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Red wine contains a potent inhibitor of phenolsulphotransferase

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Many ethanolic drinks, especially red wine, contain potent inhibitors of phenosulphotransferase. At a dilution of 1/75 from the original beverage, extracts from six types of red wine inhibited human platelet phenolsulphotransferase P by a mean of 99% and human platelet phenolsulphotransferase M by 12%. Such extracts had no significant effect on rat liver monoamine oxidase A or human platelet monoamine oxidase B. The inhibitors, which have not yet been identified, can be extracted into ethyl acetate at acid or neutral pH. Thus, they are not monoamines. Flavonoid phenols are plausible candidates. As phenolsulphotransferase M and P are involved in the metabolism of many phenols, including drugs, the inhibition of these enzymes could result in the enhancement of pharmacological potency and have important clinical consequences.

Keywords ethanol phenolsulphotransferase

Introduction

A wide range of endogenous, dietary and pharmaceutical phenols are inactivated by phenolsulphotransferase (PST) which catalyzes their conjugation with sulphate (Sandler & Usdin, 1981). In man, we have shown PST to exist in two forms (Rein *et al.*, 1981a, 1982), PST M which acts specifically on monoamines, such as tyramine, and their phenolic metabolites and PST P which inactivates phenol itself (at low concentration) and certain exogenous phenolic compounds; this subdivision has been confirmed by others (Reiter & Weinshilboum, 1982). Some drugs, such as paracetamol and salicylamide, are substrates for both (Reiter & Weinshilboum, 1982; Bonham Carter *et al.*, 1983). The two forms of the enzyme are particularly active in the intestinal wall but are also present in platelet, adrenal gland, placenta and brain (Rein *et al.*, 1981b, 1982, 1984; Sodha *et al.*, 1983). In this study we show that many ethanolic drinks, but especially red wine, contain powerful inhibitors of PST.

Methods

Samples (20 ml) of a number of different beverages (Table 1) were adjusted to pH 1 with 0.1 M HCl, extracted by shaking with 20 ml ethyl acetate in glass-stoppered tubes for 15 min and centrifuged at 900 *g* for 10 min. The supernatant organic layer was aspirated with a pasteur pipette and evaporated to dryness under nitrogen in a water bath at about 30°C before being redissolved in 2 ml of 0.01 M potassium phosphate buffer, pH 7.4. This was further diluted 1 in 100 with buffer, leading to an eventual 75-fold dilution from the original beverage in the assay mixture (see below). Water extract blanks were made following the same procedure.

A platelet suspension in sucrose was prepared as described previously (Summers *et al.*, 1982) and PST assayed using a modification (Bonham Carter *et al.*, 1981) of the method of Foldes & Meek (1973). Tyramine (final concentration 20 μM) was used as substrate for measurement of PST M and phenol (final concentration 10 μM) for PST P. The incubation mixture varied from that previously described in that it contained 20 μl enzyme preparation (pooled platelets from four individuals), 20 μl tyramine or phenol solution, 70 μl 0.01 M potassium phosphate buffer, pH 7.4, and 20 μl beverage extract in buffer, pH 7.4; 20 μl PAP^{35}S (final concentration 0.66 μM) was added at succes-

Table 1 % Inhibition of phenolsulphotransferase with extracts of various ethanolic drinks

	PST P	*PST M*	*MAO A*	*MAO B*
Red wine				
French	100	4	0	0
Italian (Chianti)	100	25	4	0
French (Claret)	100	11	5	0
Spanish	93	7	5	0
Spanish	100	3	3	0
Californian	100	22	0	0
Mean ± s.e. mean	99 ± 1.3	12 ± 4.2	2.8 ± 1.0	0
White wine				
French (Bordeaux)	67	9	2	1
Italian (Trevino)	87	4	3	0
German (Hock)	74	6	0	0
French	61	0	0	0
Mean ± s.e. mean	72 ± 6.5	4.8 ± 2.2	1.2 ± 0.9	0.25 ± 0.3
Sherry				
(1)	69	17	4	0
(2)	91	1	10	9
Mean	80	9	7	4.5
Brandy				
(1)	64	14	0	0
(2)	68	18	0	0
Mean	66	16	0	0
Whisky				
(1)	70	8	0	2
(2)	29	7	10	4
(3)	68	1	12	3
Mean	56	5.3	7.3	3
Vodka	34	4	0	3
Gin	20	3	10	0

Human platelet phenolsulphotransferase (PST) was assayed with 10 μM phenol for PST P and 20 μM tyramine for PST M. Monoamine oxidase (MAO) from rat liver homogenate was assayed with 170 μM [^{14}C]-5-hydroxytryptamine for MAO A and from human platelets with 150 μM [^{14}C]-tyramine for MAO B.

sive intervals to tubes incubated at 37°C in a water bath and the reaction terminated after 10 min as described previously (Bonham Carter *et al.*, 1981). A control containing 90 μl buffer without beverage extract was used in each assay as well as a water extract blank. Blanks where phenol or tyramine had been replaced by 20 μl water were also included. Substrate was removed by two precipitations with barium sulphate (Foldes & Meek, 1973) and the ^{35}S-labelled product was then measured in a liquid scintillation counter. Percentage inhibition was assessed by comparison with control counts after correction for water blank.

