Effect of Red Wine Ingestion on the Antioxidant Capacity of Serum

Tom P. Whitehead,1 David Robinson,2 Sharon Allaway,2,3 Jacquie Sym,2 and Ann Hale2

Aerobic metabolism in biological systems produces reactive oxygen species, and defense against such prooxidants requires antioxidant activity, e.g., predominantly vitamins C and E in serum. Recently, flavonoids (polyphenols occurring widely in plants) have been investigated in vitro for their antioxidant activity; whether they are absorbed after ingestion is not clear. Using a chemiluminescent assay of serum antioxidant capacity (SAOC), we have studied the effects in normal individuals of ingesting red wine, white wine, and high doses of vitamin C. In nine subjects who ingested 300 mL of red wine, the mean SAOC was increased by 16% after 1 h and by 11% at 2 h. The same amount of white wine produced 4% and 7% increases, respectively. The ingestion of 1000 mg (5.7 mmol) of ascorbic acid by four subjects increased their mean SAOC by 22% at 1 h and by 29% at 2 h. An in vitro comparison of red wine, white wine, and various fruit juices showed the high antioxidant capacity of red wine in addition to its ability to increase the antioxidant capacity of serum in vivo. The antioxidant effects of various flavonoids and other polyphenols were also studied.

Indexing Terms: flavonoids/plant polyphenols/ascorbate

Even under basal conditions, aerobic metabolism in biological systems creates reactive oxygen species that are prooxidants. Defense against prooxidant damage of biological molecules, such as DNA or lipids, requires both intra- and extracellular antioxidant activity. Oxidative stress or injury occurs if prooxidants are in excess (1).

In vitro studies have shown that intracellular antioxidants are predominantly enzymic, the most common being superoxide dismutase, which is a catalyst with superoxide as the substrate. Extracellular antioxidants in serum are of much lower molecular mass and include vitamin C (ascorbate), vitamin E (alpha-tocopherol) and urate, as well as several other substances present in much lower concentrations such as beta-carotene, bilirubin, and glutathione. These compounds are not catalysts, but act by breaking prooxidant chain reactions; they are consumed in the process. The major extracellular antioxidants are of dietary origin or are influenced by diet. They can, however, be supplemented by synthetic products.

Many human diseases have been associated with prooxidant damage (2), but only in the last few years has

the concept of preventing such damage by antioxidants become an important issue in the medical literature. The identification of the role of prooxidants in the oxidation of low-density lipoprotein in atherosclerosis (3) and the reduction in atherosclerotic lesions in animal models resulting from the use of antioxidants have stimulated interest.

Research into antioxidant status and administration has been mainly concerned with vitamins C and E and coronary artery disease (4, 5). The most outstanding contributions to the epidemiology of the effects of dietary supplementation with vitamin E have been the multicenter prospective studies, which showed a 40% reduction in coronary artery disease in those individuals who supplemented their vitamin E intake (6, 7).

More recently, the possible role of flavonoids acting as antioxidants has been investigated. The consumption of saturated fat in France is greater than that in the UK, but mortality from coronary artery disease in France is only about one-third of the UK rate (8). It has been suggested that the French benefit from the consumption of wine, and, because of the high flavonoid content of red as distinct from white wine, it is additionally postulated that the high consumption of red wine is responsible for this “French paradox.”

A recent in vitro study showed that the phenolic substances in Californian red wine protected low-density lipoprotein from peroxidation (9). The Dutch Zutphen Elderly Study (10) has added additional weight to the possible role of flavonoids in reducing risk of coronary artery disease. In determining five commonly occurring flavonoids in various common foodstuffs, the investigators found that tea, onions, and apples were the major sources of these flavonoids; moreover, the elderly men who consumed the largest amounts of these foods had a significantly reduced risk of coronary artery disease (10).

Here, we report our use of a recently introduced assay of serum antioxidant capacity to determine whether the consumption of red wine increases the antioxidant capacity of serum.

Materials and Methods

Serum antioxidant capacity (SAOC) was determined by means of a chemiluminescent reaction (11). Sodium perborate in the presence of horseradish peroxidase produces reactive oxygen species, and luminescence is produced if luminol is present. If p-iodophenol is also present, the light output is considerably enhanced and remains relatively constant for several minutes. If certain known antioxidants are added to the reaction, the light is suppressed until the antioxidant has been consumed, after which the light returns. The length of time

1 Department of Pathology, University of Birmingham, Birmingham B15 2TH, UK.
2 BUPA Medical Research, Battle Bridge House, 300 Grays Inn Rd, London WC1X 8DU, UK.
3 Author for correspondence. Fax Int + 44 71 837 8368. Received June 13, 1994; accepted September 30, 1994.
of suppression of light is proportional to the quantity of antioxidant added.

