Effects of food additives on leukocyte histamine release in normal and urticaria subjects

R. D. MURDOCH, PhD, Research Fellow*
M. H. LESSOF, MD, FRCP, Professor of Medicine
Guy's Campus, United Medical and Dental Schools, London

I. POLLOCK, MRCP, DCH, Research Registrar
The Cardiothoracic Institute, Brompton Hospital, London

E. YOUNG, MRCP, Senior Registrar in Dermatology
Dermatology Department, Wycombe General Hospital, High Wycombe, Bucks

Additives are included in foods for a number of reasons. Antioxidants have been used to prevent rancidity in oils and fats, preservatives to prevent fungal and bacterial growth, and colours to make foods more aesthetically pleasing, either before or after processing. Many of these compounds exert a powerful chemical or biochemical effect and some appear to be capable of exerting pharmacological effects on tissues [1] or cell systems [2, 3]. It is not therefore surprising that adverse reactions have been reported clinically in a number of separate studies [4, 5].

The evidence of IgE-mediated reactions to food additives is scanty. Weliky and Heiner [6] demonstrated the binding of tartrazine conjugates to IgE and IgD, and these conjugates were able to provoke a transient antibody response in a rat model. However, claims for the use of radioallergosorbent tests in the identification of IgE antibodies to food colourants in man [7] remain to be confirmed. Leaving aside the question of IgE-mediated reactions, there remains the question of whether asthma, urticaria, and other adverse reactions could be produced by a pharmacological mechanism, as has been claimed to be the case for aspirin [8].

The prevalence of adverse reactions to food additives has been quoted as between 0.03 and 0.15 per cent for additives in general [9] and 0.01 per cent for individual compounds such as tartrazine [10]. The most common clinical response to sulphites and other preservatives appears to be asthma [11] but azo dyes have also been implicated in a few cases of asthma [12] and have been claimed to have a role in nearly 30 per cent of patients with chronic urticaria [5]. Sodium nitrite can cause headache, skin rashes and gut symptoms [13] and is a known vasodilator. Monosodium glutamate can cause a variety of symptoms [14], possibly because the doses used cause oesophageal irritation or sodium retention. In many cases, however, the mechanism by which adverse reactions are produced remains in doubt.

Studies of aspirin-sensitive subjects have demonstrated that aspirin can provoke both histamine and leukotriene release from basophils and from nasal polyps [15], and there is evidence to suggest that this mediator release may partly depend on the inhibition of prostaglandin synthetase. Azo compounds have also been claimed to have pharmacological effects on the in vitro aggregation of platelets from normal or allergic subjects [16] and to have inhibitory actions on both prostaglandin synthetase and thromboxane activity [17]. Others have denied this claim [18].

Whatever the mechanism, mediator release can be provoked by both immunological and non-immunological events. Evidence of mediator release may therefore provide useful information. We chose to study histamine release provoked by food additives in the white blood cells of both normal and urticaria subjects, with the aim of assessing the validity of this type of test as a diagnostic tool.

Materials and methods

Subject selection

Eighteen control subjects were studied (9 male, 9 female), of whom seven (4 male, 3 female) were atopic. Control atopic subjects were defined as adults having a positive skin prick test (3 mm +) to either mixed grass pollen, house dust mite or cat fur and whose past symptoms included asthma, rhinitis or conjunctivitis. All were asymptomatic at the time of study. Non-atopic subjects were defined as having no history of atopic disease, negative skin tests and no history of allergic disease.
amongst first degree relatives. Twelve patients (5 male, 7 female) with at least a 12-week history of chronic, recurring urticaria or with angioneurotic oedema were also studied. All had symptoms which, by history alone, were not related to food additives. All the patients were symptom-free at the time of study and were defined as being atopic (3 male, 4 female) or non-atopic by the same criteria as the control subjects. Informed written consent was obtained in all cases. The study had the approval of the Guy’s Hospital Ethical Committee.

Skin prick test

Skin prick test solutions (grass pollen mix, house dust mite, cat fur, diluent control and histamine standards) were obtained from Bencards Ltd.

