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A HYPOTHESIS THAT EXOGENOUS CHEMICALS CAUSE SOME CASES OF SYSTEMIC LUPUS ERYTHEMATOSUS

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INTRODUCTION

A number of drugs have been implicated as causing a clinical illness resembling systemic lupus erythematosus (SLE). The first to be mentioned was sulfadiazine in 1945 (1). Subsequently, hydralazine was implicated as causing SLE (2) and in 1970, Perry, Tan, Carmody, and Sakamoto published their observations on a series of patients with hydralazine-induced SLE (3). With this series, the idea that exogenous chemicals could induce an illness similar to SLE was established. Furthermore, the patients who developed this adverse reaction to hydralazine were all genetic slow acetylators indicating a clear pharmacogenetic predisposition to this drug-induced disease.

PHARMACOGENETICS OF ACETYLATION

One of the pathways by which aromatic amino or hydrazino compounds is metabolized by the body is by acetylation. N-acetyltransferase in the cytosol of cells, using acetyl-coenzyme A, transfers the acetyl group to the amino or hydrazino moiety of the chemical. The rate of this reaction was first studied indirectly in man by using isoniazid (INH) as the substrate. Price Evans, Manley, and Mc Kusick observed that the concentrations of INH in the plasma of subjects 6 hours after they received a standard dose of the drug were not normally distributed. A bimodal distribution was observed with about half of the subjects having high levels and about half having low levels (4). The interpretation, confirmed in subsequent studies, was that the subjects with low levels were rapid acetylators of INH and the subjects with high levels were slow acetylators. Family studies showed that this characteristic was inherited as a Mendellian trait with the slow phenotype being the homozygous recessive while the rapid phenotype could be either homozygous or the heterozygote. Substrates for this enzyme include INH, hydralazine, sulfamethazine, dapsone, procainamide, acetylhydrazine, a caffeine metabolite, aminofluorene, beta-naphthylamine, and benzidine (5).

The frequency of slow acetylation varies greatly between different ethnic groups. A majority of Scandinavians, Egyptians, and Israelis appear to be slow acetylators. Americans of European or African ancestry are about 50% slow and

50% rapid acetylators. Only about 10% of Orientals are slow acetylators, the remainder are rapid (6-8).

HYDRALAZINE-INDUCED SLE

That hydralazine can induce an SLE-like syndrome is now well established. The pharmacogenetic predisposition of slow acetylators for this adverse drug reaction has been observed in three independent series of patients with this syndrome summarized in Table 1.

TABLE 1
SUMMARY OF STUDIES OF ACETYLATION PHENOTYPE OF PATIENTS WITH HYDRALAZINE-INDUCED SLE

		Numer of		
Investigator	Location	Slow*	Rapid**	Reference
Perry	United States	24	1	9
Strandberg, et al	Sweden	29	2	10
Boetchelor, et al	United Kingdom	29	1	11

* Slow = genetic slow acetylators
**Rapid = genetic rapid acetylators

The next question is, Why are slow acetylators predisposed to the development of hydralazine-induced SLE? Price Evans and White observed that hydralazine disappeared faster from homogenates of liver biopsied from rapid acetylators than from that from slow acetylators (12). Zacest and Koch-Weser later observed lower levels of hydralazine in rapid than in slow acetylators after the same dose (13). Reidenberg, et al, then observed that acetylation was a major "first pass" pathway of hydralazine metabolism (14), a finding subsequently confirmed by Shepherd, et al (15). Thus slow acetylators absorb a layer fraction of the dose of hydralazine as the unacetylated hydrazine compound than do rapid acetylators. This would suggest that the unacetylated compound may be the moiety initiating the lupus.

PROCAINAMIDE-INDUCED SLE

Dreyfuss, et al, were the first to observe acetylprocainamide in the urine from people taking procainamide, demonstrating that the amino group on procainamide is acetylated by man (16). Reidenberg, et al, found that this

acetylation was by the same N-acetyltransferase that acetylates hydralazine, INH, and the other drugs listed above (17). People receiving procainamide who are slow acetylators developed ANA faster and procainamide-induced SLE faster than people who are rapid acetylators (18,19). Thus, slow acetylators are predisposed to developing ANA and SLE from procainamide. This suggests that the unacetylated aromatic amine is the important part of this drug for the induction of this effect. Proof of this was obtained by giving acetylprocainamide to patients with procainamide-induced SLE and observing the SLE go into remission. This was done during clinical trials of acetylprocainamide as an antiarrhythmic drug (20-23). The acetylprocainamide was given to patients with arrhythmias who had been treated previously with procainamide and had developed the SLE syndrome. Those who had active SLE had the SLE subside. Those whose procainamide had been discontinued previously and were in remission from their SLE had their SLE remain in remission while they were taking acetylprocainamide. Clearly, this is proof that the unacetylated amino group on procainamide is responsible for the SLE.

