Means of Increasing the Tolerated Dose of Isoniazid in Mice. II. Certain Amino Acids. (23330)

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(Introduced by R. J. Huebner)
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The acute oral toxicity of isoniazid, an effective therapeutic agent in human tuberculosis, has been reported to be reduced in mice by simultaneous administration of appropriate amounts of glycine and sodium glucuronate(1), or of cycloserine(2), or glycerine(3). The present investigation extends the study to the effect of certain other amino acids on isoniazid (INH) oral toxicity in DBA mice.

Methods. The minimal lethal dose of INH by oral administration in DBA mice is 6 mg/20 g mouse (300 mg/kg). However, since glycine and sodium glucuronate permitted the survival of mice given repeated administrations of a 10 mg dose of INH, this amount of INH (500 mg/kg) was adopted for the initial tests with other amino acids. As with glycine, the amount of amino acid used for first trial was approximately 5 or 6 millimols/millimol of INH. The chemicals were weighed, mixed in one tube, and dissolved or suspended in water to such a volume that the desired concentration of each was contained in 0.5 ml for oral administration. When necessary, hydrogen ion concentration was adjusted to approximately pH 5. Due to the low solubility in water of the desired concentrations of tryptophane, leucine, isoleucine, these amino acids were unsuitable for oral administration and were omitted from the series. This study is limited to the use of water as solvent; results of investigation of other solvents will follow later. The series of experiments include (a) results of mouse survival tests with 10 mg INH and each of 26 amino acids; (b) results with 8 mg INH and 11 of these effective acids; (c) repeated oral administrations of various amounts of L-cysteine-HCl with INH; (d) blood plasma levels after a single administration of various amounts of L-cysteine-HCl and INH; and (e) *in vitro* tests on the effect of 4 amino acids on the bacteriostatic action of INH on one strain of avirulent *Mycobacterium.* Toxicity tests were made in 5 DBA mice for each of the amino acids studied in the amount that would represent a 5:1 molar ratio with INH. With the single exception of DL-aspartic acid with which one mouse died, all the other amino acids permitted 100% survival. It is of interest that a 10:1 proportionate amount of L-cysteine-HCl permitted 90% survival of a single dose.

Results. Mouse survival after a single oral administration of isoniazid with amino acid. With 10 mg of INH only 4 of these 26 amino acids were consistently able to protect 50% or more of the mice (Table I). Although in one test several of the other acids gave this result—L-serine, DL-serine, L-histidine-HCl, DL-aspartic acid, L-arginine-HCl, DL-threonine, DL-valine, DL-methionine, L-ornithine-HCl—duplication was not obtained in a second or third test. Of the 4 effective amino acids, only L-cysteine-HCl gave consistently better than 50% survival in repeated tests. In most instances increasing the amount of any of these acids beyond the 5:1 molar ratio with this dose of INH did not increase the percentage of survivors.

As also shown in Table I, a dose of 8 mg INH was tested with 11 of the 26 amino acids in at least 5:1 molar amounts, or in the case of L-cysteine-HCl with a series of 6 concentrations of from 10 to 116 mg per 20 g mouse (approximately 1 to 12 mmols/mmol INH). As might be expected, there was a significant increase in survival rate. Three of the series which were only 20% or less protective with the 10 mg dose of INH were observed to effect 50% survival when tested with 8 mg INH; and 70% or more of the mice survived with hydroxy-L-proline, L-aspartic acid, L-histidine-HCl, L-glutamic acid-HCl, and with L-cysteine-HCl in 4 different concentrations, 30, 46, 58 and 87 mg per mouse. As a conse-

quence, L-cysteine-HCl was selected for further study.

Effect of repeated administration of various amounts of L-cysteine-HCl on INH toxicity in mice. Several experiments on repeated administrations of INH with L-cysteine-HCl have shown that, with daily administrations of 8 mg INH per mouse, there was an optimal concentration of 30 to 58 mg of L-cysteine-HCl. Increasing or decreasing the amino acid concentration gave less satisfactory results. The best procedure, administration of 8 mg INH with 58 mg of L-cysteine-HCl at 72-hour intervals, permitted 60% survival after 15 doses. With 10 mg INH and 58 mg L-cysteine-HCl given at 72-hour intervals, 50% of the mice survived 7 doses. However, a similar mixture with 50 mg supplementary

TABLE I. Effect of a Single Oral Dose of Certain Amino Acids on Isoniazid Toxicity in DBA Mice.