Washed leukocyte challenge materials

Donor leukocytes were challenged with additives in Tris ACM Buffer, made up of Tris A (consisting of 120 mM NaCl, 5 mM KCl, 25 mM Tris (hydroxymethylaminomethane) and 0.03 per cent human serum albumin) together with 1 mM CaCl2 and 1 mM MgCl2, at pH 7.4. The calcium ionophore (A23187) (Calbiochem) in concentrations of 0.1, 1 and 10 µg/ml provided a non-immunological challenge. Rabbit anti-human IgE (Fc specific) was obtained from Miles Yeda and added at dilutions of 1/100, 500, 2,500, 12,500 and 62,500 to provide an immunological challenge. The food additives used were the azo colours amaranth (E123), sunset yellow (E110), carmoisine (E122), tartrazine (E102), the non-azo colours quinoline yellow (E104), green S (E142), indigo carmine (E132), the natural dye annatto (E160b), antioxidants BHA (E320) and BHT (E321), sodium benzoate (E211) and aspirin BP, at concentrations of 1, 10, 100, 1,000 ng/ml. These materials were obtained from the British Industrial Biological Research Association (BIBRA) and conformed to food grade in chemical composition.

Washed leukocyte preparation

Venous blood (60 ml) was subjected to dextran sedimentation (20 ml of blood, 5 ml of dextran 70, 6% w/v, 2 ml 0.1 M EDTA at pH 7.4) for 90 min at room temperature. The leukocyte-rich fraction was aspirated and spun at 1,100 rpm for 8 min. The supernatant was aspirated and the cell pellet washed with Tris-A-EDTA, spun at 1,100 rpm for 8 min, and the supernatant discarded. The cell pellet was then resuspended in assay buffer (Tris ACM). Three hundred µl of cells were added to 300 µl of the antigen, ionophore or additive solution and incubated at 37°C for 40 min. The reaction was stopped by centrifugation at 1,500 rpm for 10 min at 4°C. The supernatants were assayed spectrophotometrically and the results expressed as net histamine release (NHR):

\[
NHR (%) = \frac{\text{Sample} - \text{control}}{\text{Total} - \text{control}} \times 100
\]

Controls were obtained in Tris ACM alone. Totals were derived from 12 per cent perchloric acid lysis of cell aliquots.

Total serum IgE

Serum IgE levels were measured using the paper radio-immunosorbent test (Prist) (Pharmacia).

Histamine measurements

Plasma histamine was measured by a single isotope radioenzymatic assay according to the methods of Guil-loux et al. [19] and Church et al. [20], with parallel diamine oxidase digestion [21]. Total blood and washed leukocyte histamine was measured spectrophotometrically using the method of Siragian and Brodsky [22].

Lung function

Forced expiratory volume (FEV1), forced vital capacity (FVC) and the histamine provocation dose causing a 20 per cent drop in FEV1 (PC20) were measured by Cockcroft’s method [23] using a spirometer (vitalograph).

Haematology

Five hundred white cells were counted to provide a differential white cell count and to quantitate basophils.

Cortisol measurements

Plasma cortisol levels were measured at 8 a.m. to 10 a.m. by radioimmunoassay [24]. Urinary cortisol was measured by the method of Mattingly et al. [25].

Results

Spontaneous release

There was no significant difference in the circulating plasma histamine in the normal subjects, with resting levels of 0.26 ng/ml (+ 0.13 SD), and the urticaria group in remission at 0.31 ng/ml (+ 0.22 SD). Similarly, whole blood histamine did not differ significantly between the urticaria and normal groups (90 ng/ml ± 47.2 and 95.8 ng/ml ± 73 respectively).

Aliquots of cells incubated with Tris ACM alone released between 6.4 ± 0.99% of their histamine in our 11 control, non-atopic subjects, 7.6 ± 2.57% in seven control, atopic subjects, 6.34 ± 2.46% in five non-atopic urticarial subjects and 5.7 ± 3.58% in seven atopic subjects who also had recurrent urticaria. We have taken the mean ± 2 SD of this release, ie 12.1 and 12.3 per cent respectively for the urticaria and control groups, as the level of histamine release attributable to the challenge compound.

Washed leukocytes

Histamine release after the addition of azo colours (amaranth, sunset yellow, carmoisine, tartrazine) exceeded
12.3 per cent of the total cell histamine in four of 18 (22%) normal subjects and in two of 12 (16%) urticaria subjects. Results were expressed as the percentage net histamine release at different concentrations of additive, to a maximum of 100 ng/ml. There was no significant difference between the maximal release seen in the individuals with urticaria and normal subjects, and there was no obvious difference between atopic and non-atopic subjects (Fig. 1a).

