Pharmacologic Aspects of Pentamidine

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SUMMARY—Pentamidine is an aromatic diamidino compound synthesized originally for the therapy of trypanosomiasis. The pharmacologic effects of pentamidine vary, depending on its route of administration. In animals, the dominant effects have been a precipitous, transitory drop in blood pressure after injection and renal toxicity following repeated administration. To avoid the possibility of immediate toxic reactions associated with iv administration, we now usually give the drug im to humans. Further interest in pentamidine has been stimulated by its usefulness in the treatment of interstitial pneumonia caused by Pneumocystis carinii. In some patients receiving antineoplastic or immunosuppressive therapy who have superimposed P. carinii pneumonia, pentamidine may cause serious renal toxicity. Distribution and excretion studies in animals indicate pentamidine is deposited in tissues, with the greatest concentration in the kidneys, and gradually eliminated over a prolonged period. The mechanism of action of pentamidine against P. carinii or the means whereby fixation in tissues and subsequent toxicity occur have not been elucidated. Recent investigations to help clarify these points indicate that pentamidine inhibits dihydrofolate reductase in all tissues studied both in vitro and in vivo. In addition, pentamidine interacts and forms water-insoluble products with specific nucleotides and nucleic acids.—Nati Cancer Inst Monogr 43: 171-176, 1976.

Pentamidine (4,4'-diamidinophenoxypentane; Lomidine) is a member of a series of aromatic diamidino compounds synthesized in the 1930's and extensively evaluated for trypanocidal activity. The diamidino derivatives among many other classes of compounds were studied initially to find less toxic and more effective antitrypanosomal agents than the diguanidines (7-3). Based primarily on comparative therapeutic properties, attention narrowed to four aromatic 4,4'-diamidino compounds: pentamidine, stilbamidine (4,4'-stilbenedicarboxamidine), propamidine (4,4'-diamidinoxypropane), and phenamidine (4,4'-diaminodiphenyl ether). Of these compounds, pentamidine and stilbamidine have received the most extensive clinical evaluation.

Pharmacologic studies of pentamidine have been hampered by the lack of sufficiently sensitive methods for analysis of the drug in tissues and body fluids. Stilbamidine (4, 5) has been investigated more completely because of its strong fluorescence, unique among the diamidino compounds. Since 1941, pentamidine has had extensive clinical use outside the United States, primarily for the treatment of specific tropical diseases. The drug is regarded by many authorities as the agent of choice in the therapy of early African sleeping sickness (6-8), as prophylaxis against Trypanosoma gambiense (9, 10), and of value in the treatment of leishmaniasis resistant to sodium antimony tartrate and ethyl stilbene (11). The diamidines also have antibacterial properties (12) as well as activity against specific types of malaria (13) in animals.

The aromatic diamidines were tried initially by Ivády and Páldy in 1958 (14) for the therapy of interstitial pneumonitis due to Pneumocystis carinii, and their results with pentamidine were reported in 1963 (15). Subsequent evaluations have substantiated the effectiveness of pentamidine against this organism in animals (16) and man (17-22).

The clinical toxicity of pentamidine as a single agent has been well documented (12). In recent years, P. carinii pneumonia has been seen as a complication in seriously ill patients receiving antineoplastic or immunosuppressive drugs. In some of these patients, severe renal toxicity developed (21, 23, 24) after pentamidine administration.

This report briefly reviews the background and current pharmacologic knowledge of pentamidine and presents and elaborates on data showing tissue storage and elimination. Recent preliminary experimental evidence demonstrating that pentamidine is an inhibitor of dihydrofolate reductase (DHFR) is given and initial results showing the interaction of pentamidine with nucleotides and nucleic acids are discussed. These various biologic activities and properties of pentamidine should provide a greater insight into its possible therapeutic modus of action and the possible basis for the toxicity associated with the drug.

MATERIALS AND METHODS

Tissue deposition and excretion of pentamidine.—Mice were given ip injections of 10 mg pentamidine isethionate/kg body weight (calculated as base). The animals were kept in individual glass metabolism cages, and urine was collected free of feces by use of an attached anal cup (25). A few drops of 0.1 N HCl were added to the urine collection tube to maintain an acid pH. Each day the glass cage was washed with water, the final urine volume was adjusted to 15 ml/24-hour period and frozen for subsequent analysis. The feces were removed from the anal cup daily and frozen. The animals were killed at specified intervals. Organs, tissues, and feces were weighed and homogenized in five to seven times the volume of 0.1 N HCl.

