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Concentrations of Pyrazinamide Attained in Serum with Different Doses of the Drug *

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Serial concentrations of pyrazinamide in 3 volunteers showed that increasing the dose from 22 mg/kg of body-weight to 66 mg/kg resulted in substantially higher serum concentrations of the drug and a longer period of coverage (at a concentration of 25 µg/ml). Similarly, in a. study based on 6 tuberculous patients, increasing the dose of pyrazinamide from 66 mg/kg to 88 mg/kg led to appreciably higher concentrations in the serum. In neither series of tests was any acute hepatic toxicity observed. The findings suggest that it would be interesting to study the effect of doses of about 90 mg/kg of pyrazinamide in once-weekly regimens of chemotherapy for the treatment of tuberculosis.

Pyrazinamide, a potentially hepatotoxic drug, is now usually administered in daily dosages of 20 to 40 mg/kg of body-weight since larger dosages had proved toxic (McDermott et al., 1954; Morrissey & Rubm, 1959; United States Public Health Service, 1959). However, at this Centre, the drug has been given in dosages of 26 to 43 mg/kg of body-weight daily without serious clinical toxicity (Velu et al., 1961). In the course of planning a recent study at the Centre, it was proposed to give pyrazinamide (together with streptomycin and isoniazid) at once-weekly intervals, and the feasibility of administering higher doses of pyrazinamide was therefore considered. However, the administration of higher individual doses of pyrazinamide can be expected to increase therapeutic efficacy only if it results in an increase in the serum concentration of the drug. The present study was therefore designed to provide information on this subject.

PLAN AND CONDUCT OF THE INVESTIGATIONS

The investigations were undertaken in 2 stages. In the first stage, 3 volunteers were each administered 3 doses of pyrazinamide, namely, 22 mg/kg of body-weight, 44 mg/kg and 66 mg/kg, with at least 1 week between successive doses. Specimens of blood were collected 1, 3, 6 and 24 hours after the administration of the drug. In the second stage, 6 patients (whose pulmonary tuberculosis was under treatment but whose chemotherapy did not include pyrazinamide) were each administered 2 doses of pyrazinamide, namely, 66 mg/kg of body-weight and 88 mg/kg, with an interval of 1 week between the 2 doses; specimens of blood were collected 3 and 6 hours after the administration of the drug.

The free pyrazinamide content of each specimen of blood was estimated. In addition, in the study involving patients, the serum L-aspartate: 2-oxoglutarate aminotransferase (serum glutamic-oxaloacetic transaminase; SGOT) activity of the 6-hour specimens was determined in order to detect any evidence of acute liver toxicity.

METHODS

Estimation of pyrazinamide

The estimation of pyrazinamide was based on its reaction with sodium nitritopentacyanoferroate. Since pyrazinoic acid, a metabolite of pyrazinamide with very little antituberculosis activity *in vivo* (Kushner et al., 1952) or *in vitro* (Tuberculosis Chemotherapy Centre, Madras, unpublished data) also reacts with sodium nitritopentacyanoferroate, it was removed by passing the serum sample through a column of ion-exchange resin, and the resulting eluate was used for the estimation of pyrazinamide.

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Standards. Each batch of tests included the followmg standards:

(1) Sheep serum blank (in duplicate);

(2) Standard solutions: sheep serum containing 20, 40, 80, 100 and 120 µg/ml pyrazinamide.

Procedure. A 1-ml sample of the test serum was treated with 2 ml of 7.5 % trichloracetic acid and then centrifuged at 3000g for 10 minutes; 1 ml of the protein-free extract was then applied to a vertical column (diameter 8 mm, height 40 mm) of Dowex-1-8 chloride (200 to 400 mesh) prepared in water. The column was repeatedly washed with small amounts of water until two 5-ml eluates had been collected. To each eluate, 0.5 ml of 2N NaOH and 0.5 ml of nitritopentacyanoferroate reagent (Allen et al., 1953) were added, and 10 minutes later, the intensity of the resulting orange-brown complex was read in a Unicam SP 600 spectrophotometer at 500 nm, using cuvettes of 20 mm optical pathway. The sum of the optical density (OD) readings for the 2 eluates was regarded as the OD for the serum. (Eluate 1 usually contained the bulk of the pyrazinamide in serum; thus, the mean OD of 60 test sera was approximately 9 times higher for eluate 1 than for eluate 2.) The same procedure was adopted for the standard solutions and the blanks; a standard graph was drawn and the pyrazinamide concentrations in the teat sera were read off.

