2-MERCAPTO-1-(β-4-PYRIDETHYL) BENZIMIDAZOLE, AN INHIBITOR OF RNA SYNTHESIS: A REEVALUATION

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SUMMARY: The effect of 2-mercapto-1-(β-4-pyridethyl) benzimidazole (MPB) was determined in fetal rat liver explants. MPB exerted little effect upon the uptake of ^{14}C-labeled orotic acid by the fetal liver, but exerted a very pronounced inhibition of the incorporation of the precursor into TCA-insoluble, alkalilabile material. These studies justify in fetal rat liver systems the use of MPB as an inhibitor of RNA synthesis unmediated by permeability effects.

It has been reported that 2-mercapto-1-(β-4-pyridethyl) benzimidazole (MPB) reversibly inhibits nucleic acid synthesis in a variety of cells while only minimally affecting protein synthesis (1-3). Accordingly, MPB has been used as a tool in studying transcriptional mechanisms as affected by administration of various enzyme inducers to cell culture systems (4). Recently, MPB has been shown to significantly reduce nucleoside uptake into 'normal' and tumor cells (5). Furthermore, since neither the incorporation of ^{32}P-phosphate into RNA and DNA nor the pattern of labeling of RNA species was affected by MPB during short term incubations, Nakata and Bader (5) concluded that the primary action was not upon RNA synthesis. In light of these studies, some doubt has been raised about the utility of MPB as an inhibitor of nucleic acid synthesis. The present investigation was conducted to determine the effects of MPB upon permeability of ^{14}C-orotic acid in fetal rat liver explants and to ascertain its utility as an inhibitor of nucleic acid synthesis. We have found a) no detectable effect of MPB on the uptake of orotic acid into the trichloroacetic acid (TCA) soluble pool, and b) a significant decrease in the incorporation of radioactivity into the TCA-insoluble pool. Thus our studies would

support the use of MPB as an inhibitor of nucleic acid synthesis unmediated by effects upon permeability.

MATERIALS: Pregnant Sprague-Dawley rats, 18 to 20 days of gestation, were obtained from Holtzman Rat Company, Wisconsin. MPB was obtained from Aldrich Chemical Co., Milwaukee, Wis., while 6-^{14}C-orotic acid (36.5 μcuries/mmole) was purchased from Schwarz Biochem.

METHODS: A modification of the explant technique of Wicks (6) was employed and is described elsewhere (7). MPB was dissolved in dimethylsulfoxide (DMSO) and was added at 10^{-4}M to 44 hour preincubated explants in a final DMSO concentration of 0.2% (v/v). Thirty minutes prior to homogenization, ^{14}C-orotic acid (2 uc/dish) was added to the explants. Orotic acid incorporation was linear for at least twelve hours after the addition of labeled orotate to the cultures. Tissue homogenates were prepared, after rinsing the tissue several times in media, by homogenizing in 1.0 ml of cold deionized water. Cold 10% TCA (w/v, 1 ml) was added to each homogenate and the suspension maintained at 4° for several min. The precipitate was washed three times with cold 5% (w/v) TCA, dissolved in 1 ml 1N KOH and allowed to remain at room temperature for eighteen hours to complete the hydrolysis of RNA. Thereafter, 0.2 ml of conc. HCl was added to the sample on ice and after one hour, the sample was centrifuged at 3000 rpm. An aliquot of the supernatant fraction (0.5 ml) was counted in 10 ml of aquasol (New England Nuclear) at an efficiency of 75% and the A_{260} of the aliquot was determined. The combined supernatant fractions of the cold TCA washes represented the TCA-soluble pool. The TCA-soluble extracts were pooled from cultures sacrificed at each time period. The pooled samples were placed in a 90°C water bath for 1 hour to hydrolyze the pyrimidine nucleoside di- and triphosphates to mono-phosphates. The samples were then cooled and neutralized with 4N KOH to a phenol red end point. Aliquots of each sample, i.e., 2 ml, were passed through a 1x6 cm Dowex-1-formate column (X 8, 200-400 mesh) and the UMP and orotic

acid were eluted according to the method of Herbert et al. (8) with 0.2N and 0.4N ammonium formate, respectively. UMP and orotic acid were identified by their elution pattern from Dowex-1-formate columns using UV spectrophotometry with pure compounds as markers. The samples were counted in 10 ml aquasol at 75% efficiency. To normalize for variability in the amounts of tissue per dish the data have been expressed in terms of dpm of acid soluble component per RNA of liver tissue, i.e., per A_{260} TCA insoluble material.

