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ROLE OF NONSPECIFIC OXIDASES OF RAT LIVER MICROSOMES IN THE BREAKDOWN OF N_1-FURANIDYLPYRIMIDINES

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UDC 612.354:615.277.3

N_1-(3¹-Butyrolactono)-5-fluorouracil, N_1-(2¹-furanidyl)-5-trifluoromethyluracil, and N_1-(2¹-furanidyl)-5-fluorouracil are broken down in the rat in vivo with the liberation of free 5-fluorouracil. Rupture of the C–N bond in the molecule of N_1-(2¹-furanidyl)-5-fluorouracil takes place in the liver microsomes. This process is stimulated in the presence of NADPH and is inhibited by SKF-525A. All three furanidylpyrimidines studied induce differential spectra of type I in the suspension of liver microsomes, evidence of interaction of these substances with cytochrome P-450.

KEY WORDS: *N_1-furanidylpyrimidines; metabolism; nonspecific oxidases of microsomes.*

A number of N_1-furanidylpyrimidines, with antitumor properties, have been synthesized recently at the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR [3]. The question of the stability of the pseudoglycoside C–N bond in their molecule is of vital importance to the understanding of the biological properties of the N_1-furanidylpyrimidines. Depending on this factor, they may act as structural analogs of the pyrimidine nucleosides or as the corresponding pyrimidine bases. It has been shown that N_1-(2¹-furanidyl)-5-fluorouracil (Ftorafur) is not hydrolyzed by pyrimidine-nucleoside phosphorylases, which rupture the C–N bonds of natural nucleosides and of some of their closely related structural analogs [6]. These compounds are characterized by the possession of a hydroxyl group in the sugar residue of the nucleosides and the third carbon atom, necessary for binding with the enzymes [7]. In this respect, the N_1-furanidylpyrimidines differ significantly from the natural pyrimidine nucleosides.

In this investigation the possibility of rupture of the C–N bonds in the molecule of these compounds by nonspecific oxidases of the liver microsomes was studied with particular reference to three derivatives of N_1-furanidylpyrimidine.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 150-200 g. Separation of the N_1-furanidylpyrimidines from their metabolites in the samples of urine was carried out by paper chromatography [1].

Microsomes were isolated from liver homogenate (2 g tissue in 4 volumes of 0.02 M Tris-HCl buffer, pH 7.4, containing 0.15 M KCl) by the method of Cinti et al. [9]. To isolate other subcellular fractions from the liver homogenate, differential centrifugation was used [8]. The biological material was standardized in relation to a definite quantity of tissue or in relation to protein content [4].

The incubation medium for studying the mechanisms of breakdown of Ftorafur contains the following ingredients depending on the aims of the experiment (in a total volume of 1 ml):

Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 2, pp. 162-164, February, 1977. Original article submitted May 21, 1976.

TABLE 1. Degradation of Ftorafur-2-^{14}C by Microsomal Fractions of Rat Liver (M ± m)

Experimental conditions	Quantity of fluorouracil-2-^{14}C formed (in % of added substrate)
Buffer	5,8±0,6 (3)
Buffer + microsomes	7,8±0,5 (5)
Buffer + microsomes +NADPH	8,5±0,5 (5)
Buffer + microsomes + NADPH + SKF-525A	6,6±0,5 (5)

Note. Number of experiments shown in parentheses.

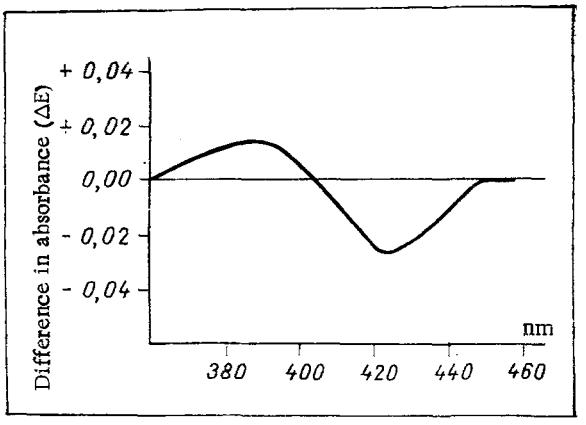

Fig. 1. Differential spectrum of rat liver microsomes induced by Ftorafur.

