Acta Derm Venereol (Stockh) 1993; 73: 362–364

Evaluation of Phototoxic Properties of Some Food Additives: Sulfites Exhibit Prominent Phototoxicity

BERNADETTE EBERLEIN-KÖNIG, THOMAS BERGNER, STEFAN DIEMER and BERNHARD PRZYBILLA

Dermatology Clinic and Polyclinic of the Ludwig-Maximilian University, Munich, Germany

Additives are used widely to enhance the quality of food products. To identify possible phototoxic properties, 13 food additives (benzoic acid, sodium benzoate, 4-hydroxybenzoic acid, 4-hydroxybenzoic acid methyl ester, 4-hydroxybenzoic ethyl ester, 4-hydroxybenzoic acid propyl ester, p-hydroxybenzoic acid n-butyl ester, benzyl alcohol, sorbic acid, potassium sorbate, propionic acid, sodium disulfite and sodium sulifte) were evaluated in vitro by means of a photohemolysis test using suspensions of human erythrocytes. Irradiation was performed with various light sources differing with regard to their spectral irradiance. Sodium sulfite and sodium disulfite induced photohemolysis up to almost 100%, the effect depending on the concentration of the compounds and UV dose administered. Radiation rich in UVB was most effective; a sunlight-simulating lamp induced photohemolysis to a lesser degree. All other substances tested did not cause significant photohemolysis. As sulfites are frequently encountered, they may contribute to UVB sensitivity. The clinical significance of these findings has to be established by further work. ***Key words: Photohemolysis; UV.***

(Accepted May 3, 1993.)

Acta Derm Venereol (Stockh) 1993; 73: 362–364.

B. Eberlein-König, Dermatologische Klinik und Poliklinik der Ludwig-Maximilians-Universität München, Frauenlobstr. 9–11, D-80337 München, Germany.

Food additives, e.g. smoke, alcohol, vinegar, oil or spices, have been used for centuries to enhance the quality of food products. In the last decades, significant technological advances in food processing have been accompanied by a fulminant increase of such additives. Today, more than 2,500 compounds are intentionally added to foods to produce a desired effect (1). Thus, humans are exposed to food additives on a large scale.

Only a few studies have addressed possible phototoxic effects of food additives. Some dyes (rose bengal, erythrosine, phloxine) have been found to inactivate yeast cells due to a photodynamic action (2). It has also been reported that photoactivated tartrazine causes hemolysis (3). A phototoxic compound may induce skin reactions, and phototoxicity can be regarded as a prerequisite of photoallergenicity. Even more important, it has been suggested that chronic exposure to phototoxic compounds may be related to photocarcinogenesis. These considerations prompted us to assess in vitro the phototoxic potential of various food additives, which are mainly used to inhibit microbiological growth and to prevent enzymatic and nonenzymatic discoloration.

MATERIAL AND METHODS

UV sources

Irradiations were performed with the following lamps: 1) UVASUN 5000 (Mutzhas, Munich, Germany), emitting in the range of 320–460 nm (maximum approx. 375 nm); UVA irradiance at a distance of 40 cm was 42 mW/cm^2; 2) TL 20 W/12 light bulbs (Philips, Hamburg, Germany) with a main emission between 275–365 nm (maximum approx. 315 nm); irradiance was 1.0 mW/cm^2 for UVB and 0.4 mW/cm^2 for UVA at a distance of 40 cm; 3) SOL 3 sunlight-simulating lamp equipped with H2 filter (Hönle, Martinsried, Germany) with a main emission between 290–800 nm (broad maximum between approx. 400–700 nm); irradiance was 0.95 mW/cm^2 for UVB and 10.5 mW/cm^2 for UVA at a distance of 40 cm.

Dosimetry

UVA or UVB intensity (or dose) was measured with an integrating instrument (Centra-UV, Osram, Munich, Germany).

Test substances

Tests were performed with sodium benzoate, potassium sorbate, benzoic acid, sodium disulfite, sodium sulfite, sorbic acid (Merck, Darmstadt, Germany), benzyl alcohol, p-hydroxybenzoic acid n-butyl ester (Sigma, St. Louis, USA), 4-hydroxybenzoic acid, 4-hydroxybenzoic acid methyl ester and 4-hydroxybenzoic acid ethyl ester, 4-hydroxybenzoic acid propyl ester and propionic acid (Roth, Karlsruhe, Germany). The purity of sodium disulfite and sodium sulfite was 98% as declared by the manufacturer. Test substances were dissolved in distilled water or methanol and further diluted in TCM buffer (3 g Tris, 0.3 g KCl, 7 g NaCl, 0.147 g $CaCl_2 \times 2\ H_2O$, 0.2 g $MgCl_2 \times 2\ H_2O$, dissolved in 1000 ml distilled water, pH 7.4; 280 mosm/kg). Absorption spectra were recorded with an UV-VL spectrophotometer UV 2100 (Shimazu, Tokyo, Japan).