Over the same dose range the non-azo colours (Fig. 1b) green S, quinoline yellow and indigo carmine produced considerably less histamine release. However, a similar number, four of 18 (22%) of normal subjects released histamine, of whom three released non-significant amounts. In the urticaria subjects there was no difference in histamine release levels between the azo and non-azo colours. Histamine release of over 12.3 per cent was demonstrated in two subjects (16%).

Annatto (Fig. 2a), a natural food colour, released histamine in only one urticaria subject, whose leukocytes reacted to the highest dose used, and one normal subject whose cells showed a 43 per cent histamine release at the very low concentration of 10 ng/ml. The two antioxidants BHA and BHT (Fig. 2b) released histamine from the
had low levels of benzoate and azo histamine release of Fig. release, Class subjects Table histamine release white cells of four of 18 normals and 13 who reacted in subjects sensitive). One only released substantial amounts at this dose level.

One subject, denoted by an asterisk in Figs 1, 2 and 3, had low levels of spontaneous histamine release (4%) but proved to be exceptionally reactive to some azo and non-azo compounds as well as to annatto, BHA and BHT, benzoate and aspirin.

The number of reactors releasing more than 12.3 per cent histamine to the individual compounds in each class of additive can be seen in Table 1. The results confirm that as individual compounds the azo colours are more reactive than non-azo colours and annatto, while sodium benzoate and aspirin release more than 12.3 per cent histamine in 20 and 16.6 per cent respectively.

Of the 30 subjects studied, seven of the 12 urticaria sufferers did not react to a single compound with release greater than 12.3 per cent. Two reacted to benzoate and aspirin alone whilst of the other three, one reacted to aspirin and BHT, one to both azo colours and non-azo colours, and one patient reacted to nine separate compounds. Of the 18 subjects who did not have urticaria, eight did not react to any compound. Six reacted to one compound with greater than 12 per cent release, two reacted to two compounds, one to four and one to seven. There was no correlation between reactivity to aspirin and tartrazine, or benzoate and aspirin, and no suggestion that age or sex might predispose to greater histamine release.

Reproducibility

Four subjects who reacted were rechallenged to test the reproducibility of the system. In all cases, subjects reacted only to those compounds which had previously caused histamine release, although the magnitude of the response varied as did the optimum concentration of additives. In three non-reactors who were rechallenged the lack of response was confirmed in the second test.

Anti-IgE and ionophore challenge

There was no significant difference in the dose response or maximum response to these agents in either group.

white cells of four of 18 normals and two urticaria subjects who reacted in a dose dependent manner with maximal release of 13 to 30 per cent.

Sodium benzoate (Fig. 3a) showed a similar rate of histamine release (4/18 normals). Four out of 12 urticaria subjects released histamine, one of whom showed a histamine release of nearly 70 per cent. When pharmacological levels of aspirin were added to the separated white cells (Fig. 3b) the cells of three out of 12 (non-aspirin sensitive) urticaria suffers reacted to 1,000 ng/ml by releasing 13-30 per cent of their histamine content. Five out of 18 (27.7%) of normal subjects reacted similarly, but only two subjects released substantial amounts at this dose level.

One subject, denoted by an asterisk in Figs 1, 2 and 3, had low levels of spontaneous histamine release (4%) but

Table 1. Histamine release from the washed leukocytes of 30 subjects on exposure to food additives or aspirin.

| Class          | Compound       | Healthy subjects (Total 18) | Urticaria subjects (Total 12) |
|---------------|----------------|-----------------------------|------------------------------|
| Azo colours   | Amaranth       | 2                           | 2                            |
|               | Sunset yellow  | 1                           | 1                            |
|               | Carmoisine     | 3                           | 2                            |
|               | Tartrazine     | 3                           | 1                            |
| Non-azo colours | Green S      | 0                           | 1                            |
|               | Quinoline yellow | 1                       | 0                            |
|               | Indigo Carmine | 1                           | 1                            |
| Naturally derived | Annatto    | 1                           | 1                            |
| Anti-oxidants | BHA           | 1                           | 1                            |
|               | BHT            | 4                           | 1                            |
| Other         | Sodium benzoate | 3                          | 3                            |
|               | Aspirin        | 2                           | 3                            |

Fig. 3. Net percentage leukocyte histamine release in 18 normal (hatched) and 12 urticaria (dotted) subjects in response to (a) sodium benzoate and (b) aspirin at concentrations of 1,000 ng/ml. Significant release was taken as the mean + 2 SD of the spontaneous release, ie 12% of total.
Neither group showed any eosinophilia or basophilia. There was no correlation between blood cortisol or total blood histamine levels and basophil numbers, although high blood cortisol levels (>800 nmol/l) were accompanied by a low blood histamine, suggesting that there may nevertheless have been an effect on basophil function. There were no statistical differences between skin test histamine reactivity in any of the four groups.

