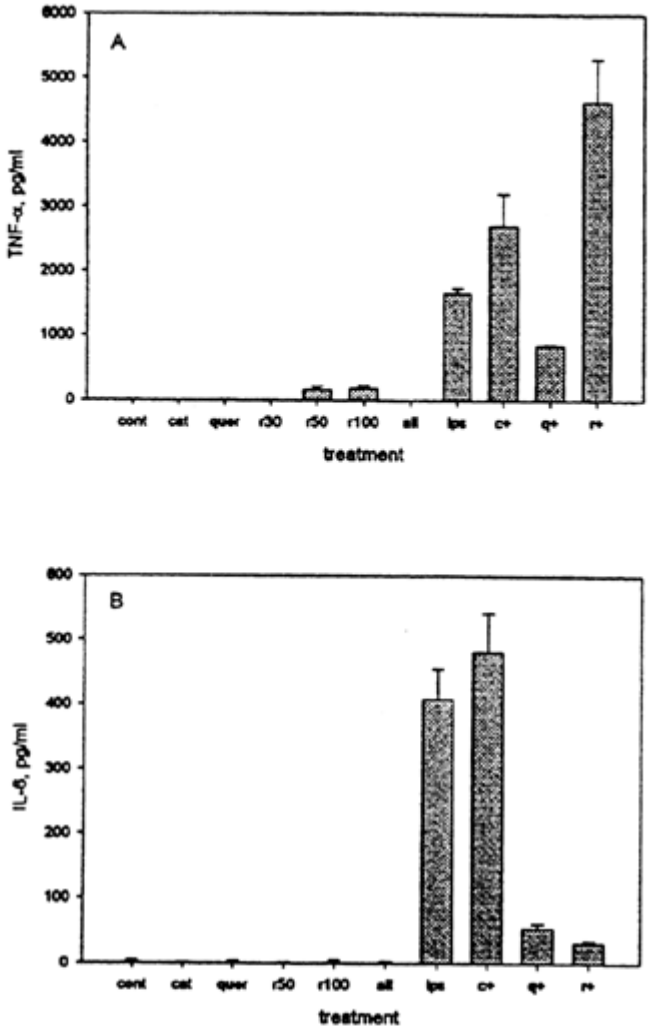

Figure 1. Effect of wine phenolics on cytokine production. RAW 264.7 macrophages were cultured in the presence of catechin (C;1200 μM), quercetin (Q; 200 μM), resveratrol (R; 30, 50 or 100 μM) or a combination of all phenolics and/or LPS (10 ng/ml). After 24 hours, supernatants were collected and the concentrations of cytokine determined by ELISA. Values are means ± standard error, N=3. Panel A: TNF-α . Similar trends were observed in 4 separate experiments. Values for Cont, C, Q and All were below the limits of detection. Panel B: IL-6. Similar results were obtained in a second, independent experiment. Values for Cont, C, Q, R and All were below the limits of detection.

TNF-*a* and enhanced LPS-dependent expression by 300% (Figure 2). Thus, while the polyphenolics found in wine can act as antioxidants; in some assays, our results do not support the view that the role as an antioxidant is through an inhibition of the activation of NF-*k*B. Instead these compounds must have a more selective action on genes activated by stimulants such as LPS by a mechanism that occurs after NF-*k*B activation. Future studies will be needed to determine whether macrophages in the intact animal respond in a similar fashion.

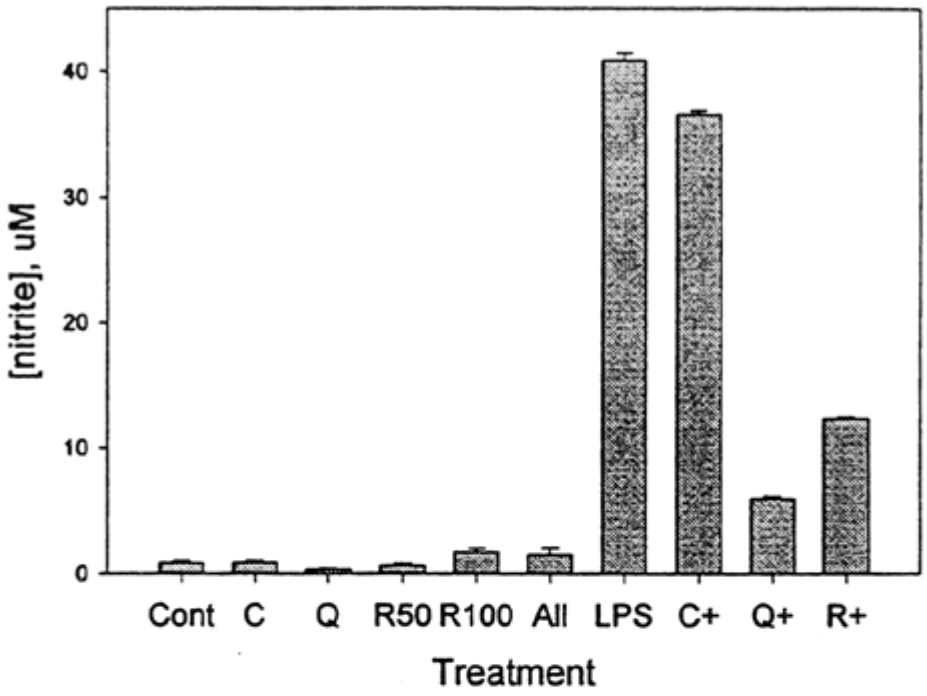

Figure 2. Effect of wine phenolics on NO production. RAW 264.7 macrophages were cultured in the presence of catechin (C; 1200 µM), quercetin (Q; 100 µM), resveratrol (R; 50 or 100 µM) or combination of all phenolics (All) and/or LPS (10 ng/ml). After 24 hours, the concentration of NO in the supernatants was determined as nitrite by the Griess reaction, as described in Methods. Values are means ± standard error, N=3. Similar results were obtained in a second, independent experiment.

REFERENCES

1. Shigenaga, M.K. , Hagen, T.M. and Ames, B.N. (1994) Proc. Nail. Acad. Sci. USA 91:10771-10778.

2. Ames, B.N., Shigenaga, M.K., and Hagen, T.M. (1993) Proc. NatI. Acad. Sci. USA 90:7915-

7922.

3. Gronbaek, M., Deis, A., Sorensen, T.I.A., Becker, U., Borch-Johnsen, Muller, C., Schnohr P., and Jensen, G. (1994) British Med. J. 308:302-308.

4. Gronbaek, M., Deis, A., Sorensen, T.I.A., Becker, U., Schnohr P., and Jensen, G. (1995) British Med. J. 310:1165-1169.