Methods

The photohemolysis test was performed as previously described (4). Briefly, suspensions of human erythrocytes from at least three donors or correspondingly prepared erythrocyte-free samples were incubated with the test substances at concentrations of 10^{-5}, 10^{-4} or 10^{-3} mol/l. Both substance-free erythrocyte samples (blanks) as well as samples containing the test substances were exposed to 0, 25, 50 or 100 J/cm^2 UVA from the UVASUN 5000 apparatus, to 0, 100 (37.5), 200 (75), 400 (150), 800 (300) or 1600 (600) mJ/cm^2 UVB (UVA) from the TL/12 light bulbs or to 0, 5 (0.45), 25 (2.26), 50 (4.52) J/cm^2 UVA (UVB) from the SOL 3 lamp. 100% hemolysis was obtained by exposure of the erythrocytes to distilled water. Supernatants were recovered by centrifugation. After incubation with Drabkin's solution (Sigma, St. Louis, USA), hemolysis was determined by reading of absorbance at 550 nm with a MR 700 Microplate® reader (Dynatech AG, Denkendorf, Germany). Hemolysis was calculated for each radiation dose on the basis of the absorbance data according to the formula:

$$\%\ \text{hemolysis} = 100 \times \frac{(\text{test sample}) - (\text{blank}) - (\text{erythrocyte-free sample})}{(100\%\ \text{hemolysis}) - (\text{blank})}$$

Results are given as mean. In order to exclude equivocal results, only photohemolysis >5% was regarded to be a meaningful positive finding.

The pH of the test samples was measured with a pH-meter (Orion, model SA 520, Colora, Munich).

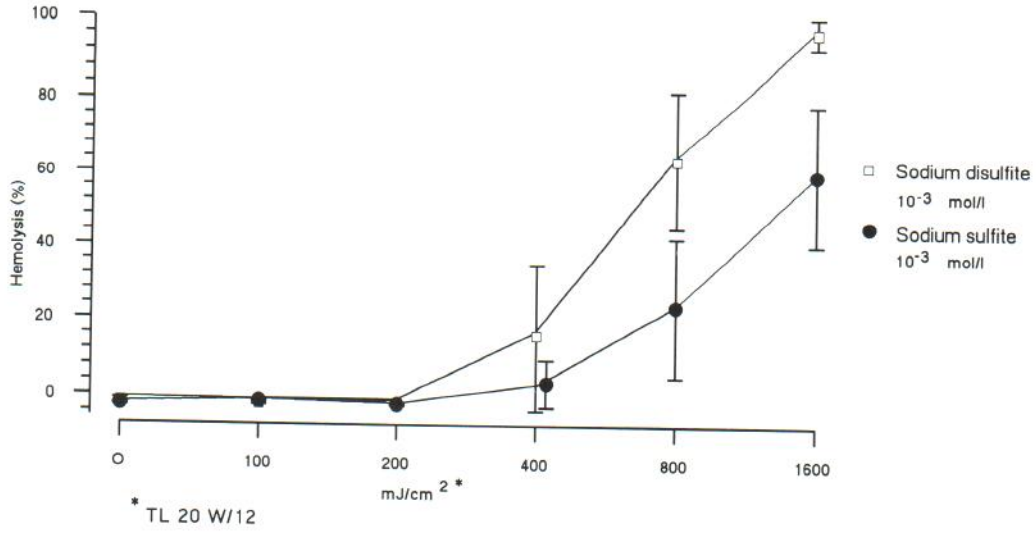

Fig. 1. Photohemolysis (mean ± SD) induced by sodium disulfite (10^{-3} mol/l) or sodium sulfite (10^{-3} mol/l) and radiation rich in UVB ($n = 6$).

RESULTS

At exposure to the TL/12 lamps, sodium sulfite and sodium disulfite in the 10^{-3} mol/l concentration caused a UV dose-dependent hemolysis up to 64.1% or 99.7%, respectively (Fig. 1). A phototoxic effect also at 10^{-4} mol/l was found for sodium disulfite with two out of three erythrocyte suspensions from different donors. Irradiation with the SOL 3 lamp in the presence of sodium disulfite also induced photohemolysis (Fig. 2), whereas exposure to the UVASUN 5000 apparatus had no such effect. No hemolysis occurred when erythrocytes prepared as indicated above were incubated with sodium sulfite or sodium disulfite (10^{-3} mol/l) preirradiated with the maximum of the radiation doses used. Changes of the pH values of solutions with sodium sulfite or sodium disulfite before and after exposure were <0.2. None of the other 11 compounds tested exhibited a significant phototoxic action in this assay.