Estimation of SGOT

The SGOT activity was estimated by the procedure described by the Sigma Chemical Company (1961), and expressed in Karmen Units.

RESULTS

Concentrations of pyrazinamide in the serum

Volunteers. The serum concentrations of pyrazinamide produced in volunteers by different doses of the drug are presented in Table 1 and summarized in the upper half of Table 3. The mean concentrations at 1 hour were 19, 24 and 44 µg/ml with the doses of 22, 44 and 66 mg/kg of body-weight, respectively. A similar pattern was observed with, the results at 3, 6 and 24 hours–namely, the mean concentration being substantially higher with the 66 mg/kg dose than with the 22 mg/kg dose. Further, with each of the 3 doses, the mean concentration was highest at 3 hours, being 27 µg/ml with the 22 mg/kg dose, 52 µg/ml with the 44 mg/kg dose and 70 µg/ml with the 66 mg/kg dose.

TABLE 1

SERUM CONCENTRATIONS OF PYRAZINAMIDE IN VOLUNTEERS

Volunteer	Weight (kg)	Dose of pyrazinamide (mg/kg)	Serum concentration of pyrazinamide (µg/ml) after following number of hours:			
			1	3	6	24
1	74	22	45	38	31	11
		44	65	59	55	22
		66	48	76	59	21
2	61	22	21	36	31	7
		44	**6**	**45**	**39**	11
		66	**60**	**96**	**58**	21
3	75	22	**7**	**15**	**12**	7
		44	**35**	**52**	**46**	11
		66	29	48	54	16

It is known that a large proportion of the bacterial population in wild strains of tubercle bacilli is inhibited *in vitro* by pyrazinamide in a concentration of 25 µg/ml (Tripathy, 1966). In this study, it was estimated (from a graphical representation of the data, which is not presented here) that this concentration was attained after about 3 hours with the 22 mg/kg dose, after a little over 1 hour with the 44 mg/kg dose and under 1 hour with the 66 mg/kg dose; these levels were maintained for approximately 4, 15 and 19 hours, respectively. Thus, increasing the dose of pyrazinamide from 22 mg/kg to 66 mg/kg resulted in a longer period of coverage with the drug.

All the 24-hour specimens contained pyrazinamide in measurable quantities; further, the concentrations with the 44 and 66 mg/kg doses were above 10 µg/ml in all 3 volunteers (Table 1). This suggests that pyrazinamide persists in the body for a long time and is metabolized slowly, an inference which is confirmed by the finding that the half-life of the drug (computed from the serum concentrations at 6 and 24 hours after the administration of the 66 mg/kg dose) was approximately 12 hours for all 3 volunteers.

In summary, increasing the dose of pyrazmamide from 22 mg/kg of body-weight to 66 mg/kg resulted in higher serum concentrations of the drug between 1 and 24 hours, an approximately proportionate increase in the highest concentration attained, and a

TABLE 2
SERUM CONCENTRATIONS OF PYRAZINAMIDE IN PATIENTS

Patient	Weight	Dose of pyrazinamide (mg/kg)	Serum concentration of pyrazinamide (µg/ml) at the following number of hours after administration of the test dose:	
			3	6
1	34	66	91	94
		88	142	170
2	38	**66**	109	95
		88	107	113
3	40	66	81	70
		88	129	138
4	36	**66**	63	72
		88	59	121
5	43	66	91	77
		68	134	127
6	50	**66**	91	112
		88	64	123

longer period of coverage with a concentration of 25 µg/ml.