Lung function studies showed no significant difference in histamine PC_{20} between the urticaria groups and normals (11.8 mg/ml ± 7.2 and 12 mg/ml ± 5.8 respectively). Similarly, no significant difference was seen in FEV_{1} of 3.4 l/min ± 0.99 and 3.5 l/min ± 0.61, respectively, in the two groups.

Discussion

One of the major criticisms of in vitro studies of mediator release induced by allergens, drugs or food additives is that the concentrations used for test purposes may not correspond to the concentrations attained by natural exposure. In calculating the concentrations to be used in our tests we therefore estimated a maximum daily ingestion by assuming a high intake of those foods which contain the maximum declared level of additives. By adding such items together [26] it seems possible to achieve an intake of 100 mg/day of azo colours, 50 mg/day of non-azo colours, 200 mg/day of antioxidants and 1 g/day of benzoate products. If acetylsalicylic acid (aspirin) is taken as a drug its effects are known to differ from those of sodium salicylate, which is often found in foods, but having decided to look for maximal effects we have used aspirin in our studies. The quantity of this product taken does not usually exceed 2 g daily.

Toxicity studies [27] suggest that only two per cent of azo and non-azo compounds are absorbed intact across the gut, but that the other compounds were almost totally absorbed. If these substances were taken as a single bolus, diffused through a circulating blood volume of 5 litres and were neither metabolised nor excreted, the maximum possible concentration could reach 400 ng/ml for the azo colours, 200 ng/ml for the non-azo colours, 20 µg/ml for the antioxidants, and 100 µg/ml for benzoates and aspirin. Such predicted levels are known to be achieved after single doses of aspirin, since a dose of 650 mg can give maximum plasma levels of 50–60 µg/ml, 90 minutes after ingestion. Similarly, the azo colours may well be within the range of 1–2 per cent intact absorption, suggested by the preliminary results of urine excretion studies after an oral dose of tartrazine (R. Walker, 1987 unpublished). The spontaneous release of histamine was at a low level in all cases, and an upper limit of normals was taken to be the mean ± 2 SD, ie 12.3 per cent. In attempting to assess the pharmacological effects of food additives on the white cells of healthy subjects, it was clear that the large majority of normal subjects either do not react to the maximum concentrations encountered during normal exposure or release less than 10 per cent of their histamine (well below the level of 12.3 per cent histamine release). A minority released larger quantities, and although this was unaccompanied by symptoms, we cannot rule out the possibility that the release of histamine or other mediators might potentiate other dietary or metabolic effects and so lead to symptoms in a susceptible minority.

The release of a single mediator, histamine, from circulating blood basophils in vitro may not necessarily correlate with the in vivo effects on intestinal mast cells or on other tissues. Nor can it reflect the secondary consequences of histamine release in the gut, its influence on mucosal permeability and absorption, or the more complex results that might be provoked by delayed absorption or by tartrazine metabolites [27]. Our studies should therefore be seen as part of a larger examination of food additives, in which their effects in vivo have also been studied [28]. Nevertheless, the ability to provoke histamine release, which we have demonstrated, was a consistent one, both in normal subjects and in those with urticaria. All subjects showed a consistent pattern of response and, in tests spread out over a six week period, either repeatedly failed to react to specific food additives or consistently showed significant histamine release.

The leukocyte histamine release in response to challenge with azo and non-azo colours, antioxidants, benzoate or aspirin was never associated with symptoms following ingestion and could not have been predicted by atopic status or the presence of urticaria. Histamine release showed no correlation with age, sex, resting levels of plasma or blood histamine, or spontaneous histamine release during incubation, or responsiveness to anti-human IgE or calcium ionophore. If we are indeed dealing with a pharmacologically mediated reaction, there must be other, as yet unidentified, dietary or metabolic factors which determine whether this response is accompanied by adverse clinical effects.