Monoamine oxidase (MAO) A inhibition was monitored using rat liver homogenate with [^{14}C]-5-hydroxytryptamine as substrate (Glover *et al.*, 1982) and MAO B inhibition using human platelets with [^{14}C]-tyramine as substrate (Summers *et al.*, 1982).

Results

Reconstituted ethyl acetate extracts from the beverages listed in Table 1 were first investigated to identify the presence of any PST substrate, by incorporating them into the assay system

with ^{35}PAPS but without added substrate. None showed any activity. However, when added to assay systems together with either phenol or tyramine, many extracts showed potent inhibition (Table 1). PST P was always inhibited more than PST M and red wine extracts, in particular, were the most powerful. All red wines, except one, caused 100% inhibition of PST P at the dilution employed. The white wines also resembled each other and were similar to brandy and whisky in their inhibitory ability. The two sherries were rather more potent than the white wine but individual variation clearly existed between the different brands in each category. Vodka and gin caused the least inhibition. At the 1/75 dilution used here, none of the drink extracts had any significant effect on MAO A or B. Although the inhibitor was most efficiently extracted at pH 1, extracts made from the beverages without prior pH adjustment showed a similar pattern.

Discussion

The major chemical difference between red and white wine lies in their flavonoid phenolic content contributed from grape skin and seeds during fermentation. A typical red wine contains 1,200 mg/l and a white wine 50 mg/l (Singleton & Noble, 1976). By their chemical nature, these may well have been responsible for the inhibition of PST we have observed. The degree of inhibition bore no relationship to the ethanol content of the original beverage and, indeed, ethanol would have evaporated during the extraction procedure. This inhibitor(s), as yet unidentified, must be acidic or neutral rather than basic for it is more easily extractable at acid pH. Inhibition cannot, for instance, be attributable to a phenolic monoamine such as tyramine, which would not be extractable at acid pH. It is possible that acidic phenols are substrates as well as competitive inhibitors of PST. The barium acetate assay method used here does not always work well for acids. However, no substrate activity was obtained using red wine extracts in an ecteola-cellulose resin assay method either (unpublished observations).

The potency of some of the PST inhibitors in beverages, shown in Table 1, is remarkable. Half a litre of red wine diluted 1/75 in 40 l of body water could, theoretically, result in 100% inhibition of all the PST P in the body. A similar volume of red wine diluted to about 4 l by the contents of stomach and intestine might largely or wholly inhibit gastrointestinal PST M. Even as little as 50 ml might totally inhibit PST P in this site. Of course, it remains to be established whether this degree of inhibition also occurs *in vivo*. Phenols of the flavonoid family such as catechins and anthocyanins can be absorbed by human gut (Griffiths, 1982), in contrast with such larger molecular weight polyphenols as the tannins in tea, which are not readily absorbed (Singleton, 1981). However, much remains to be understood of the absorption, metabolism and distribution of these compounds in man.

If red wine causes significant inhibition of PST *in vivo* the clinical implications may be substantial. A large number of phenolic compounds are metabolized by sulphation, particularly at low concentration (Mulder, 1982) or in individuals with low glucuronidating ability (Caldwell *et al.*, 1980). Phenol itself can act as a co-carcinogen (Bakke & Midtvedt, 1970) and other related phenols, possibly of dietary origin, may also have this property. The toxic threshold for many other common drugs, such as paracetamol or isoprenaline, could be lowered by concomitant administration of red wine. Certain subjects in the population who are genetically poor metabolizers (Idle *et al.*, 1983) are particularly at risk from adverse drug reactions. The group of patients we observed with dietary migraine and a deficit of PST P (Littlewood *et al.*, 1982) may well fall into this category. Many migrainous patients report that red wine, in particular among alcoholic drinks, can provoke an attack (Glover *et al.*, 1984). It is possible that the compounds causing inhibitory activity of the type noted here also play a role in this.

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References

Bakke, O. M. & Midtvedt, T. (1970). Influence of germ-free status on the excretion of simple phenols of possible significance in tumour promotion. *Experientia*, **26**, 519.