Figure 1 shows the result of adding a synthetic toco-pherol Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; supplied by Aldrich Chemical Co., Poole, Dorset, UK) to this reaction. For Trolox, ascorbic acid, and urate there is a linear relationship between t (the time taken to reach 10% of the original signal) and the amount of antioxidant present; all three substances give an equimolar response with the same type of curve (11). If serum is added to the reaction, the light is suppressed, but recovery is slower and the luminescence does not return to its original level. Instead, the value at recovery is ~50% lower (Fig. 2), due to the quenching effect of serum proteins (11).

The between-batch precision (CV) of the method was ±5% with hydroquinone as the test substance. The reference interval (mean ± 2 SD) for men is 447 ± 120 μmol/L, and for women is 381 ± 160 μmol/L (12).

The six women and four men who took part in this study were ages 20–50 years. None was on routine medication, and all had given informed consent. Before each test, they avoided drinking wine for one week, but otherwise kept to their normal diet. They came to the laboratory in the morning having fasted overnight. Tests were carried out on different days, and subjects taking part in more than one test took at least 1 week between tests. All subjects except one had fasting values for SAOC within the reference range.

The tests performed were as follows:

1. Four women ingested 1000 mg (5.7 mmol) of ascorbic acid with water. Blood was collected before ingestion and at 1 and 2 h after.
2. Nine subjects (four men, five women) drank 300 mL of red wine (1990 Chateau du Juge Bordeaux) over a period of 30 min. Blood was collected before, and 1 and 2 h after, that period.
3. Three subjects (one man, two women) ingested 300 mL of a white wine (1992 Chateau du Juge Bordeaux) over a period 30 min. Blood was taken before, and 1 and 2 h after, ingestion.
4. Two subjects (one man, one woman) acted as controls, blood being taken from them at hourly intervals during the morning.

Results

The results of the various tests are shown in Table 1. Within the first hour after ingestion of ascorbic acid, mean SAOC values rose from 473 to 576 μmol/L, and then rose further (to 610 μmol/L) in the second hour. After ingesting red wine, all subjects showed an increase in SAOC at 1 and 2 h, but the responses were variable. The mean concentration increased from 486 to 572 μmol/L in the first hour, then in the next hour it fell to 540 μmol/L. For both ascorbic acid and red wine, the mean concentrations at 1 and 2 h postingestion were significantly greater than the mean basal values (paired t-test; P <0.005). Mean SAOC in the three subjects drinking white wine rose from 425 to 443 μmol/L in the first hour, and then rose further to 454 μmol/L in the second hour. These increases were not statistically significant. The two control subjects did not show any significant change.

Although subjects' weights were not available, we noted that one woman with observable obesity gave the highest increase in SAOC. Thus the response does not appear to be explicable on the basis of consumption per unit body weight.

Adding to the chemiluminescent reaction 20 μL of a 1:500 dilution of the red wine given to the study subjects demonstrated its powerful antioxidant capacity. Based on use of Trolox as a calibrator, the capacity was equivalent to 17 000 μmol/L. The ascorbate present in the 300 mL of red wine ingested was <50 mg (0.3 mmol). Analysis of nine red wines from various sources indicated a variation from 10 000 to 20 700 μmol/L, with a mean of 15 400 μmol/L (Table 2). Four white wines from differ-
Table 1. Changes in total serum antioxidant capacity.

<table>
<thead>
<tr>
<th>Subject (sex)</th>
<th>Basal</th>
<th>+1 h</th>
<th>+2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>473</td>
<td>576</td>
<td>610</td>
</tr>
<tr>
<td>(± SD)</td>
<td>± 57</td>
<td>± 45</td>
<td>± 83</td>
</tr>
</tbody>
</table>

After consuming 1000 mg (5.7 mmol) of ascorbic acid

1 (F) 535 621 (16)* 699 (31)*
2 (F) 508 599 (18) 650 (28)
3 (F) 433 568 (31) 580 (34)
4 (F) 417 517 (24) 510 (22)
Mean 473 576 (22)* 610 (29)*
(± SD) ± 57 ± 45 ± 83