IDIOPATHIC SLE

Since it appeared that genetic slow acetylators were predisposed to the development of hydralazine-induced or procainamide-induced SLE, it seemed reasonable to see if there was an excess of genetic slow acetylators among patients with idiopathic SLE. Early studies by Godeau, et al, and Reidenberg and Martin indicated that this was the case (24,25). Subsequently, investigators in 15 locations around the world measured the acetylator phenotypes of 435 patients with idiopathic SLE (5,19). There were 240 slow acetylators and 195 rapid acetylators instead of 211 slow and 224 rapid acetylators expected ($P<0.01$). Thus, there is a small excess of slow acetylators among patients with idiopathic SLE. This suggests that some cases of this disease are caused by chemicals in the environment or diet that are substrates for N-acetyltransferase.

CASES OF CHEMICALLY-INDUCED SLE

Tartrazine

A patient developed lupus while taking a dosage form of procainamide that contained tartrazine. The medication was stopped and the lupus went into remission. Subsequent rechallenge with tartrazine caused photosensitivity, arthralgias, and myalgias (26). Thus tartrazine rather than procainamide is the likely chemical causing this patient's illness.

Tartrazine is a yellow azo food dye. It is widely used in foods and drug products. When taken orally, azo reduction products are excreted in the urine (27-30). Thus, tartrazine, and by analogy, perhaps the other azo food dyes, can be dietary sources of amines.

L-canavanine

Dietary l-canavanine has been found to cause SLE. The original observation was that a man eating alfalfa seeds during a clinical trial of these as a cholesterol-lowering agent developed pancytopenia, ANA, antibodies to double-stranded DNA, and splenomegaly. When the alfalfa seeds were stopped, the abnormalities subsided (31). Further research revealed that monkeys fed alfalfa seeds developed SLE and that this effect could also be produced by adding l-canavanine to the diet instead of the alfalfa (32,33). Subsequently, 2 cases were reported of women with SLE in remission who had exacerbations of their disease after they started eating alfalfa tablets (34).

Canavanine is a naturally ocurring amino acid that is present in leguminous plants (35).

Hydrazine

A laboratory technician who worked with hydrazine developed lupus after she worked with this chemical for a number of months. Interrupting exposure lead to remission of her symptoms. Reuse of the chemical in her work lead to exacerbation of the lupus. Detailed experimental studies produced further evidence implicating hydrazine as causing her illness. In addition, the patient was a member of a family that had a very high frequency of asymptomatic antibodies to nuclear constituents, suggesting additional genetic factors predisposing to this illness (36).

OTHER CHEMICALS

A number of other drugs have been implicated as causing some cases of SLE. Sulfonamides, PAS, practolol, acebutolol, nitrofurantoin, and chlorpromazine are among these. Each is either an amine or can be metabolized to an amine (37).

The anticonvulsants can also cause SLE. These are not amines. Captopril in high dosage induces ANA (38). It is a sulfhydryl containing compound but not an amine. Recently, we have learned that propylthiouracil, the sulfur-containing antithyroid compound can cause Coombs' positive hemolytic anemia and antinuclear antibody in cats. Substituting propyluracil, in which an oxygen atom has replaced the sulfur, for the propylthiouracil permitted all of these abnormalities to return toward normal (39). This implicated the sulfur moiety as important for this effect.

CONCLUSION

It is clear that a variety of drugs can cause a syndrome very similar to, if not the same as, some cases of idiopathic SLE. Many of these drugs are amines

and the amino group on procainamide has been shown to be the essential moiety for this adverse drug effect. Since adverse drug effects are really cases of chemically-induced disease in which the chemical is known, it is likely that other chemicals in the same chemical class as the drug can also cause the same adverse effects. Many of the drugs that can cause SLE are amines or hydrazines. Chemicals that are not drugs: canavanine, hydrazine, and tartrazine have also caused cases of SLE. These are an amino acid, a hydrazine, and a precursor of an amine. It would seem likely that other cases of idiopathic SLE are, in fact, due to other dietary or environmental chemicals in this class. The small excess of genetic slow acetylators among patients with this disease supports this concept.