Bonham Carter, S. M., Glover, V., Sandler, M., Gillman, P. K. & Bridges, P. K. (1981). Human platelet phenolsulphotransferase: separate control of the two forms and activity range in depressive illness. *Clin. Chim. Acta*, **117**, 333–334.

Bonham Carter, S. M., Rein, G., Glover, V., Sandler, M. & Caldwell, J. (1983). Human platelet phenolsulphotransferase M and P: substrate specificities

and correlation with *in vivo* sulphoconjugation of paracetamol and salicylamide. *Br. J. clin. Pharmac.*, **15**, 323–330.

Caldwell, J., Davies, S. & Smith, R. L. (1980). Interindividual differences in the conjugation of paracetamol with glucuronic acid and sulphate. *Br. J. Pharmac.*, **70**, 112P–114P.

Foldes, A. & Meek, J. L. (1973). Rat brain phenolsulfotransferase-partial purification and some properties. *Biochim. Biophys. Acta*, **327**, 365–373.

Glover, V., Liebowitz, J., Armando, I. & Sandler, M. (1982). β-Carbolines as selective monoamine oxidase inhibitors: *in vivo* implications. *J. Neural Transmiss.*, **54**, 209–218.

Glover, V., Littlewood, J., Sandler, M., Peatfield, R., Petty, R. & Rose, F. C. (1984). Dietary migraine: looking beyond tyramine. In *Progress in Migraine Research 2*, ed. Rose, F. C., pp. 113–119. London: Pitmans.

Griffiths, L. A. (1982). Mammalian metabolism of flavonoids. In *The Flavonoids. Advances in Research*, eds. Harborne, J. B. & Mabry, T. J., pp. 681–718. New York: Chapman Hall.

Idle, J. R., Oates, N. S., Shah, R. R. & Smith, R. L. (1983). Protecting poor metabolizers, a group at high risk of adverse drug reactions. *Lancet*, **i**, 1388.

Littlewood, J., Glover, V., Sandler, M., Petty, R., Peatfield, R. & Rose, F. C. (1982). Platelet phenolsulphotransferase deficiency in dietary migraine. *Lancet*, **i**, 983–986.

Mulder, G. J. (1982). Conjugation of phenols. In *Metabolic Basis of Detoxication*, eds. Jakoby, W. B., Bend, J. R. & Caldwell, J., pp. 247–269. New York: Academic Press.

Rein, G., Glover, V. & Sandler, M. (1981a). Phenolsulfotransferase in human tissue: evidence for multiple forms. In *Phenolsulfotransferase in Mental Health Research*, eds. Sandler, M. & Usdin, E., pp. 98–126. London: Macmillan.

Rein, G., Glover, V. & Sandler, M. (1981b). Sulphate conjugation of biologically active monoamines and their metabolites by human platelet phenolsulphotransferase. *Clin. Chim. Acta*, **111**, 247–256.

Rein, G., Glover, V. & Sandler, M. (1982). Multiple forms of phenolsulphotransferase in human tissues: selective inhibition by dichloronitrophenol. *Biochem. Pharmac.*, **31**, 1893–1897.

Rein, G., Glover, V. & Sandler, M. (1984). Characterization of human brain phenolsulphotransferase. *J. Neurochem.*, **42**, 80–85.

Reiter, C. & Weinshilboum, R. M. (1982). Acetaminophen and phenol: substrates for both a thermostable and a thermolabile form of human platelet phenol sulfotransferase. *J. Pharmac. exp. Ther.*, **221**, 43–51.

Sandler, M. & Usdin, E. (1981). *Phenolsulfotransferase in Mental Health Research*. London: Macmillan.

Singleton, V. L. (1981). Naturally occurring food toxicants: phenolic substances of plant origin common in foods. *Adv. Food Res.*, **27**, 149–242.

Singleton, V. L. & Noble, A. C. (1976). Wine flavour and phenolic substances. In *Phenolic, Sulfur and Nitrogen Compounds in Food Flavors*, eds. Charalambous, G. & Katz, I., pp. 47–70. American Chemical Society Symp. Series No. 26.

Sodha, R. J., Glover, V. & Sandler, M. (1983). Phenolsulphotransferase in human placenta. *Biochem. Pharmac.*, **32**, 1655–1657.

Summers, K. M., Brown, G. K., Craig, I. W., Littlewood, J., Peatfield, R., Glover, V., Rose, F. C. & Sandler, M. (1982). Platlet monamine oxidase: specific activity and turnover number in headache. *Clin. Chim. Acta*, **121**, 139–146.

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