Pentamidine analysis.—The procedure of Waalkes and DeVita (26) was used for the assay of pentamidine. Pentamidine was extracted under basic conditions from plasma, urine, and tissue samples into organic solvents. After this extraction and the subsequent concentration of the pentamidine into acid, the reaction between pentamidine, glyoxal, and benzaldehyde to form a fluorescent end product was performed in basic medium following, in general, a modification of the method for aromatic dia-
midines developed by Jackson et al. (27). After extraction of this product into organic solvents, the final determination was made with an Aminco-Bowman spectrophotofluorometer.

Duplicate samples were analyzed in all cases. Samples of urine or tissues, identical samples with known amounts of pentamidine added to determine recovery, standard pentamidine solutions alone, and a reagent blank were run through the entire procedure.

DHFR studies.—Aromatic diamidines included pentamidine isethionate (a generous gift of May and Baker, Ltd., Dagenham, England, through Rhodia, Inc., New York, N.Y.), stilbamidine, and propamidine (Chemistry Branch, Division of Cancer Treatment, National Cancer Institute). Other chemicals and their sources were: folic acid and methotrexate (Lederle Laboratories, Pearl River, N.Y.), NADPH (Sigma Chemical Co., St. Louis, Mo.), and pyrimethamine (2,4-di amino-5-(p-chlorophenyl)-6-ethyl-pyrimidine) (Burroughs Wellcome and Co., Research Triangle, N.C.). Dihydrofolate (DHF) was prepared from folate by the dithionate method of Futterman (28) as modified by Blakley (29) and stored under nitrogen at −68 ° C.

Liver or kidney from male Sprague-Dawley rats was used as the source of DHFR. The enzyme was purified to homogeneity by affinity chromatography as described.3

Enzyme assay.—Activity of DHFR was measured by spectrophotometric method, with the decrease in absorbance used that occurs at 340 nm when NADPH and DHF are converted to NADP and tetrahydrofolate, respectively (30). A value of 12,000 was used for the molar extinction change at 340 nm which accompanies the reaction (31). Unless otherwise indicated, the standard assay mixture contained a final volume of 1 ml: 100 nm Tris-HCl buffer, pH 7.0; 1 mm KCl; 0.10 mm NADPH; 0.05 mm DHF containing 1 mm 2-mercaptoethanol, and enzyme.

Enzyme activity was assayed at 27 ° C with a Gifford Model 2400-S multiple absorbance recorder; the decrease in absorbance at 344 nm was recorded automatically at 5-second intervals. Enzyme activity was expressed as μmoles of substrate reduced per hour per volume of enzyme solution.

In vivo study.—Male Sprague-Dawley rats (300 g; 3/ group) were given ip injections of saline (control), pentamidine (20 mg/kg calculated as free base), pyrimethamine (10 mg/kg), or methotrexate (10 mg/kg). Twenty-four hours later, the animals were killed. The kidneys were dissected rapidly and homogenized in saline (1 g/10 ml). The homogenate was centrifuged for 1 hour (17,000 Xg) at 0 ° C and the supernatant used for assay of DHFR as previously described. Protein concentration was determined by the method of Lowry et al. (32).

RESULTS

As shown in text-figure 1, after ip injection into mice, pentamidine was deposited in tissues and gradually excreted over a prolonged time. From the amount of pentamidine per gram tissue, the greatest amount was found in the kidneys with the next highest amount in the liver as compared with the remaining tissues of the animal. The relative rate of release of pentamidine from each site with time appeared similar.

After administration of pentamidine to mice, the drug was excreted predominantly in the urine (text-fig. 2) but also was found in the feces. The relative total amounts in urine and feces were similar at each time interval studied: approximately 4 to 1, respectively. Again, prolonged storage in tissues was evident with secondary delayed excretion.

The results summarized in text-figure 3 demonstrate that pentamidine inhibited the activity of liver DHFR.


Text-figure 1.—Amount of pentamidine in mouse kidneys, livers, and all other tissues at intervals after ip injection of 10 mg/kg. Average of 3 animals for each time point. Values expressed as pentamidine base.

