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DETECTION OF FREE RADICAL INTERMEDIATES DURING ISONIAZID AND IPRONIAZID METABOLISM BY ISOLATED RAT HEPATOCYTES.

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The two hydrazine derivatives Isoniazid and Iproniazid are widely used the therapy of tuberculosis and depressive syndromes respectively and have been indicated as responsible for causing hepatic injury (1).

Both the compounds follow the same metabolic pathway, primarly consisting in the splitting between the iso-nicotinic ring and the hydrazinic group, which in the case of Isoniazid has been previously acetylated (2). Acetyl and isopropyl-hydrazine, released from acetyl-Isoniazid and Iproniazid respectively, are then metabolized by the cytochrome P_{450}-dependent monoxygenase system to reactive intermediates that are postulated to be free radicals (2).

The identification of highly reactive free radicals is now made possible by the use of spin trapping agents, as phenyl-butyl nitrone (PBN), which reacts with the radicals to form stable nitroxide adducts, detectable by electron spin resonance (e.s.r.) spetroscopy (3).

Materials and Methods.

Isolated hepatocytes and liver microsomes were prepared from phenobarbital-induced rats, as previously described (4), and incubated at 37° C for 30 min with 1 mM Isoniazid, Iproniazid or acetyl and isopropyl-hydrazine and in the presence of 25 mM PBN (Aldrich-Europe, Bersee, Belgium). At the end of the incubation the PBN-radical adducts were extracted as in (4) and analyzed using a Brucker 200 D 12/10 e.s.r. spectrometer.

Metal-catalized oxidation of acetyl and isopropyl-hydrazine was performed according to Augusto et al. (5). Isopropyl-hydrazine was synthetized as described by Gever and Hayes (6).

Results and Discussion.

Following the treatment of isolated hepatocytes with Isoniazid and Iproniazid, free radical adducts of PBN are clearly detectable. The nitroxide hyperfine splitting constants are $\alpha^N = 14.4$ $\alpha^H = 2.47$ for Isoniazid and $\alpha^N = 14.9$, $\alpha^H = 2.49$ for Iproniazid. Similar e.s.r. spectra are also evident when acetyl or isopropyl-hydrazine are added instead of the parent compounds to isolated hepatocytes (Table 1).

The e.s.r. spectra obtained from liver cell preparations are also consistent with those observed following Cu^{2+}-catalized oxidatin of both acetyl and isopropyl-hydrazine in the presence of PBN (Table 1), indicating that the two systems produce the same type of free radicals.

Table 1: Hyperfine splitting constants of the PBN-adducts produced during the biological activation or the chemical oxidation of acetyl and isopropyl-hydrazine.

	e.s.r. spectra hyperfine splitting constants (Gauss)			
	isolated hepatocytes		chemical oxidation	
	α^N	α^H	α^N	α^H
Acetyl-hydrazine	14.3	2.47	14.4	2.53
Isopropyl-hydrazine	14.9	2.49	15.0	2.49

The key role played by the microsomal monoxygenase system in the free radical activation of the above hydrazines is indicated by the decrease in the e.s.r. signal intensity due to the addition of cytochrome P_{450} inhibitors such as SKF 525 A and 1-naphtyl-isothiocyanate (2,7).

Table 2: Effect of cytochrome P_{450} inhibitors on the intensity of the e.s.r. signal produced by rat liver microsomes incubated with acetyl-hydrazine.

Treatment	e.s.r. signal intensity (arbitrary units)	% inhibition
Acetyl-hydrazine 0.5 mM	28 ± 1.4	-
" " + SKF 525 A 1 mM	18 ± 1.6	37%
" " + 1-naphtyl-isothiocyanate 0.1 mM	5 ± 0.5	82%

The data here presented confirm the hypothesis that free radical intermediates are produced by the hepatic monoxygenase system during Isoniazid and Iproniazid metabolism.
Concerning the chemical structure of the radical trapped, the analogies between the spectral features of the PBN adducts formed by the two drugs and those produced by acetyl or isopropyl-hydrazine either in isolated hepatocytes or during Cu^{2+}-mediated oxidation, suggest that the acetyl and isopropyl groups might be involved in the radical formation. This interpretation is consistent with the reported presence of ^{14}C-labelling of hepatic proteins, following rat treatment with both ^{14}C-acetyl and isopropyl-hydrazine (2).

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References.

1) H.J. Zimmerman in: "Hepatotoxicity" pp. 418-510, Appleton-Century-Crofts, New York, (1978).

2) J.A. Timbrell, Drug Metabolism Reviews, 10, 125 (1979).

3) E.G. Janzen in: "Free Radicals in Biology" (Ed. W.A. Pryor) vol.4, pp. 116-128, Academic Press, New York (1980).

4) E. Albano, K.A.K. Lott, T.F. Slater, A. Stier, M.R.C. Symons and A. Tomasi, Biochem. J., 204, 693 (1982).

5) O. Augusto, P.R. Ortiz de Montellano and A. Quintanilha, Biochem. Biophys. Res. Commun., 101, 1324 (1981).

6) G. Gever and K. Hayes, J. Org. Chem., 14, 813 (1949).

7) B. Testa and P. Jenner, Drug Metabolism Review, 12, 1 (1981).