0.5 ml of homogenate or of a suspension of microsomes corresponding to 200-400 ml of the original tissue, 0.2 μmole Ftorafur-2-^{14}C (0.57 mCi/μmole), 1 μmole NADPH (Reanal), 1 μmole SKF-525A (Smith, Kline, and French Laboratories). Incubation was carried out for 60 min at 37°C in a Warburg apparatus. The reaction was stopped by heating to 100°C for 5 min. After centrifugation, the supernatant fraction of the incubation medium was analyzed by paper chromatography.

The radioactivity of areas of the chromatograms corresponding to mobility of the reference substances was determined with an SL-30 (Intertechnique) liquid scintillation counter, using toluene scintillator.

Differential spectra of the microsomes were photographed by the method of Schenkman et al. [1] on a Specord UV-VIS recording spectrophotometer.

EXPERIMENTAL RESULTS AND DISCUSSION

After intraperitoneal injection of N_1-(3'-butyrolactono)-5-fluorouracil in a dose of 500 mg/kg and of N_1-(2'-furanidyl)-5-trifluoromethyluracil in a dose of 750 mg/kg into the rats, besides the original substances, the urine of the animals was found to contain free 5-halogen pyrimidine derivatives, 5-fluorouracil and 5-trifluoromethyluracil, respectively. The writers showed previously [2] that Ftorafur is also degraded to the free base. After intravenous injection of radioactive Ftorafur into rats in a dose of 60 μCi/kg, 6% of this preparation was excreted in the urine in 24 h as a metabolite — radioactive 5-fluorouracil. Consequently, for all the derivatives of N_1-furanidylpyrimidine studied, hydrolysis of the pseudoglycoside C–N bond was a characteristic feature in experiments in vitro. The liberation of the 5-halogen pyrimidine derivatives as a result of this process ought undoubtedly to be reflected in their biological activity.

An essential problem is that of the identity of the tissue in which N_1-furanidylpyrimidines are metabolized and by what mechanism. By the use of a chromatographic method it was

shown that during incubation of Ftorafur for 1 h at 37°C with blood plasma and 20% homogenates of various rat tissues, degradation of the preparation was observed only in liver homogenate. The total quantity of radioactive fluorouracil formed in that case reached 10.70 ± 1.42%. Menawhile, during spontaneous degradation of Ftorafur in activated liver tissues the quantity of radioactive fluorouracil was only 5.82 ± 0.55%. These results indicate that rupture of the pseudoglycoside C—N bond is an enzymic process and takes place in the liver tissues.

During fractionation of liver homogenate from rats receiving radioactive Ftorafur into subcellular fractions much of the radioactivity was found to be located in the microsomes (28%) The system of nonspecific oxidases located in the microsomes is known to be the site of metabolism of most xenobiotics, including several antitumor substances [10]. According to the results now obtained, these enzymes evidently also are concerned in the metabolism of N_1-furanidylpyrimidines. First, it was shown that radioactive 5-fluorouracil is formed during incubation of Ftorafur not only with homogenates, but also with microsomes of the liver. Characteristically degradation of the compound to 5-fluorouracil is stimulated in the presence of NADPH, a coenzyme for nonspecific oxidases (Table 1). Conversely, on the addition of SKF-525A, known to be an inhibitor of microsomal oxidation of xenobiotics, to the incubation medium the process was retarded. Second, all three N_1-furanidylpyrimidines investigated, namely Ftorafur, N_1-(2'-furanidyl)-5-trifluoromethyluracil, and N_1-(3'-butyrolactono)-5-fluorouracil, induce differential spectra of type I with a characteristic maximum at 385 nm and a minimum at 420 nm in a suspension of rat liver microsomes. The differential spectrum of the microsomes in the presence of Ftorafur in a concentration of 0.18 mM is given in Fig. 1. Changes of this sort in the optical properties of the microsomes point to the formation of complexes of these compounds with the protein moiety of the oxidized form of cytochrome P-450 — the terminal region of the nonspecific oxidase system of the liver microsomes. The formation of such a complex is an essential condition for the metabolism of xenobiotics by microsomal oxidases [5].

It was thus shown in the case of three derivatives of N_1-furanidylpyrimidine that rupture of the pseudoglycoside C—N bond in the molecule of these compounds observable in vivo takes place in the liver through the participation of the nonspecific oxidases of the microsomal fraction.

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