Text-figure 2.—Relative amounts of pentamidine recovered, expressed as percent at various intervals in feces, urine, and tissue after ip injection of 10 mg/kg. Actual recovery of the total dose of pentamidine administered was 100% for 1, 18, and 41 hours and 87% for 90 hours. Average of 2 animals for each time interval.
the degree of inhibition being proportional to the quantity of the compound incubated with the enzyme. On a molar basis and under identical conditions, propamidine was a more effective inhibitor than pentamidine, whereas methotrexate, particularly, and also pyrimethamine were considerably more potent than either pentamidine or propamidine. Because of its strong absorbance at 340 nm, stilbamidine could not be included in these studies.

The data in table 1 demonstrate that, 24 hours after administration of pentamidine, methotrexate, or pyrimethamine, DHFR activity was significantly reduced in the kidneys of treated animals in contrast to those of untreated controls. The effects of methotrexate and pyrimethamine were compared.

**DISCUSSION**

Much of the data on the biologic effects of pentamidine were published during the 1940's shortly after its trypanocidal activity had been demonstrated. Pentamidine has been studied and administered almost exclusively by iv, sc, or im injection. Probably the most extensive physiologic and pharmacologic study of the aromatic diamidines was reported by Wien (33) in 1943. In mice the LD50 by the iv route was 28 mg/kg and by the sc route, 64 mg/kg. For both mice and rabbits, the acute toxic effects appeared similar. Death was attributed to respiratory failure associated with general depression of the central nervous system, occasional febrile clonic convulsions, and lowering of body temperature. In larger animals, after iv injection, the most profound effect appeared to be on the blood pressure. All four aromatic diamidines studied after iv injection produced a marked transient fall in blood pressure in anesthetized cats and decerebrated animals. The effect was reduced or abolished by calcium but only partially inhibited by atropine. The drop in blood pressure was attributed to peripheral vasodilatation and not to a direct cardiac or central nervous mechanism.

Injection (iv) of pentamidine (10 mg/kg) into cattle, sheep, and goats (24) produced shock, sometimes fatal. Marked venous congestion, flabby heart, and evidence of either or both kidney or liver damage were found on postmortem examination. With lower, better tolerated doses, kidney damage accompanied by rising blood urea nitrogen (BUN) was noted before evidence of hepatotoxicity. Blood sugar levels did not change in animals eventually dying, though BUN rose 50–100%.

Pathologic examination of the kidneys of rabbits treated with large doses (20 mg/kg) of propamidine and stilbamidine revealed cloudy swelling, desquamation, and fatty degeneration of the renal convoluted tubules (12). Repeated doses, equivalent to therapeutic levels in man, caused no histologic changes. By either the sc, im, or iv routes, similar though transient impairment of renal function was observed after maximally tolerated doses. Some animals died suddenly from uremia after a single injection of a large dose of diamidine. Repeated administration of pentamidine to young rats inhibited growth. Inflammatory changes were noted frequently at the site of injection (33).

Wien et al. (35) evaluated various metabolic changes produced in animals by the diamidines. Blood sugar levels were increased by pentamidine but only at, or near, toxic doses. Repeated administration was associated with depletion of liver glycogen. Elevations of blood urea and non-protein nitrogen levels occurred in some instances on doses which did not influence blood sugar levels. With toxic doses, cloudy swelling of the kidneys and fatty degeneration of the liver were produced. Repeated administration of therapeutic doses caused no change in blood elements, but, with toxic doses in guinea pigs, leukocytosis with increase in polymorphonuclear cells preceded death.

Amdides also have other biologic properties which may contribute to their pharmacologic and toxicologic effects. In 1944, Blaschko et al. (36, 37) found diamidines, particularly pentamidine and propamidine, inhibited liver histaminase, though distinct species differences were noted. In 1949, MacIntosh and Paton (38) reported specific organic bases, including pentamidine, released tissue histamine. That same year Gemmill (39) published data indicating pentamidine as well as other amidines inhibited anaerobic glycolysis of glycogen to lactate in muscle extracts.

Excretion, distribution, and quantitative disposition studies for pentamidine were hindered initially, due to a lack of sufficiently sensitive assay procedures. Unique to the aromatic diamidines, stilbamidine is a strongly fluorescent compound. This characteristic is secondary to its central double-bond structure not present in the other diamidines. Consequently, tissue distribution studies of stilbamidine could be done in animals by use of this fluorescent property. In these animals (4, 5), prolonged intense fixation to renal and hepatic tissue was found. Subsequent studies (40) with 14C-labeled stilbamidine confirmed these results with evidence of residual compound for 6 months or more. Stilbamidine was eliminated both in urine and feces, with no radioactivity found in exhaled respiratory carbon dioxide. Although to a lesser degree, prolonged storage of the compound was also found in the lungs and heart. All observers noted an apparent absence of a circulating form of the diamidine in the blood.