	Isoniazid—10 mg		Isoniazid—8 mg	
Amino acid	Amino acid, mg*	No. mice surviving / No. mice tested	Amino acid, mg*	No. mice surviving / No. mice tested
0		0/20		1/35
L-Cysteine-HCl	116	0/10	116	3/10
"	87	6/10	87	9/10
"	58*	56/75	58	20/20
"			46*	20/20
"			30	9/10
"			10	6/10
L-Glutamic acid-HCl	100	16/30	100	7/10
"	66*	11/20	53*	17/20
D-Glutamic acid	60	4/10		
DL-Glutamic acid-H_2O	60*	0/10		
L-Aspartic acid	48*	10/20	48	4/10
"			39*	7/10
DL-Aspartic acid	48*	5/30	48	10/20
"			39*	4/10
Hydroxy-L-proline	48*	10/20	39*	7/10
L-Arginine-HCl	76*	10/29	76	9/20
"			61*	6/10
L-Histidine-HCl	68*	8/30	68	14/20
"			55*	8/10
DL-Methionine	54*	6/26		
Acetyl-DL-methionine	96	15/30	70	11/20
"	70*	4/30	56*	26/40
L-Ornithine-HCl	61*	6/30		
" -diHCl			60*	13/20
DL-Ornithine-HCl	61*	2/10	49*	5/10
DL-Serine	38*	6/21		
L-Serine	38*	6/30	38	0/20
"			30*	10/20
DL-Threonine	43*	6/20		
DL-Valine	43*	6/20		
DL-Alanine	32*	2/10		
Beta-Alanine	32*	2/10		
DL-Citrulline	63*	2/10		
L-Proline	42*	2/10		
Glycine	27*	2/20		
L-Cystine	88*	1/10		
L-(+)-Lysine-HCl	67*	1/20		
DL-Homocystine	97*	0/ 6		
L-Tyrosine	66*	0/10		

* Indicates amt/20 g mouse to give a ratio of 5 mMols of amino acid/mMol isoniazid.

sodium glucuronate permitted 50% of the mice to survive 10 doses. This endpoint is in contrast to 70% survival after 15 doses of a mixture of 10 mg INH with 50 mg glycine and 50 mg sodium glucuronate(1). The difference may be partially accounted for by the excess of glycine (a 10:1 molar ratio), but the toxicity of L-cysteine-HCl *per se* would preclude the repeated use of such an amount of this acid. Thus, whereas a single administration of 8 mg INH alone permitted survival of only 2 of 70 animals, when supplemented with L-cysteine-HCl (58 mg), up to 15 doses given twice weekly were tolerated by 60% of the mice.

Blood plasma concentrations. Blood plasma concentrations of INH in mice were determined at different time intervals after combined INH-cysteine administration. The method of INH assay was the "pyridyl" procedure(4). In a control test, no interference in the INH color reaction was observed when L-cysteine-HCl was present. The highest level obtained was 7.8 mg% of INH in the plasma 1 hour after administration of a mixture of 8 mg INH and 58 mg L-cysteine-HCl. There was but little difference in the concentrations after either 8 or 10 mg INH with 58 mg L-cysteine-HCl either at the 2-hour interval—4.5 and 5.0 mg% respectively—or at the 24-hour interval—0.6 and 0.3 mg% respectively. However, the use of supplementary sodium glucuronate (50 mg) with 10 mg INH and 58 mg L-cysteine-HCl resulted in a lower 2-hour level and a somewhat higher 24-hour level—3.3 and 1.1 mg%. This latter observation may indicate an explanation for the longer survival time in this group than in the mice without glucuronate. As reported earlier, a control of 5 mg INH alone in DBA mice showed a 30-minute level of 6.5 mg%, a 2-hour level of 2.9 mg%, and little or no detectable amount after 24 hours. Hence it would appear that the INH-L-cysteine-HCl mixture produced somewhat higher levels which persisted longer than those produced by the highest tolerated dose of INH alone.