RESULTS AND DISCUSSION: MPB rapidly inhibited orotic acid incorporation into RNA in rat fetal liver explants (Figure 1). Within the first 30

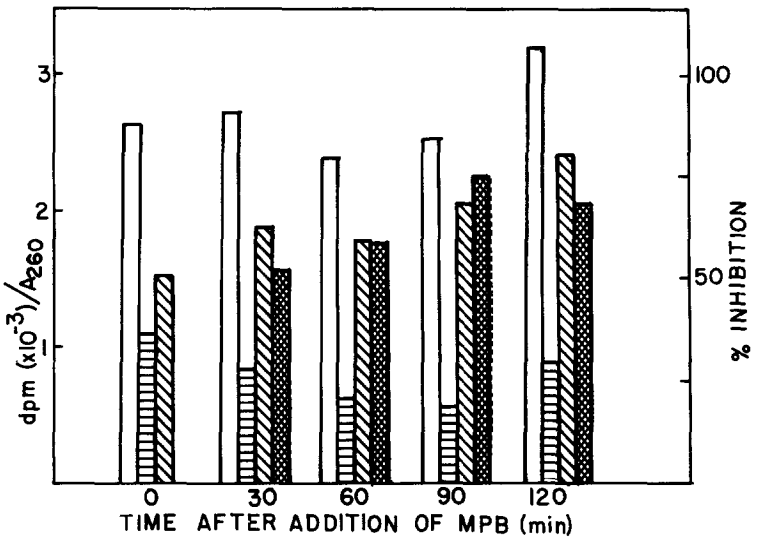

Figure 1: The effect of MPB on RNA synthesis and on radioactivity in the acid-soluble pool after ^{14}C-orotic acid administration to fetal liver explants. MPB (10^{-4}M) was added to 44 hr preincubated fetal liver explants. Thirty minutes prior to sacrifice ^{14}C-orotic acid, 2 μcuries per dish was added. The total incorporation into the TCA-soluble fraction (see Abscissa) of the fetal liver explants was determined ( ). The UMP ( ) and orotic acid ( ) components were separated on Dowex-1-formate and the radioactivity of each component was determined. These values have been expressed (left ordinate) as dpm incorporated per absorbancy at 260 nm (A_{260}) of the TCA-insoluble material, i.e., RNA, to normalize for differences in the amount of tissue added per dish. The % inhibition of incorporation of labeled orotic acid into TCA-insoluble material, alkali-labile material (RNA) is expressed on the right ordinate ( ). The mean (range) of orotic acid incorporated into RNA in at least 10 control cultures was 189 (168-208) dpm/A_{260}.

min after the addition of MPB to cultures, RNA synthesis was inhibitied by 52%. Maximum inhibition, i.e., 75%, was observed 90 min after the addition of MPB. Neither the total radioactivity in the acid soluble pool nor the radioactivity of the orotic acid component of the acid soluble pool was appreciably affected at these times (expressed per A_{260} RNA for purposes of normalization). The radioactivity in the UMP pool decreased after MPB treatment in this experiment and in a duplicate experiment, but the decrease (30 min) was not enough to account for the inhibition of the incorporation of the precursor into RNA. In this regard, Summers and Mueller (9) observed that although RNA synthesis was inhibited, transcription of DNA by RNA polymerase was unaffected by MPB treatment.

The utilization of metabolic inhibitors of nucleic acid synthesis as tools in culture systems and in conjunction with labeled precursors administered in vivo necessitates observing the effects of these inhibitors on incorporation as well as their effect upon the cellular uptake of the radioactive precursor. With MPB, the reversible inhibitor of RNA synthesis, this scrutiny is of primary importance since MPB, as in the case of high levels of heterologous nucleotides (10), has been shown to inhibit the uptake of nucleosides into cells (5). The present investigation justifies the utilization of MPB as an inhibitor of RNA synthesis in fetal liver explants with ^{14}C-orotic acid as the precursor of RNA synthesis. No significant alterations of permeability of the labeled precursor were observed although at later times, i.e., 60 or 90 min, an inhibition of the conversion of intracellular orotic acid to uridine nucleotides was apparent. At the earlier time, i.e. 30 min, this inhibition was insufficient to account for the blockade in RNA synthesis by MPB.

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