The urinary excretion of stilbamidine and several of its derivatives, after repeated daily administration, was also
studied in animals with fluorescence used for assay (41). An initial delay in excretion was found until apparent tissue saturation had occurred. This finding was thought to represent and confirm the tissue deposition and fixation.

Launoy et al. (42, 43) in 1960 studied the elimination of 14C-labeled pentamidine in both mice and rats. A sample of radioactive compound was synthesized with the 14C label on the terminal amidine carbon, and a second sample with the 14C label in the central carbon chain. With either compound sample, a similar excretion pattern was found for mice, which suggested that pentamidine was eliminated intact without metabolic change. It was calculated that approximately half the injected dose in mice had been eliminated within 5 days. No attempt was made to analyze the drug separately in urine and feces. A major portion of the labeled compound was found in the kidneys and liver, and a much smaller amount dispersed in all other tissues, though the blood appeared devoid of radioactivity. In a longer term study in rats, the investigators found prolonged tissue storage and delayed elimination of pentamidine.

With an improved fluorimetric assay procedure, a pharmacologic study of pentamidine was done in 1970 (44). This included disposition, distribution, and excretion of the drug in mice and plasma levels and urinary excretion in patients with neoplastic disease undergoing treatment for P. carinii pneumonia. Only a single ip injection (10 mg/kg) was given to the mice. The results and conclusions from these experiments were similar to those published by Launoy et al. (42, 43), which indicated pentamidine is excreted unchanged with prolonged tissue fixation. It is apparent that pentamidine is stored (text-fig. 1) in tissues, predominantly renal, and gradually disappears with time. Analyses of other mouse organs in addition to kidneys and liver, including lungs, spleen, abdominal and pelvic tissues, brain, skin, and skeleton, indicated the kidneys, and secondarily the liver, contained the greatest concentration. Essentially no compound was found in the brain. Pentamidine excretion (text-fig. 2) occurs primarily by way of the kidneys, with lesser total amounts present in the feces. In a more recent study in rats, pentamidine, 36 hours after a single ip injection (10 mg/kg), was present in all anatomic areas of the kidneys when grossly dissected into cortex, medulla, and collecting ducts. Quantitatively, the greatest amount was in the cortical area, with actual levels of 37, 29, and 26 µg/g of tissue, respectively. Pentamidine in the urine for each 6-hour period of a day was determined in 7 patients with suspected or proved P. carinii pneumonia. The dosage was 4 mg/kg/day given im for 10–12 days. On this regimen, the plasma levels were low (0.3–0.5 µg/ml), did not rise appreciably immediately after injection, remained essentially the same throughout each 24-hour period, and did not increase with succeeding days of treatment. If the plasma level rose, the usual time was 1 hour after the injection. The highest levels were in patients with an elevated BUN. The amount of pentamidine in the urine for each 6-hour period of a day was determined. For 5 patients, of the total excreted in the urine during the 24 hours after the injection of pentamidine, half to two-thirds occurred during the first 6 hours after administration of the drug. The amount excreted in the urine each day was approximately 20% or less of the daily dose. No attempt was made to determine the possible amount of drug excreted by way of the gastrointestinal tract. After cessation of therapy, the duration of the urinary excretion of pentamidine was determined for 3 patients. Decreasing amounts of the compound were detected up to 6–8 weeks after termination of the drug therapy. The evaluation of the effect of pentamidine on renal function was complicated by other factors. Several patients with increases in BUN during pentamidine therapy also were receiving nephrotoxic antibiotics. For those patients with an increase in BUN, the peak elevation occurred 8–14 days after initiation of pentamidine therapy, with return to normal levels between days 17 and 30. No other significant or consistent hepatic effects and no changes in blood sugar levels were noted.

