METABOLISM OF HETEROCYCLIC COMPOUNDS IN MOUSE LIVER MICROSONES

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The metabolic transformation of nitroheterocyclic compounds (NHCC) in liver microsomes and tumor (Ehrlich ascites carcinoma, EAC) microsomes of mice was investigated in rather great detail in our study of two drugs from the classes of nitroacridine (nitracrine) [7] and nitrofuran (quinifur) [2, 5] derivatives. The data of the studies indicted above are evidence that the structure of NHCC substantially influences their microsomal metabolism. For example, nitracine (compound XI, table 1) is rapidly consumed during incubation both with liver and with EAC microsomes in the presence of NADPH, although the composition of the metabolites differs in the two cases [7]. On the contrary, quinifur (compound V) is rapidly broken down in liver microsomes but is stable in the tumor microsomes. We also found that although V is a rather effective radiosensitizer of tumor cells in culture, it is ineffective in noncontact methods of administration, which we attribute to its metabolic inactivation in the liver [2]. XI possesses antitumor activity both \textit{in vivo} and \textit{in vitro} [7]. Thus, the metabolism of NHCC in the liver can play a vital role in the plant of its therapeutic effectiveness, and this role depends on the structure of the drug. As a result of an investigation of the kinetics of the metabolic transformation of V, we arrived at the conclusion that several different mechanisms participate in this process [5, 6].

(1-XIII)
This work is a part of our investigations aimed at establishing the correlation between the metabolism and biological action of xenobiotics. Its concrete goal was to establish the influence of the structure of the indicated compounds on the kinetic parameters of their microsomal metabolism.

MATERIALS AND METHODS

All the compounds used in the work, with the exception of acridine (XII) and XI, were synthesized by N. M. Sukhova (see Table 1). The authenticity of their structure was established by the methods of IR and NMR spectroscopy. XI (known in Poland under the name of ledacrine) was a gift from Prof. Ch. Radzikowski (Poland, Wroclaw). Hydroxylamine-2,2,6,6-tetramethyl-4-hydroxy-piperidine (HA) and the corresponding stable nitrosoyl radical (HA') were synthesized by Dr. L. A. Krinitskaya (Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow). The remaining reagents, as well as the methods of isolation of mouse liver microsomes (SHK strain, females, weighing 18-20 g), preparation of aerobic and anaerobic microsomal incubation mixtures, and measurement of the rates of oxygen uptake using a Clark electrode were described earlier [5].

The microsomal incubation mixtures contained: a suspension of microsomes in 0.1 M Na phosphate buffer (pH 7.4) with concentration of microsomal protein 1 mg per ml of mixture, components of the NADPH-regenerating mixture (10 mM glucose-6-phosphate and 1 activity unit/ml of glucose-6-phosphate dehydrogenase). In the anaerobic experiments the mixtures contained supplementary components of the oxygen-absorbing mixture (75 mM glucose and 18 activity units each of glucose oxidase and catalase). These experiments were conducted in a hermetically sealed cuvette (of the Tunberg cuvette type). After the addition of the oxygen-absorbing mixture, the cuvette was purged with argon, exposed for 3-4 min until all the oxygen was absorbed (according to the data of polarographic monitoring), and the metabolism of the xenobiotics being studied was triggered by the addition of NADP from a branch in the lid of the cuvette. Spectral and kinetic measurements of the metabolic consumption of the compounds were performed in quartz spectrophotometric cuvettes (optical path length from 0.1 to 1 cm) on a specord M-40 spectrophotometer (German Democratic Republic). Since not one of the compounds investigated here is consumed in the microsomes nor affects the rate of uptake of O\textsubscript{2} by microsomes in the absence of NADPH (which we verified in control experiments), 0.1 ml of the solution of the compound studied was added to the working cuvette before the beginning of the reaction; moreover, its initial concentration in the incubation mixture was usually 30 \mu M. The same amount of the solvent (or 0.1 M Na phosphate buffer for II, III, V, VII, XI, or ethanol for I, IV, VI, VIII--X, XII, XIII; see Table 1) was added to the control cuvette. Then the differential spectrum of the added compound was recorded, and its metabolism was triggered by adding 0.1 ml of a solution of NADPH (a source of NADPH in the presence of the NADPH-generating system). The NADPH concentration was constant in the process of incubation at 0.1 mM. Simultaneously, the same volume of this solution was added to the reference cuvette. The kinetics of the metabolic consumption of the xenobiotic was recorded by three methods: by continuous recording of the change in the optical density at one wavelength (usually in the region of the longest-wave absorption maximum of the original molecule or metabolite) (Fig. 1), with the aid of automatic recording of the differential spectrum after equal time intervals (Fig. 2a, b, c, d), and by recording of the values of the optical density at the wavelengths of the major absorption maxima of the investigated compounds and its metabolites after equal time intervals.