Patients. The findings in patients are presented in detail in Table 2, and summarized in the lower half of Table 3. The mean concentrations at 3 and 6 hours were 86 µg/ml and 85 µg/ml with the 66 mg/kg dose, and 105 µg/ml and 131 µg/ml, respectively, with the 88 mg/kg dose. Thus, the administration of a higher dose of pyrazinamide resulted in appreciably higher mean serum concentrations of the drug.

The mean serum concentrations at 3 and 6 hours after the dose of 66 mg/kg of body-weight were similar, suggesting that the drug was slowly absorbed and that the absorption continued beyond 3 hours. This is confirmed by the finding of a higher mean concentration at 6 hours (131 µg/ml) than at 3 hours (105 µg/ml) with the 88 mg/kg dose.

TABLE 3
MEAN SERUM CONCENTRATION OF PYRAZINAMIDE IN VOLUNTEERS AND IN PATIENTS

	No. of subjects	Dose of pyrazinamide (mg/kg)	Mean serum concentration of pyrazinamide (µg/ml) at the following number of hours after administration of the test dose:			
			1	3	6	24
Study in volunteers	3	22	19	27	23	8
		44	24	52	46	14
		66	44	70	57	19
Study in patients	**6**	**66**		86	85	
		88		105	131	

SGOT activity in patients

No acute hepatic toxicity was observed in any of the 6 patients. Further, these patients had SGOT values ranging from 21 to 40 units in the 6-hour blood specimens collected after the first dose of pyrazinamide, and from 29 to 45 units in similar specimens collected after the second dose. These values are within the ranges of 6 to 51 units (Tuberculosis Chemotherapy Centre, Madras, 1966) and 14 to 54 units (Ramakrishnan et al., 1968) [1] observed in tuberculous patients on admission to treatment at this Centre.

DISCUSSION

The estimation of pyrazinamide in biological fluids is usually based on the reaction of the drug with sodium nitritopentacyanoferroate in strongly alkaline medium, giving an orange-brown complex whose colour intensity can be determined spectrophotometrically at a wavelength of 500 nm (Allen et al., 1953; Caccia, 1957). Biological fluids containing pyrazinamide are likely to contain pyrazinoic acid, a metabolite which also reacts with sodium nitritopentacyanoferroate, producing a similar colour complex. Estimates of pyrazinamide in biological fluids, based on its reaction with sodium nitritopentacyanoferroate, would therefore indicate the *total* content of pyrazinamide and pyrazinoic acid. The latter has been shown to have very little antituberculosis activity in mice (Kushner et al., 1952) and in *in vitro* tests on Löwenstein-Jensen medium and on acidified Löwenstein-Jensen medium (Tuberculosis Chemotherapy Centre, Madras, unpublished data). Consequently, a method has been adopted which eliminates the pyrazinoic acid and estimates only the pyrazinamide content of the serum.

[1] See article on p. 775 of this issue.

Our investigation with volunteers has shown that increasing the dose of pyrazinamide from 22 mg/kg of body-weight to 66 mg/kg resulted in an approximately proportionate increase in the highest pyrazinamide concentration attained, and in longer periods of coverage with a concentration of 25 µg/ml. Similarly, our study in patients has shown that increasing the dose of pyrazinamide from 66 mg/kg of body-weight to 88 mg/kg resulted in appreciably higher serum concentrations. There is, therefore, a pharmacological basis for expecting that pyrazinamide administered in doses of 66 mg/kg or 88 mg/kg of body-weight would be more effective therapeutically than when administered in doses of 22 mg/kg or 44 mg/kg of body-weight. Also, the absence of acute hepatic toxicity with the doses of 66 mg/kg and 88 mg/kg in the study in patients is encouraging for the use of high-dosage pyrazinamide on a *once-weekly* basis. Finally, Ramakrishnan et al. (1968) have shown that none of the 19 patients who received approximately 70 mg/kg of body-weight of pyrazinamide on a once-weekly basis for 24 weeks had any evidence of hepatotoxicity. On the basis of all these findings, a large-scale investigation of the efficacy of a once-weekly regimen of high-dosage pyrazinamide plus high-dosage isoniazid plus streptomycin has been undertaken, the results of which will be reported later.