Neither the mechanism of action of pentamidine nor its toxic effects on the kidney have been fully explained. It has been suggested that pentamidine acted through interference with aerobic glycolysis (45). Hawking and Smiles (4), investigating the trypanocidal action of stilbamidine, noted the fixation of the drug to nucleoproteins. In patients with multiple myeloma, both stilbamidine and pentamidine induce selective basophilic granulations in myeloma cells. These granulations (46, 47) have been reported to be made up of a combination of RNA and diamidine, which suggests the possibility of incorporation of these compounds into nucleic acid components within the cell. In more recent studies (48), pentamidine has been reported to inhibit in vitro synthesis of DNA, RNA, phospholipid, nucleotides, and protein in cells of a murine ascites tumor. In clinical studies, Robbins et al. (49) reported megaloblastosis of the bone marrow in a patient treated with pentamidine. This was accompanied by lowered serum folate levels. From these clinical observations, Robbins (50) suggested that pentamidine might act as a direct inhibitor of folate metabolism in a manner similar to the effect of methotrexate. However, Frenkel et al. (16) showed that pretreatment with folic acid did not influence the therapeutic efficacy of pentamidine in rats infected with P. carinii.

To clarify some of these actions of pentamidine, in particular the bone marrow megaloblastosis following its administration, experiments were initiated to examine the possible effect of pentamidine, propamidine, pyrimethamine, and methotrexate on DHFR. The last compound was used for comparative purposes as a classical example of a chemical inhibitor of DHFR. Both pentamidine and propamidine in vitro inhibit DHFR (text-fig. 3), but on a molar basis the activity is considerably less than that observed with methotrexate or pyrimethamine. Twenty-four hours after administration of pentamidine to rats, DHFR activity in kidney extracts was reduced (table 1) and was similar to the effects of methotrexate and pyrimethamine. In contrast to the results obtained by the in vitro experiments, the apparent relative amount of pentamidine required to inhibit DHFR to an extent similar to methotrexate seemed considerably less. This, however, may be due to the more prolonged storage of pentamidine in the tissues after in vivo administration.

Also in process are in vitro studies with human tissues, including normal liver and kidneys, leukemic spleen, and erythrocytes obtained from a patient with polycythemia vera as sources of DHFR. The results to date are similar to those shown in text-figure 2. In addition, investigations to clarify the nature of the individual inhibition are being done and will be reported in detail elsewhere. The results

4 Waalkes TP, Slawsky R, Adamson R: Unpublished observations.

5 Makulu DR, Waalkes TP: Unpublished observations.
of these experiments indicate that the inhibition is competitive with respect to the enzyme substrate and dihydrofolate and noncompetitive with respect to the coenzyme NADPH.

In a further attempt to elucidate the biologic interactions of pentamidine, studies of the effects of the aromatic diamidines on nucleic acids have been made (57). By in vitro experiments, pentamidine was found to react with isolated nucleic acids to form water-insoluble precipitates at neutral pH. This effect was observed with all types of DNA and RNA of bacterial, fungal, or mammalian origin. In subsequent studies, aromatic diamidines were found to interact and precipitate with all nucleic acids, nucleotide triphosphates, and nucleotide diphosphates, but not with nucleotide monophosphates, nucleosides, free nucleic acid bases, and non-nucleic acid diphosphates, such as fructose diphosphate, or with inorganic phosphates. This finding suggests that the nucleotide diphosphate molecule is the minimum nucleic acid structure necessary for interaction with aromatic diamidines.

It is clear that pentamidine in animals is deposited and stored for prolonged periods in tissues and that probably in humans a similar process occurs. The maximum concentration in the kidneys associated with prolonged tissue fixation would appear to establish ideal conditions for renal malfunction, particularly in seriously ill patients. The interaction of pentamidine with nucleic acids and its inhibition of DHFR might well play roles, both in its renal toxicity and its ability to eradicate the *P. carinii* organism. The precise mechanism of the deposition of pentamidine in tissues has not been completely clarified, but its ability to react and form precipitates with nucleic acids and nucleic acids and to bind and inhibit DHFR suggests these types of combinations within tissues may be important factors. Such molecular interactions could be the mechanism whereby the maximum deposition of pentamidine occurs in the kidneys, followed by gradual release and excretion predominantly in the urine.

The importance of DHFR in intrinsic cellular reactions also leads to speculation that pentamidine may be effective against *P. carinii* by virtue of its ability to inhibit this important enzyme system. Although no enzymatic studies have been carried out directly on the *P. carinii* organism, recent experiments have indicated the DHFR of an extract of *T. cruzi* is exquisitely sensitive to pentamidine. Previous work (52) indicates that various DHFR inhibitors, including methotrexate, are also active inhibitors of the enzyme from extracts of trypanosomes. Nevertheless, in vivo, no known DHFR inhibitor had been effective against trypanosomes. This therapeutic failure is speculated to be secondary to plasma protein binding and poor transport into the parasites, but pentamidine apparently has no such restrictions.

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