Since drugs in other chemical classes can also cause SLE, it is likely that other kinds of chemicals in addition to amines can cause this disease. Further research is needed to evaluate this possibility.

REFERENCES

1. Hoffman BJ (1945) Arch Dermatol Syph 51:190
2. Morrow JD, Schroeder HA, Perry HM Jr (1953) Circulation 8:829
3. Perry HM, Tan EM, Carmody S, Sakamoto A (1970) J Lab Clin Med 76:114
4. Price Evans DA, Manley KA, Mc Kusick VA (1960) Br Med J 2:485
5. Reidenberg MM, Drayer DE (in press) In: Lahita R (ed) Systemic Lupus Erythematosus. John Wiley and Sons, New York
6. Lunde PKM, Frislid K, Hansteen V (1977) Clin Pharmacokinet 2:182
7. Weber WW, Hein DW (1979) Clin Pharmacokinet 4:401
8. Weber WW, Hein DW (1985) Pharmacol Rev 37:26-79
9. Perry HM (1973) Am J Med 54:58
10. Strandberg I, Boman G, Hassler L, Sjoquist F (1976) Acta Med Scand 200:376
11. Batchelor JR, Welsh KI, Tinoco RM, et al (1980) Lancet 1:1107
12. Price Evans DA, White TA (1964) J Lab Clin Med 63:394
13. Zacest R, Koch-Weser J (1972) Clin Pharmacol Ther 13:420
14. Reidenberg MM, Drayer DE, De Marco AL, Bello CT (1973) Clin Pharmacol Ther 14:970
15. Shepherd AMM, Ludden TM, McNay JL, Lin MS (1980) Clin Pharmacol Ther 28:804
16. Dreyfuss J, Bigger JT Jr, Cohen AI, Schreiber EC (1972) Clin Pharmacol Ther 13:366
17. Reidenberg MM, Drayer DE, Levy M, Warner H (1975) Clin Pharmacol Ther 17:722
18. Woosley RL, Drayer DE, Reidenberg MM, et al (1978) N Engl J Med 298:1157
19. Reidenberg MM (1983) Amer J Med 75:1037-1042
20. Roden DM, Reele SB, Higgins SB, et al (1980) Am J Cardiol 46:463
21. Lahita R, Kluger J, Drayer DE, et al (1979) N Engl J Med 301:1382
22. Stec GP, Lertora JJL, Atkinson AJ Jr., et al (1979) Ann Intern Med 90:799
23. Kluger J, Drayer DE, Lahita R, Reidenberg MM (1981) Ann Intern Med 95:18

24. Godeau P, Aubert M, Imbert JC, Herreman G (1973) Ann Med Interne 124:181
25. Reidenberg MM, Martin JH (1974) Drug Metab Dispos 2:71
26. Pereyo N (1980) The Schock Letter 30:1
27. Jones R, Ryan AJ, Wright SE (1964) Fd Cosmet Toxicol 2:447-452
28. Ryan AJ, Roxon JJ, Sivayaverojana A (1968) Nature 219:855
29. Honohan T, Enderlein FE, Ryerson BA, Parkinson TM (1977) Xenobiotica 7:765-774
30. Roxon JJ, Ryan AJ, Wright SE (1967) FD Cosmet Toxicol 5:367-369
31. Malinow MR, Bardana EJ Jr, Goodnight SH Jr (1981) Lancet i:615
32. Bardana EJ Jr, Malinow MR, Houghton DC, et al (1982) Amer J Kidney Dis 1:345-352
33. Malinow MR, Bardana EF Jr, Pirofsy B, et al (1982) Science 216:415-417
34. Roberts JL, Hayashi JA (1983) New Engl J Med 308:1361
35. Bell EA (1960) Biochemical J 75:618-620
36. Reidenberg MM, Durant PJ, Harris RA, et al (1983) Am J Med 75:365
37. Uetrecht JP, Woosley RL (1981) Clin Pharmacokinet 6:118
38. Reidenberg MM, Case DB, Drayer DE, et al (1984) Arthritis Rheum 27:579-581
39. Aucoin DP, Peterson ME, Hurvitz AI, et al (1985) J Pharmacol Exper Ther 234:13-18

Discussion - The amine hypothesis of SLE.

