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# U.S.-JAPAN COOPERATIVE MEDICAL SCIENCE PROGRAM

## Proceedings of
## First Workshop on the Pharmacology of Antileprotic Drugs

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The first Workshop on the Pharmacology of Antileprotic Drugs, organized by the U.S. Leprosy Panel of the United States-Japan Cooperative Medical Science Program, Geographic Medicine Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, was held 12 November 1969, at the National Institutes of Health. The proceedings were convened under the chairmanship of C. C. Shepard (National Communicable Disease Center) who discussed the endpoint of activity of 4,4′-diaminodiphenyl sulfone (dapsone, DDS) in the mouse foot pad test system, using inocula of strains of *Mycobacterium leprae* from treated and untreated leprosy patients. From available data it was concluded that the minimal effective concentration of DDS in the plasma of the mouse or man is substantially less than 10 ng./ml. and may be in the range of 1.0 to 2.5 ng./ml. These results emphasized the need for procedures for measuring DDS in this range. Current fluorometric procedures, which are limited to sensitivities of approximately 10 ng. DDS/ml. of plasma, were discussed by A. J. Glazko (Parke-Davis) and J. H. Peters (Stanford Research Institute). New modifications of the earlier fluorometric technic now allow the simultaneous determination of DDS and its monoacetylated conjugate, MADDS. Peters described numerous tests that demonstrated the validity if the fluorometric procedures. In addition, studies of interference by or contributions of numerous known and suspected metabolities of DDS in man and animals testified to the specificity of the methods. These compounds included various conjugates (N-glucuronide, N-sulfates) and several N-hydroxylated and ring-hydroxylated derivatives of DDS and MADDS. Other approaches to the problem of measurement of DDS at nanogram levels were discussed by Harris (New England Nuclear) and H. P. Burchfield (Gulf South Research Institute). The former described attempts to apply the double-isotope derivatization technic using $^{35}S$-labeled DDS and either $^{14}C$- or $^{3}H$-labeled acetic anhydride. Disadvantages were high cost per assay and a lower limit of sensitivity at 10 ng DDS or MADDS. Burchfield described procedures for converting DDS and MADDS to haloderivatives that could be separated via gas-liquid chromatography and detected by electron capture. Interference by plasma constituents and reagents, however, is still a problem that must be solved before the high sensitivities inherent in these methods can be realized. One of his observations may prove useful for the fluorometric determination of the repository form of DDS, 4,4′-diacetyldiaminodiphenyl sulfone (DADDS). This compound is separated from DDS and MADDS in the extraction procedures used by Glazko or Peters, but adequate sensitivity for its measurement was not obtained because of low inherent fluorescence. Burchfield reported that if DADDS is reduced ($LiAlH_4$) to the corresponding diethyl derivative, fluorescence yield per unit weight is increased approximately ten-fold.

In a review of earlier work on the disposition of DDS in man, Peters emphasized that orally administered DDS is almost

completely absorbed from the gut and distributed about evenly throughout the body. Only liver and kidneys exhibit moderately higher amounts than other tissues. Metabolites of DDS reported by earlier workers include an N-glucuronide and an N-sulfate of DDS and MADDS. After the standard therapeutic dose of 100 mgm. DDS, average peak plasma levels of DDS of about 2 μgm./ml. were found. These levels are about 1,000-fold higher than the minimal inhibitory concentration of DDS extrapolated from mouse foot pad tests. Therefore, lower doses of DDS may yield equivalent therapeutic effects in man, but as yet this has not been clearly established. From current studies, using fluorometric procedures, Peters concluded that, in parallel with the known polymorphic acetylation of isoniazid and sulfamethazine in man, DDS is similarly acetylated to MADDS and, therefore, rapid and slow acetylators of DDS are demonstrable in human populations. However, DDS is different from isoniazid and sulfamethazine in that rapid or slow acetylator subjects cannot be detected by the half-time disappearance from plasma or by proportions of DDS and MADDS in the urine. Furthermore, MADDS is deacetylated to DDS by man, whereas neither acetylisoniazid nor acetylsulfamethazine is converted back to the parent drug in human subjects. Rapid and slow acetylators can only be ascertained by simultaneous measurements of both DDS and MADDS in plasma. Of 14 subjects who were phenotyped using isoniazid and sulfamethazine, seven rapid acetylators exhibited a mean plasma MADDS:DDS ratio of 0.95 (range, 0.56 to 1.7); seven slow acetylators gave a mean of 0.20 (range, 0.14 to 0.33). Both types of subjects deacetylated oral MADDS, and no difference between rapid and slow acetylator phenotypes for deacetylation could be detected. Four separate tests of acetylator phenotype for DDS, separated by 2 to 4 weeks, were performed in 3 rapid and 3 slow acetylators. The results showed that individual acetylation characteristics for DDS are stable and reproducible.

