Vol. 90, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 12, 1979 Pages 721-725

A PROTEIN-BOUND FORM OF THIOACETAMIDE IN LIVER NUCLEOLI

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Received July 30,1979

SUMMARY: The cellular distribution of ^{35}S from ^{35}S- thioacetamide was determined in rabbit liver subcellular fractions following its in vivo administration. Of the various fractions isolated, only the nucleolar fraction contained ^{35}S counts that were insoluble in 10% trichloroacetic acid but soluble in trichloroacetic acid if the fraction was treated with trypsin but not RNase or DNase. These results demonstrate that a protein bound form of thioacetamide is present in the nucleolus following in vivo administration of this drug.

The administration of thioacetamide, a known hepatotoxin and hepatocarcinogen has been shown to cause specific changes in the nucleolus; an increased synthesis of guanine and cytosine rich 35S and 45S RNA occurs with a concomitant decrease in ribosomal RNA in the cytoplasm (1,2).

In this investigation, ^{35}S from ^{35}S-thioacetamide was found to bind to liver nucleoli in a form which is insoluble in 10% TCA but soluble in TCA if the nucleolar fraction undergoes trypsin digestion prior to precipitation with TCA.

MATERIALS AND METHODS

New Zealand female rabbits weighing 2 kg each were injected intraperitoneally with 10 ml of isotonic sodium chloride containing 90 mg of thioacetamide labeled with 0.25 mCi of ^{35}S. Food was removed from cages 16 hours prior to injection. At 2 hours after the administration of ^{35}S-thioacetamide, the animals were killed by a sharp blow on the head and livers were removed and chilled on ice. Each liver was homogenized in 0.25M sucrose containing 0.02M Tris-acetate buffer pH 8.3 and 10^{-4}M EDTA. The homogenate was fractionated by differential centrifugation to obtain separate fractions of the mitochondria and its supernatant (3). Nuclei were reisolated after treatment with detergent (4) and fractionated into nucleolar, deoxyribonucleoprotein and nuclear sap fractions (1). The microsomal fraction was treated with sodium deoxycholate to isolate the ribosomal fraction from the microsomal protein fraction (6).

Abbreviations: TCA, trichloroacetic acid

0006-291X/79/190721-05$01.00/0

Duplicate 1 ml samples of each subcellular fraction were each subjected to a precipitation with 5 ml 10% TCA. The supernatant was removed and the precipitate was dissolved in 1.0 ml distilled water. These precipitate preparations and 1.0 ml aliquots from each supernatant were then counted to determine TCA insoluble and soluble counts, respectively. Total ^{35}S counts were determined from additional 1 ml samples of each subcellular fraction.

Enzymic digestion of each subcellular fraction was carried out with individual treatments of trypsin (2x crystallized, Sigma Chem. Co), bovine pancreatic ribonuclease (RNase, 5x crystallized, Sigma Chem. Co) and bovine pancreatic deoxyribonuclease (DNase, type III Sigma Chem. Co), each at a final concentration of 4 mg/ml. Tryptic digestion was carried out in the presence of 0.1M Tris-acetate buffer pH 7.8 while the RNase and DNase digestions were performed in the presence of 0.1M sodium acetate buffer pH 5.0 containing 0.1M magnesium sulfate. After 16 hours incubation at 25°C, each of the enzymes was added to their appropriate control tubes and then the 1 ml digested samples and their controls were immediately subjected to precipitation with 5 ml 10% TCA. Effects of digestion were then determined by measuring the ^{35}S counts in the supernatant.

RESULTS AND DISCUSSION

The differential distribution of ^{35}S counts among liver subcellular fractions of ^{35}S-thioacetamide treated rabbits is given in Table I together with the pattern of ^{35}S counts that resulted from a TCA precipitation of each fraction with and without digestion by trypsin, RNase and DNase respectively. The largest number of counts was found in the cell sap. Ninety three percent of the nucleolar ^{35}S counts were precipitated by TCA while the majority of the ^{35}S counts in the remaining fractions were found to be TCA soluble. About 25% of the ^{35}S in the deoxyribonucleoprotein fraction were also precipitated by TCA but these counts may represent a nucleolar contamination of the deoxyribonucleoprotein fraction.

