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Metabolism of N^6-(Δ^2-isopentenyl)adenosine in rat liver

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N^6-(Δ^2-ISOPENTENYL)ADENOSINE (IPAR) is a modified ribonucleoside which has been found in tRNA of plants, micro-organisms and mammalian tissues.[1] In rats treated with toxic doses, IPAR produces transient lymphoid depression and hepatoxicity as well as antiproliferative effects in intestinal mucosa, bone marrow and regenerating liver.[2] Although the antitumor activity of IPAR is slight, complete but short-lasting bone marrow remissions have been obtained with IPAR in one patient with acute promyelocytic leukemia.[3] Studies designed to correlate cytotoxic effects of IPAR with early biochemical lesions induced by the drug indicate that the hepatoxicity in rats may be related to the rapid and prolonged inhibition of incorporation of ^{14}C-phenylalanine into liver protein.[4] This inhibition appeared to be relatively specific for liver, since spleen, thymus and small intestine were almost unaffected.[4]

IPAR is a substrate for adenosine kinase[5] and is also an inhibitor of cellular multiplication in culture.[6] In mammalian systems such as leukemia L1210, sarcoma 180, mammary carcinoma TA3 and canine kidney, IPAR has been reported to be phosphorylated to the 5′-monophosphate level[6,7] and is suggested to exert its inhibitory effects in that form.[6,8]

This report describes a study of the levels of phosphorylation of IPAR-8-^{14}C in rat liver.

A DEAE-cellulose column chromatographic method[9] has been employed for the separation of liver ribonucleotides present in the acid-soluble fraction of a rat treated i.v. with labeled adenosine. The results of such a chromatographic separation are shown in Fig. 1. In this chromatogram, four sharp and symmetrical ^{14}C-labeled ribonucleotide peaks were identified as IMP, AMP, ADP and ATP. The results also show the presence of one large labeled nucleoside peak, AR (Fraction 11–22), and two other peaks not yet identified (Fractions 5–10 and 24–34). No detectable labeling of guanosine nucleotides was observed. Other peaks shown in this chromatogram which are not ^{14}C-labeled are eluted in the general area of pyrimidine nucleotides. Identification of labeled peaks was based on their distinct 280/260 nm absorbance

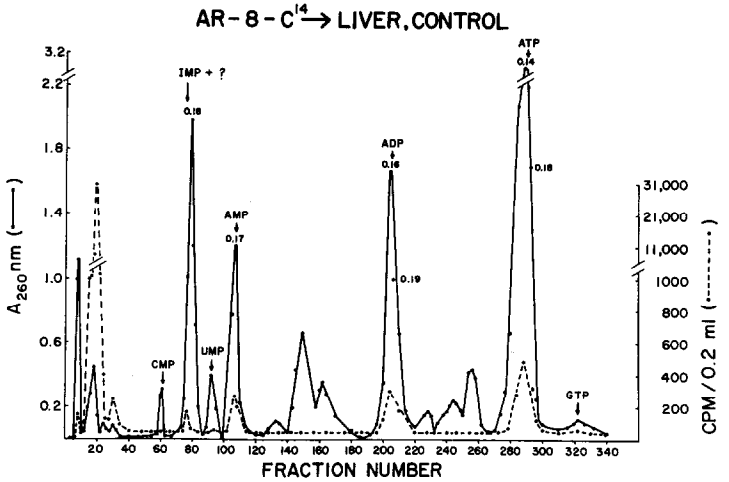

FIG. 1. DEAE-cellulose column chromatography of the acid-soluble fraction of rat liver. A rat was injected i.v. with 25 μCi adenosine-8-^{14}C (52 μCi/μmole), and 15 min later the animal was sacrificed and the liver extracted as described in Methods. Fractions 70–90 represent a heterogenous peak consisting of IMP and one other component, probably TMP. The arrow indicates the elution position of IMP. Small quantities of deoxyribonucleotides present in tissue extracts were not identified in this system and are presumably eluted with their ribonucleotide counterparts. ●——●, Absorbance at 260 nm; ●------●, cpm/0·2 ml fraction. The absorbance ratio (280/260 nm) is indicated above the adenosine and inosine nucleotide peaks.

ratios, on elution positions on the calibrated DE-52 columns, and by their R_f values on PEI cellulose plates.[9] Recoveries of radioactive materials were 90–95 per cent of the sample applied to the column. Molar ratios of ATP/ADP were close to those reported in the literature.[10] Specific activities of the labeled fractions were in the order AR > AMP > ADP > ATP. In contrast, the amounts of nucleotides with absorbance at 260 nm were related as ATP > ADP > AMP > AR.