Urinary excretion of DDS and MADDS and their derivatives was examined by combining the newer fluorometric procedures with earlier technics for measuring DDS and MADDS derivatives. Acid-labile DDS and MADDS derivatives were measured after mild acid hydrolysis; DDS conjugates were measured after strong acid hydrolysis. No differences in excretion patterns during 120 hours and after 100 mgm. DDS could be discerned in rapid and slow acetylators. Mean per cent of the dose of DDS as DDS was 9.1; as MADDS, 0.5; as acid-labile DDS, 16.1; as acid-labile MADDS, 2.0; as acid-hydrolyzable DDS, 31.7; and as total DDS derivatives, 59.6. Excretion of the first four categories was essentially the same after an equimolar dose of MADDS, but excretion of acid-hydrolyzable DDS and total DDS derivatives were 10 to 14 per cent higher during 120 hours after ingestion. Control studies showed that the acid-labile fractions could only be derived from N-glucuronide conjugates. The only known compounds that contribute to the acid-hydrolyzable DDS conjugates were N-sulfates of DDS and MADDS. Glazko also observed that urinary excretions of total DDS derivatives were higher in human subjects receiving 100 mgm. MADDS than in those receiving 100 mgm. DDS. He also described semiquantitative results obtained by paper chromotography on the excretion of metabolites by various animals receiving 2 to 7 mgm. DDS/kg., intramuscularly. In mice, the major excretory products were DDS, DDS-N-glucuronide, DDS-N-sulfate, and small quantities of the same MADDS conjugates. In rat urine, DDS, MADDS, and N-glucuronides and N-sulfates of both DDS and MADDS were the major products. Dogs excreted only DDS and the above two DDS conjugates, but no MADDS or MADDS conjugates. Large amounts of the N-glucuronides of DDS and MADDS, but only small amounts of other compounds, were found in rabbit urine. Rhesus monkeys excreted large quantities of DDS, MADDS, MADDS-N-glucuronide and MADDS-N-sulfate, with relatively smaller amounts of the other derivatives. In human urine the major products were DDS and DDS-N-glucuronide. Substantial quantities of more polar metab-

olites (di-N-glucuronide or di-N-sulfate?) were also found.

Peters reported studies wherein fluorometric procedures were employed to determine plasma levels of DDS and MADDS in mice, rabbits, and rhesus and squirrel monkeys receiving 1.0 mgm. DDS or 1.2 mgm. MADDS/kgm. In mice, no MADDS was detectable 1 hour after intraperitoneal administration of either drug. Therefore, no acetylation of DDS could be demonstrated and deacetylation of MADDS to DDS was extremely rapid and complete. Half-time disappearance of DDS from plasma was 2.6 hours after DDS and 2.4 hours after MADDS administrations. Rabbits, previously phenotyped as rapid and slow acetylators of sulfamethazine, exhibited different plasma ratios of MADDS to DDS after intravenous DDS that also characterized them as rapid and slow acetylators of DDS. But mean half-time disappearance of DDS from plasma in the two phenotypes (51 and 58 minutes) was nearly the same. After MADDS administration, DDS levels indicated that both types of rabbits were poor deacetylators of MADDS, with no difference between them. Rhesus monkeys were found to acetylate more extensively than any other species examined, exhibiting mean plasma ratios of MADDS to DDS of 7:1. Very low levels of DDS were found after MADDS, indicating only limited deacetylation. These animals cleared the compounds from the plasma approximately six times faster than did man. Squirrel monkeys exhibited capacities to acetylate DDS and to deacetylate MADDS that were similar to those in rapid acetylator human subjects, although clearance of the compounds from the circulation was about three times faster in these simians than in man. Examination of patterns of products in urine collected concurrently with the plasma samples from the monkeys showed that excretion by rhesus monkeys was quantitatively similar to that by man, with the exception that in the former more MADDS and acid-labile MADDS conjugates were found. In contrast to man and rhesus monkeys, squirrel monkeys excreted only small amounts of all classes of DDS compounds after either DDS or MADDS injection. In this species, total DDS derivatives amounted to $\frac{1}{3}$ to $\frac{1}{8}$ of the total found in the other primates.

W. T. Colwell, Jr., (Stanford Research Institute) reviewed earlier work on the structure-activity relationships of the sulfones as antituberculous and antimalarial drugs. The minimal structural requirements for activity are apparently the following: (a) one unsubstituted 4-amino group; (b) the second 4-amino group may be replaced by electronically similar groups such as a hydroxyl group; (c) the second 4-amino group may be in the 2 or 3 position. These variations are not inconsistent with the suggestion that DDS and its analogs act through the incorporation of the drug into a folic acid analog, wherein the 4-aminobenzoic acid moiety is replaced by DDS.

More recent work was described by N. E. Morrison (Leonard Wood Memorial), who has developed a rapid preliminary screen for testing antileprotic activity using *Mycobacterium sp.* 607. It was reported that replacement of the second amino group of DDS with other functional groups results in loss of activity. An exception was that replacement with a hydroxyl group yielded a relatively active compound, suggesting that hydrogen bonding may be the essential role of the second amino group of DDS. Monosubstitution of the second amino group of DDS with acetyl, hydroxyethyl or formyl did not cause a marked loss of activity. In general, substituted sulfonamides containing heterocyclic residues exhibit cross-resistance to DDS-resistant organisms. Other antimycobacterial and antimalarial drugs did not exhibit significant cross-resistance. Of this group, rifampin and the "rimino-phenazines" B.663 and B.1912 were of particular interest. Addition of human serum albumin (5 mgm./ml.) to the incubation test media resulted in a 4-fold decrease in the minimal inhibitory concentration of DDS, with smaller decreases in this parameter for MADDS and monoformyl DDS. It was suggested that protein-binding plays a role in the transfer of drugs through the highly lipophilic outer surface of the mycobacterial cell.