A.L. de Weck

I wonder whether you could elaborate a little bit on the immunological aspects. Is there any indication that the slow acetylators might have more anti-amine or anti-hydralazine antibodies which could cross-react with DNA? And, in your lymphocyte studies, do you have any evidence that these amines could immunomodulate T suppressor cells and in this way lead to the formation or increased formation of antinuclear antibodies?

M.M. Reidenberg

We do not know what antibody correlates with disease activity. In the case of procainamide-induced lupus we know that the titer of the various antibodies to nuclear constituents does not correlate with symptoms, and that the titers do not decrease when symptoms go away after the procainamide is stopped. There is evidence that procainamide binds covalently to macromolecules when given to animals, so one of the hypotheses is that procainamide serves as a hapten. However, we immunized rabbits with a procainamide-bovine serum albumin conjugate. The rabbits got sick but they did not get lupus. The hypothesis for the l-canavanine is that it mimicks one of the components of nuclear protein and is sufficiently foreign to induce the production of antibodies, but the proof of that is lacking. The other possibility, and the one I favor, is that procainamide acts pharmacologically on the immune system. Let me mention some data that supports this hypothesis. Acetylprocainamide is partly deacetylated in vivo so that all of our patients treated with acetylprocainamide had in fact, low levels of procainamide in their blood. These levels of procainamide were well under 1 µg/ml at the time when their lupus was going into remission. One patient whose arrythmia was resistant to acetylprocainamide received increasing doses of the drug so that at one point his procainamide serum level was above 1 µg/ml, and then he began to get some arthralgias and some fatigue. When we reduced the dose so that the procainamide serum level fell below 1 µg/ml, these arthralgias and the fatigue went away. I would think that constant procainamide levels of around 0.5 µg/ml would be quite

sufficient to serve as a hapten in somebody already sensitized, and since these levels were associated with remission of disease, it seems more likely that a pharmacological mechanism was operating, rather than procainamide functioning as a hapten. The other possibility is that procainamide and other amines are oxidized to hydroxylamines and then react with or oxidize nuclear macromolecules making them antigenic without covalently binding to them. Under these circumstances one could conceive of a mechanism by which one needed enough of the amine for this minor oxidative pathway to operate and produce the abnormal protein.

O. Pelkonen

The success or failure of the type of studies that you describe are totally dependant on the reliability of the method used to differentiate the various phenotypes. Do you know of any environmental factor that may affect acetylation phenotyping?

M.M. Reidenberg

The environmental factors that affect the rate of acetylation of a test compound are simply the amount of other amines present. For instance, if isoniazid half life is used as a marker, it can be slowed by the simultaneous administration of large doses of procainamide.

L.F. Prescott

It seems to me that your findings cannot all be explained by one unified hypothesis. On the one hand, you have a very clear dose-time acetylator status relationship with procainamide and on the other you postulate that tartrazine can produce the same syndrome, when this is a compound that is administered in very small amounts, as a food additive. Furthermore, from the chemical structure of tartrazine one would expect that it would be excreted very rapidly from the body, so that the exposure would be very small. In this case an immunological reaction rather than a dose-dependent pharmacological effect would seem more likely.

M.M. Reidenberg

Tartrazine is actively metabolized by the intestinal bacteria, so that subjects receiving it excrete metabolites of tartrazine rather than tartrazine itself. This compound was originally

thought to be non absorbable because of the sulphonic acid but the evidence in the rat, and to a limited extent in man, is that aromatic amines circulate in the body after oral administration of tartrazine. On the other hand, it seems clear that there is some immunological susceptibility to the disease. Probably many factors play a role in amine-induced SLE. In the case of procainamide the dose of the amine is so large that this becomes a predominant factor, whereas with some other compounds a factor of extreme sensitivity should be taken into account, in view of the very small doses that induce the disease.

P. du Souich

I have a comment on factors that appear to influence the acetylation reaction. Some years ago we were able to show that treatment of rabbits with Freund adjuvant increased the rate of acetylation, and so do corticosteroids, both in slow and fast acetylators.