When trypsin digestion preceded TCA precipitation, the majority of the counts in the nucleolar fraction were found to be TCA soluble. A similar effect was observed with the deoxyribonucleoprotein fraction while no change in solubility was observed when these fractions were treated either with DNase or RNase. The distribution of counts in the remaining fractions was not affected by their enzymic digestions, thus the number of counts precipitated by TCA was the same for both untreated and enzymatically treated fractions.

TABLE 1

Distribution of ^{35}S in subcellular fractions isolated from livers of ^{35}S-thioacetamide treated rabbits (counts/g liver)

fractions	TOTAL	TCA ppt	TCA soluble	TCA soluble after digestion by trypsin	RNase	DNase
Nucleoli	301.3	278.8	4.8	217.9	3.8	2.1
Deoxyribonucleoprotein	113.8	28.8	83.8	111.3	81.3	82.5
Nuclear sap	315.6	37.5	277.5	235.0	297.6	282.5
Mitochondria	1662.5	158.8	1537.5	1625.0	1475.0	1500.0
Ribosomes	460.0	97.5	362.5	362.5	350.0	356.3
Microsomal protein	5287.5	352.5	5031.3	5280.0	4600.0	4775.0
Cell sap	93150.0	5075.0	88000.0	86125.0	86125.0	8600.0

The procedures for the isolation of the subcellular fractions and their subsequent enzymic digestions were as described in Materials and Methods. The above counts were determined for 1 ml samples of the following preparations: the total subcellular fraction, the 10% trichloroacetic acid precipitate (TCA ppt), each resuspended in 1 ml of distilled water and the 10% trichloroacetic acid supernatant (TCA soluble) with and without enzymic digestions. Each 1 ml sample was suspended in 15 ml of Aquasol (New England Nuclear) in a scintillation vial and counted within an accuracy of less than 5%. Each result represents an average of two experiments. The TCA soluble counts of the untreated fractions were equivalent to control values of the enzymic digestions; the average of these data is presented as one column.

The precipitation of nucleolar ^{35}S by TCA and the solubilization of these counts in the TCA after trypsin digestion suggests that thioacetamide or a possible metabolite of this drug exists in the nucleoli in a protein bound form.

It has been suggested that the nucleolar changes in RNA synthesis during thioacetamide treatment may result from the activation and release of latent lysosomal RNase which enters the nucleoli and results in a breakdown of ribosomal RNA precursors. The degradation of the precursors would cause the depletion of ribosomal RNA in the cytoplasm and this depletion would cause an increase in nucleolar RNA synthesis by a loss of a feedback control between the cytoplasmic and nucleolar ribosomal RNA (7). The present data suggest that thioacetamide has a direct effect on nucleoli.

The hepatotoxins, carbon tetrachloride and acetoaminophen, have been shown to be activated by a cytochrome P-450 mediated reaction and are able to bind covalently to microsomal proteins (8,9,10). Thioacetamide also appears to be activated by cytochrome P-450 (11) and the main metabolite found in the plasma and urine during drug treatment is thioacetamide sulfine (12). This metabolite inhibits the activities of mixed-oxidase enzyme and δ-aminolevulinic acid synthetase and is more active than thioacetamide in inducing necrosis (11,12,13).

In addition to the changes in nucleolar RNA synthesis thioacetamide has been shown to inhibit the induction of tyrosine aminotransferase by hydrocortisone (13) and to increase the level of albumin messenger RNA (14,15,16).

Whether the thioacetamide binding protein(s) play a role in the synthesis of nucleolar RNA is yet to be determined. Its isolation and identification, however, may contribute towards a better understanding of the events that occur during the processing of ribosomal and messenger RNA.

Acknowledgements: This work was supported by grants from PSC-BHE Research Award Program of The City University of New York, the Alma Toorock Fund for Cancer Research and Fundacao de Amparo a Pesquisa do Estado de S.Paulo, Brazil.

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