Tests of the activity of various sulfones against *M. leprae* inocula in the mouse foot

pad system were discussed by L. Levy (PHS Hospital, San Francisco). He found the following, fed in concentrations equimolar to 0.01 DDS, were inactive: sulfone, 4-aminodiphenyl sulfone, as well as other compounds unrelated to those discussed earlier. Active compounds were the sulfide and sulfoxide analogs of DDS, 4-amino-4′nitrodiphenyl sulfone and 3,-4′diaminodiphenyl sulfone. It was suggested that the first three may be activated by metabolism in the mouse. The activity of MADDS was equal to that of DDS, but results of studies on the metabolism of MADDS in the mouse indicate that this derivative is deacetylated completely to DDS. Numerous other derivatives are currently under investigation. Shepard added the observation that the diformyl derivative of DDS (DFD) was found to be slightly more active than DDS. Recent studies on DFD (an effective antimalarial, especially against chloroguanide-resistant plasmodia) were also summarized by R. Rozman (WRAIR). In man, DFD labeled in the formyl groups with $^{14}C$ yields high amounts of $^{14}CO_2$ in the expired air (45-50% of the dose after several days), indicating extensive deformylation and oxidation of the labeled residue to $CO_2$. In these studies, the very small amount of $^{14}C$-labeled compounds excreted in the urine suggested extensive, if not complete, deformylation of DFD. DDS is formed since, as Shepard indicated, DDS levels after DFD are similar to those found after DDS administration. However, volunteers have tolerated as much as 4,600 mgm. DFD/day without untoward effects. Since DDS in such doses would produce severe hemolysis, the simple conversion of all administered DFD to DDS could not be occurring. Yet, Glazko reported that the pattern of urinary excretion of compounds by subjects receiving DFD was similar to that in subjects receiving DDS with the exception that very small amounts of monoformyl-DDS were found in the former. DFD could not be detected.

Glazko also described studies of plasma levels of DDS following intramuscular injection of the repository forms of DDS, DADDS and CI-608 [4,′4‴-(*p*-phenylenebismethylideneimino-*p*-phenylenesulfonyl)-bisacetanilide] to man. Absorption rate from the site of injection was an inverse function of particle size of DADDS preparation. The more water-soluble CI-608 yielded two-fold higher DDS levels than did DADDS during two weeks after administration, but the levels from both were similar after four weeks and longer.

Therapeutic trials of DADDS (225 mgm. intramuscularly, every 77 days) now in progress in Venezuela, Malawi, South Korea, New Guinea and San Francisco were described by Shepard and Levy. Early results suggest that in some patients receiving DADDS, killing of bacilli proceeds more slowly than under standard DDS therapy, but critical tests of bacillary counts and of viability in the mouse foot pad system were not completed at the time of the workshop. A chemoprophylactic study using DADDS is also in progress in Ponape. Some of these trials have been in progress long enough to expect critical information during the next year.

The historical development of the "riminophenazines" (r-alkyl) as antituberculous and antileprotic drugs was described by Colwell. The most useful, B.663 [2-(*p*-chloroanilino)- 3-isoprophlimino- 5-(*p*-chlorophenyl)-3, 5-dihydrophenazine] has the advantage of being active against DDS-resistant *M. leprae*. Levy reported that B.663 inhibited multiplication of *M. leprae* in the mouse foot pad test system when administered at a dose of 0.001% in the diet. Lower doses were ineffective. Using colorimetric procedures, he reported that B.663 accumulated in the tissues, less than 1 per cent of the ingested dose being excreted in the urine. No metabolites of B.663 were detected. Based on earlier observations that administration of 200 mgm. B.663/week was fully therapeutic in tests in Sungei Buloh, various therapeutic regimens ranging from 200 mgm./day to 1,200 mgm. once every four weeks are currently under study in Cebu.

A survey of various potential methods for the measurment of B.633 in biologic fluids was presented by Burchfield. The most promising was gas-liquid chromatography using an electron capture detector.

In the last presentation, Shepard re-

viewed other drugs besides DDS and B.663 found to be bactericidal by the kinetic method in the mouse foot pad test system. They are sulfadimethoxine, ethionamide, rifampin, streptovaricin and cephaloridine. Preliminary results indicate that rifampin is active in leprosy of man. However, the high cost of this drug may preclude its extensive use. He emphasized that most antimalarial drugs are not active as antileprotic agents. Combination drug tests are now in progress in the mouse foot pad system, but these may not be needed in man because of the unusually low occurrence of DDS-resistant organisms in leprosy patients (about 1 per 1,000).