The absorbance spectrum of sodium disulfite is shown in Fig. 3. The absorbance peaks at 209 nm. Sodium sulfite showed a similar absorbance spectrum (data not shown).

DISCUSSION

Sodium sulfite as well as sodium disulfite exerted prominent phototoxic effects in this in vitro assay, sodium disulfite being the more phototoxic agent. This is probably a concentration effect, as the disulfite ion almost completely separates into its two constituting sulfite units in aqueous solutions (5) according to the following equation:

$$S_2O_5^{2-} + H_2O \rightleftharpoons 2\ HSO_3^-.$$

The other agents tested were not phototoxic in the photohemolysis test used.

The action spectrum of sulfite phototoxicity seems to lie predominantly in the UVB range. Although the highest UVB dose administered with SOL 3 was 4.5 J/cm^2, irradiation with this source yielded lower photohemolysis than 1.6 J/cm^2 UVB from the TL/12 light bulbs. This may be a photoinhibition due to the other wavelengths emitted by the SOL 3 lamp, possibly attributable to a photodegradation of sulfite or sulfite-induced photoproducts. No photohemolysis was observed after irradia-

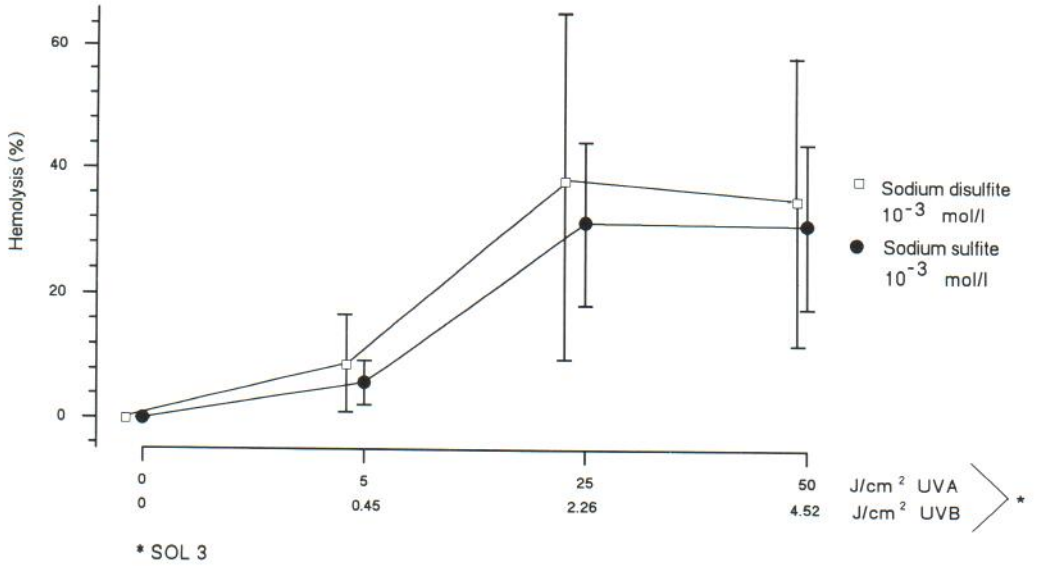

Fig. 2. Photohemolysis (mean ± SD) induced by sodium disulfite (10^{-3} mol/l) or sodium sulfite (10^{-3} mol/l) and radiation with solar simulating lamps ($n = 6$).

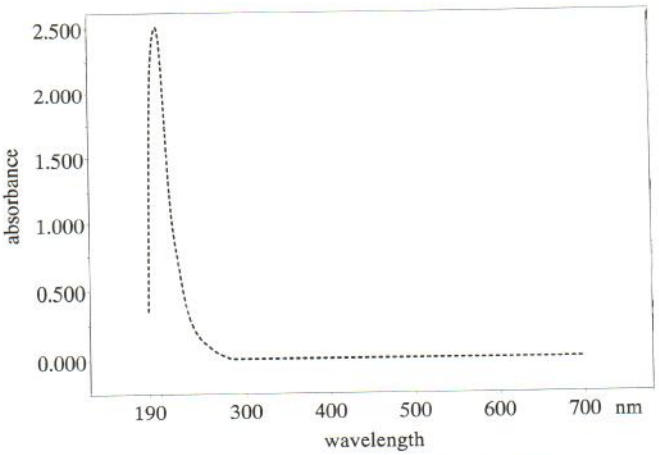

Fig. 3. Absorbance spectrum of sodium disulfite (10^{-3} mol/l).

tion with the UVASUN apparatus, which is rich in UVA and free of UVB. This is notable, as most phototoxic substances have their action spectrum in the UVA range (2).