After consuming 300 mL of red wine

5 (F) 456 720 (58) 576 (26)
6 (M) 480 588 (22) 600 (25)
7 (F) 540 696 (29) 576 (7)
8 (F) 492 516 (5) 516 (5)
9 (M) 636 696 (9) 684 (8)
10 (F) 504 564 (12) 540 (7)
11 (F) 441 488 (11) 467 (6)
12 (M) 484 527 (9) 527 (9)
13 (M) 337 354 (5) 378 (12)
Mean 486 572 (18)* 540 (11)*
(± SD) ± 80 ± 119 ± 86

After consuming 300 mL of white wine

14 (M) 470 474 (1) 502 (7)
15 (F) 445 460 (3) 460 (3)
16 (F) 361 396 (10) 401 (11)
Mean 425 443 (4) 454 (7)
(± SD) ± 57 ± 42 ± 51

Controls

17 (F) 453 445 (−2) 484 (7)
18 (M) 653 617 (−6) 653 (0)
Mean 553 531 (−4) 569 (3)
(± SD) ± 141 ± 122 ± 120

*Numbers in parentheses give the percentage change from the basal value.

Table 2. Total antioxidant capacity in various fluids.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Antioxidant capacity, µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red wine (9 varieties)</td>
<td>1000–20 727 (Mean 15 437, SD 3432)</td>
</tr>
<tr>
<td>White wine (4 varieties)</td>
<td>872–1284 (Mean 1106, SD 189)</td>
</tr>
</tbody>
</table>

Apple juice 7890
Orange juice 2000
Grape juice 680

All fruit juices were freshly squeezed.

Table 2 also gives for comparative purposes the antioxidant capacity of three freshly prepared fruit juices. Each juice was diluted 100-fold with distilled water, and 20 µL was used in the assay calibrated with Trolox. Only one sample of each fruit juice was analyzed, but all were found to contain some antioxidant capacity, predominantly because of their ascorbate content. Apples are known to have a high flavonoid content.

The number of different phenols already identified in red wines is well >100 and includes both flavonoid and nonflavonoid phenols. This high phenolic content is due to the incorporation of the grape skins into the fermenting grape juice during production. The grape skins are rich in phenols, some of which are water soluble, but many only becoming so as the alcohol concentration increases. White wine making does not involve the grape skins, but only the fermented grape juice.

The polyphenols in red wine are known to include quercetin; in fruits and vegetables, myricetin and apigenin also occur. These three polyphenols are flavonoids. Quercetin, and three nonflavonoid polyphenols known to occur in red wine—namely, hydroquinone, gallic acid, and 1,2,3-trihydroxybenzene—were studied for their antioxidant capacity. To the luminescent reaction was added 20 µL of a 50-fold dilution of a 10 mg/100 mL solution in 100 mL/L ethanol, and the reaction monitored.

Figure 3 shows the resulting curve for each substance. Also shown is the curve produced when all four polyphenol solutions were mixed in equal proportions, along with that produced by 20 µL of a 100-fold dilution of a typical red wine.

All of these substances showed antioxidant activity, but the shape of the graph of photon output against time for each substance varied. Despite the differences in response of the individual polyphenols, in combination they gave a curve very similar in shape to that produced by red wine.

Discussion

The role of polyphenols, particularly the flavonoids, in human nutrition has been the subject of dispute since the first reports on guinea pigs cured of scurvy symptoms by citrin, a mixture of flavonoids occurring in lemons, published in 1937 (13). Quercetin, a commonly occurring flavonoid usually present in red wine, is claimed by some authors to have important anticarcinogenic properties (14) and by others to be toxic because of its enzyme inhibitory and mutagenic properties (15). There is also some dispute in the literature as to whether quercetin is absorbed (16).

The current wide interest in the possible role of pro-
oxidants in common disease states and the recognition of the flavonoids and other polyphenols as possessing antioxidant capability require a consideration of those foodstuffs containing such substances, predominantly fruits and vegetables, in terms of their role in preventing oxidative stress.

An important issue is the absorption of such substances and their role in the action of low-molecular-mass extracellular antioxidants. We conclude that antioxidants occurring in red wine do significantly raise the SAOC after consumption, and support the suggestion that the ingestion of red wine may be a contributory factor to the “French paradox.” The precise mechanism by which this increase in SAOC is achieved, and the particular polyphenols involved, are not specifically identified by this investigation but may be a fruitful source of further work.

References