Acknowledgements

This article is based on work which forms part of a collaborative study carried out at Guy's Hospital (M. H. Lessof, R. D. Murdoch, I. Pollock), Brompton Hospital (Dr Warner), Wycombe General Hospital (Drs Wilkinson and Young) and the British Industrial Biological Research Centre (Drs Gangoli, Miller, Nicklin and Lake). We would like to thank Mr N. Virk and Mrs S. Thorpe for their technical and haematological assistance and Mrs J. Crisp for kindly preparing the manuscript.

This article records part of a multicentre study of food additive intolerance commissioned by the Ministry of Agriculture, Fisheries and Food.

References

1. Hedman, S. E. and Anderson, R. G. G. (1981) Acta Pharmacol et Toxicol. 48, 101.
2. Hedman, S. E. and Anderson, R. G. G. (1984) Acta Pharmacol et Toxicol. 52, 153.
3. Safford, R. J. and Goodwin, B. F. J. (1984). International Journal of Immunopharmacology, 6, 233.
4. Juhlin, L., Michaelsson, G. and Zetterstrom, O. (1972). Journal of Clinical Allergy and Immunology, 50, 92.
5. Juhlin, L. (1981). *British Journal of Dermatology*, **104**, 369.
6. Weliky, N. and Heiner, D. C. (1980) *Clinical Allergy*, **10**, 375.
7. Brighton, W. E. (1987) IgE antibodies to four food colours. Communication to Journées Franco-Britanniques d’Asthmologie, Montpellier. 25-26 Feb.
8. Asad, S. I., Youlten, L. J. F. and Lessof, M. H. (1983) *Clinical Allergy*, **13**, 459.
9. Commission of the European Communities (1981). Report of a working group on adverse reactions to ingested additives, 111 EN. Brussels: Commission of the European Communities.
10. Poulsen, E. (1980) Danish report on allergy and intolerance to food ingredients and food additives. Toxicology Forum. Aspen: Colorado.
11. Stevenson, D. D. and Simon, R. A. (1987) *Journal of Allergy and Clinical Immunology*, **68**, 26.
12. Freedman, B. J. (1977) *Clinical Allergy*, **7**, 407.
13. Moneret-Vautrin, D. A., Einhorn, C. and Tisserand, S. (1980) *Annales de la Nutrition et de l’Alimentation*, **34**, 1125.
14. Smith, S. J., Markandu, N. D., Rottellar, C., Elder, D. M. and MacGregor, G. A. (1982) *British Medical Journal*, **285**, 1205.
15. Kaliner, M., Wasserman, S. I. and Austen, K. F. (1973) *New England Journal of Medicine*, **289**, 277.
16. Gallager, J. S., Splansky, G. L. and Bernstein, I. L. (1980) *Clinical Allergy*, **10**, 683.
17. Cesarani, R., Colombo, M., Robuschi, M. and Bianco, S. (1978) *Prostaglandin and Medicine*, **1**, 499.
18. Gerber, J. G., Payne, N. A., Oelz, O., Nies, A. S. and Oates, J. A. (1979) *Journal of Allergy and Clinical Immunology*, **63**, 289.
19. Guilloux, L., Hartmann, D. and Ville, G. (1981) *Clinica Chimica Acta*, **116**, 269.
20. Church, M. K., Pao, GJ-K. and Holgate, S. T. (1982) *Journal of Immunology*, **129**, 2216.
21. Belcher, N. G., Murdoch, R. D., Dalton, N. et al. (1987) *American Review of Respiratory Disease* (In press).
22. Siraganian, R. P. and Brodsky, M. J. (1976) *Journal of Allergy and Clinical Immunology*, **57**, 525.
23. Cockcroft, D. W., Killian, D. N., Mellon, J. J. A. and Hargreaves, F. (1977) *Clinical Allergy*, **7**, 235.
24. Polyethylene glycol assisted second antibody radioimmunoassay. Modification of St. Thomas’s Method. Personal communication from M. J. Wheeler.
25. Mattingly, D., Dennis, P. M., Pearson, J. and Cope, C. L. (1964) *Lancet*, **ii**, 1046.
26. Fisher, C. (1986) Personal communication.
27. Jones, R., Ryan, R. J. and Wright, S. E. (1964) *Food and Cosmetic Toxicology*, **2**, 447.
28. Murdoch, R. D., Pollock, I. P. and Naeem, S. (1987) *Journal of the Royal College of Physicians of London*, **21**, 257.

© Crown copyright 1987