Effect of certain amino acids on INH bacteriostatic action. Following a procedure previously described(1), 4 of the series of amino acids, acetyl-DL-methionine, DL-serine, L-glutamic acid-HCl, and L-cysteine-HCl, were selected for an *in vitro* study of their possible effect on inhibition by INH of the growth of an avirulent *Mycobacterium* strain B103. These 4 acids in *in vivo* toxicity tests ranged in effectiveness from 13% to 100% (Table I). Yet none of them in 5:1 molar ratios altered the inhibition endpoint of INH, 5 to 10 μg; nor were they *per se* inhibitory of growth of this microorganism. In addition, in 3 tests L-cysteine-HCl supplemented with sodium glucuronate in the proportions used in the repeated administrations experiment had no effect on the INH bacteriostatic action.

Discussion. A comparison of the results obtained here with L-cysteine-HCl and those with glycine shows that *per se* cysteine is more effective as a means of increasing the tolerated dose of INH. Thus with 10 mg INH, glycine permits only 10% survival, whereas L-cysteine-HCl permits 74% survival; and correspondingly with 8 mg INH, the survival rates are 50% and 100% respectively. Thus, in the 5:1 molar ratio in mice, L-cysteine-HCl alone permits tolerance of an INH dosage which would require supplementary sodium glucuronate if glycine were the amino acid of choice. These results would further indicate that many of the amino acids might prove effective in reducing the acute toxicity of the LD_{50} dose of 5 mg INH, or even reducing the toxic manifestations due to repeated administrations of a sublethal dose.

The mechanism by which the animal is able to tolerate toxic drugs when amino acids are given concomitantly has been discussed by various workers. Martin and his collaborators(5), employing amino acids to reduce the toxic effect in mice of sulfa drugs and various anthelmintics, considered the effect a prophylactic one. Ragno(6), who found L-glutamic acid efficacious in INH therapy of certain human tuberculous infections, suggested that its effectiveness might be due to its combination with the ammonia released from INH *in vivo*. Support for this suggestion is found in the recent reports of Greenstein and collaborators(7) on metabolism of amino acids in rats, with the statement that

L-arginine is an efficient means of detoxifying ammonium acetate; whereas the present study suggests that arginine is not adequately effective with INH. Possibly amino acids may counteract the inhibitory effect of INH on such enzymes as diphosphopyridinenuclease, or on the diphosphopyridine nucleotide itself.

One clinical report has indicated that use of simultaneous glycine and sodium glucuronate with INH did not control peripheral neuritis, but in 2 tuberculous patients markedly reduced frequency of convulsive seizures induced by the prolonged INH therapy and thus permitted uninterrupted treatment(8). Since L-cysteine-HCl may be effective due to a different mechanism than glycine and sodium glucuronate, it is conceivable that its use might benefit those INH-treated patients in whom toxic reactions are not counteracted by glycine and sodium glucuronate.

Summary and conclusions. Twenty-six amino acids have been tested for their ability to reduce the toxic effect of isoniazid (INH) in mice. Eleven of these—L-cysteine-HCl, L-glutamic acid-HCl, L-histidine-HCl, L-aspartic acid, hydroxy-L-proline, L-ornithine-diHCl, acetyl-DL-methionine, L-arginine-HCl, DL-aspartic acid, L-serine, and DL-ornithine-HCl—in appropriate concentration (a 5:1 molar ratio) permitted at least 50% survival of DBA mice given an 8 mg lethal dose of INH per 20-g mouse (400 mg/kg). Optimal results were obtained with single or repeated administration of 58 mg of L-cysteine-HCl (2900 mg/kg) with 8 mg INH. This dosage yielded a maximum blood plasma level of 7.8 mg% 1 hour after administration, as contrasted with 6.5 mg% following a 5 mg dose of INH alone. No interference by 4 amino acids, L-cysteine-HCl, L-glutamic acid-HCl, DL-serine, or acetyl-DL-methionine, was noted in the INH bacteriostatic test on one strain of avirulent *Mycobacterium* B103. From this study it would appear that L-cysteine-HCl, and perhaps other amino acids, are as effective as glycine and sodium glucuronate, previously reported, in increasing the tolerated dose of INH in DBA mice.

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Bile Constituents of the Opossum *Didelphys marsupialis virginiana.** (23331)

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In the study of gallstone disease the usual laboratory animal is not satisfactory because of low concentration of cholesterol in bile of the animals (Table I). It would be advantageous to find an animal which excretes a large amount of cholesterol in the bile and in high concentration. Wibler(6) reported significantly high cholesterol content in opossum gallbladder bile. This report is based on the several constituents of the gallbladder and hepatic bile of the opossum. Bile acids in opossum gallbladder bile were first studied by Haslewood and Wootton(7), and cholic acid

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