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RÉSUMÉ

La pyrazinamide est habituellement donnée à des doses quotidiennes de 20 à 40 mg par kilogramme de poids corporel. Envisageant de l'adrninistrer hebdomadairement, en association avec la streptomycine et l'isoniazide, les auteurs ont étudié l'évolution des concentrations sériques du produit en fonction des variations du dosage.

Dans un premier stade, trois volontaires ont reçu chacun 3 doses hebdomadaires successives de 22 mg/kg, 44 mg/kg ou 66 mg/kg de pyrazinamide. Des échantillons de sang ont été prélevés 1, 3, 6 et 24 heures après la prise du médicament. En portant la dose de 22 mg à 66 mg/kg, on a obtenu des concentrations sériques plus élevées entre la 1re et la 24e heure, une hausse approximativement proportionnelle de la concentration maximale et une persistance accrue de la concentration de 25 µg/ml.

Dans un deuxième stade, 6 malades atteints de tuberculose pulmonaire ont reçu chacun 2 doses de 66 mg/kg ou de 88 mg/kg de pyrazinamide à une semaine d'intervalle. Trois et 6 heures après la prise du produit, les concentrations sériques moyennes ont atteint 86 et 85 µg/ml dans le premier cas et 150 et 131 µg/ml lorsque le dosage était porté à 88 mg/kg. Aucun signe d'hépatotoxicité aiguë n'a été observé chez ces 6 malades et les valeurs de la transaminase glutamique-oxalacétique sont restées dans des limites normales.

En raison de son intérêt thérapeutique et de son innocuité, l'administration d'environ 90 mg/kg de pyrazinamide dans les schémas de traitement hebdomadaire mérite d'être envisagée. Des recherches à ce sujet sont actuellement en cours au Centre de chimiothérapie de la tuberculose, à Madras, Inde.

REFERENCES

Allen, W., Aronovic, S., Brancone, L. & Williams, J. (1953) *Analyt. Chem.,* **25,** 895

Caccia, P. A. (1957) *Amer. Rev. Tuberc.,* **15,** 105

Kushner, S., Dalalian, H., Sanjurjo, J. L., Bach, J. L., Jr, Safir, S. R., Smith, V. K., Jr & Williams, J. H. (1952) *J. Amer. chem. Soc.,* **74,** 3617

McDermott, W., Onnond, L., Muschenheim, C., Deuschle, K., McCune, R. & Tompsett, R. (1954) *Amer. Rev. Tuberc.,* **69,** 319

Morrissey, J. F. & Rubin, R. C. (1959) *Amer. Rev. resp. Dis.,* **80,** 855

Ramakrishnan, C. V., Janardhanam, B., Krishnamurthy, D. V., Stott, H., Subbammal, S. & Tripathy, S. P. *(1968) Bull. Wld Hlth Org.,* **39,** 775-779

Sigma Chemical Company (1961) *Sigma techn. Bull.,* 505

Tripathy, S. P. (1966) *Sensitivity test for pyrazinamide.* In: *Proceedings of the 21st Tuberculosis and Chest Diseases Workers' Conference held February 1966, at Calcutta,* Delhi, Navchetan Press, p. 272

Tuberculosis Chemotherapy Centre, Madras (1966) *Bull. Wld Hlth Org.,* **34,** 483

United States Public Health Service (1959) *Amer. Rev. Tuberc.,* **80,** 371

Velu, S., Andrews, R. H., Angel, J. H., Devadatta, S., Fox, W., Jacob, P. G., Nair, C. N. & Ramakrishnan, C. V. (1961) *Tubercle (Edinb.),* **42,** 136