The UV-induced autoxidation of SO_3^{2-} to SO_4^{2-} includes the intermediate $SO_3^{\cdot-}$, $SO_4^{\cdot-}$ and $SO_5^{\cdot-}$ radicals generated during a radical chain reaction (5,6). The sulfite radical ($SO_3^{\cdot-}$) was shown to react readily with free polyunsaturated fatty acids (PUFA) (7,8), but it seems to be rather non-reactive to PUFA micelles (8) and therefore, probably, also to cell membranes. So, $SO_4^{\cdot-}$ or $SO_5^{\cdot-}$ could be responsible for membrane damage. To clarify the exact nature of the sulfite-dependent phototoxic reaction, photoproducts induced by UVB irradiation will have to be identified as well as their reaction with membrane components.

A variety of sulfiting agents have been employed for centuries in food processing. These agents are currently used to sanitize food containers and fermentation equipment to reduce microbial spoilage of food and to prevent enzyme-catalyzed oxidative discoloration and non-enzymatic browning during preparation, storage and distribution of foods (1). As antioxidants, they are added to e.g. fruits, vegetables, potatoes, shellfish, beer and wine. Disulfites are also used as preservatives in drugs. Estimations are that 2–3 mg of sulfites is consumed each day by the average citizen in the United States, and an additional consumption of 5–10 mg of sulfite per day occurs in wine and beer drinkers (9).

To our knowledge, up to now phototoxic effects of sulfites have not yet been reported. Phototoxicity has been studied in one model system, photohemolysis, which of course limits the conclusions that can be drawn regarding clinical significance. However, sulfites are ubiquitous contactants. So, encountered not only as preservatives, but also as airborne pollutants, their phototoxic potential may be biologically relevant. The question arises whether sulfite phototoxicity increases UVB sensitivity and thus even augments the risk of photocarcinogenesis. Such effects have been proposed to be possible sequels of the ingestion of photosensitizing food (10). Further in vitro and in vivo studies are needed to clarify these issues.

ACKNOWLEDGEMENTS

This research was supported by Deutsche Forschungsgemeinschaft (Br 147/56–2) and Bayerisches Klimaforschungsprojekt (BayFORKLIM). The authors want to thank Mrs. Hilke Nyhuis for skilled technical assistance.

REFERENCES

1. Branen AL, Davidson PM, Salminen S. Food additives. New York and Basel: Marcel Decker Inc., 1990.
2. Iwamoto Y, Tominaga C, Yanagihara Y. Photodynamic activities of food additive dyes on the yeast Saccharomyces cerevisiae. Chem Pharm Bull Tokyo 1989; 37: 1632–1634.
3. Merville MP, Decuyper J, Lopez M, Piette J, Van De Vorst A. Phototoxic potentialities of tartrazine: screening tests. Photochem Photobiol 1984; 40: 221–226.
4. Przybilla B, Georgii A, Bergner T, Ring J. Demonstration of quinolone phototoxicity in vitro. Dermatologica 1990; 181: 98–103.
5. Gmelins Handbuch der Anorganischen Chemie. 8th edn., Vol. 9, part B2, Weinheim: Verlag Chemie, 1960: 397–613.
6. Hayon E, Treinin A, Wilf J. Electronic spectra, photochemistry and autoxidation mechanisms of the sulfite-bisulfite-pyrosulfite systems; the $SO_2^{\cdot-}$, $SO_3^{\cdot-}$, $SO_4^{\cdot-}$ and $SO_5^{\cdot-}$ radicals. J Am Chem Soc 1972; 94: 47–57.
7. Lizada MCC, Yang SF. Sulfite-induced lipid peroxidation. Lipids 1981; 16: 189–194.
8. Erben-Russ M. Pulsradiologische Untersuchungen radikalischer Reaktionen mehrfach ungesättigter Fettsäuren in wäßriger Lösung. Dissertation, Universität München, 1991.
9. Settipane GA. Adverse reactions to sulfites in drugs and food. J Am Acad Dermatol 1964; 10: 1077–1080.
10. Schlatter J, Zimmerli B, Dick R, Panizzon R, Schlatter C. Dietary intake and risk assessment of phototoxic furocumarins in humans. Food Chem Toxicol 1991; 29: 523–30.