The column chromatographic separation of labeled ribonucleosides and ribonucleotides present in rat liver acid-soluble fractions at 4 hr after i.v. administration of IPAR-8-^{14}C (50 μCi/μmole)[11] is shown in

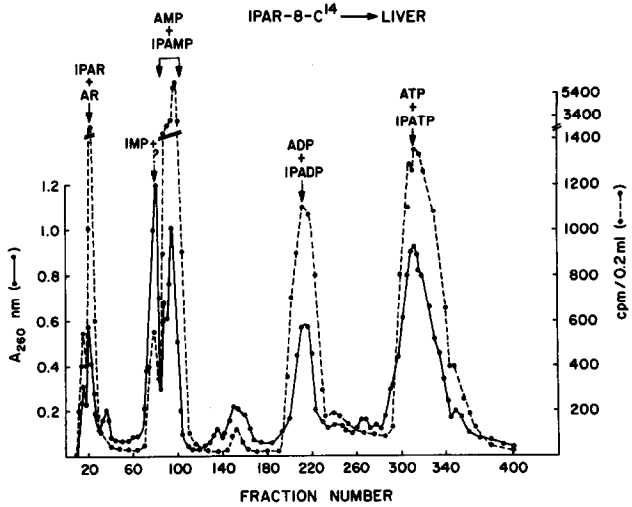

FIG. 2. Chromatographic profile of the acid-soluble fraction of rat liver on DEAE-cellulose column 4 hr after an i.v. administration of 15 μCi IPAR-8-^{14}C. Other conditions are as outlined in Fig. 1.

Fig. 2. The radioactive peak eluted first (Fractions 10–30) was a mixture of AR and IPAR. Four radioactive ribonucleotide peaks were eluted from the DE-52 column in positions corresponding to IMP, AMP, ADP and ATP. Phosphorylation of IPAR to ribonucleoside mono-, di- and triphosphates was suggested first by the broad, dissymmetrical elution patterns of the peaks, indicating the presence of more than one component, and second, by the higher 280/260 nm absorbance ratios than those expected for pure adenosine nucleotides. The 280/260 nm absorbance ratios for the pooled fractions under each peak were 0·52 for the monophosphates, 0·23 for the diphosphates and 0·38 for the triphosphates.

For the identification of IPAR-8-^{14}C metabolites, labeled ribonucleotide peaks eluted from the DE-52 column were pooled, concentrated and taken up in 0·1 M Tris–HCl buffer, pH 10·0, containing 5 mM $MgCl_2$. Four units of alkaline phosphatase (calf intestine, 380 U/mg) were added per 10 A_{260} units of ribonucleotides and the mixture was incubated at 37° for 1·5 hr followed by the addition of four more units of the enzyme and the reaction was terminated 1·5 hr later at −70°. Digestions to the ribonucleoside monophosphate level were carried out as follows: 10 A_{260} units of the dried pooled labeled fractions were dissolved in 0·1 M Tris–HCl, pH 8·6, containing 5 mM $MgCl_2$. To this solution, 0·5 mg snake venom phosphodiesterase (10 mg/ml) was added and incubated at 37° for 16 hr. Chromatographic separation of the enzymatically digested samples was carried out on Silica gel in doubly deionized water and by ascending chromatography on Whatman No. 3 mm paper with *n*-butanol–acetic acid–water (20:3:7).[12] The R_f values for the enzymatically digested samples and those of commercially obtained samples are listed in Table 1. After alkaline phosphatase treatment of each of three labeled nucleotide peaks (AMP, ADP, ATP), there were two radioactive spots identified as AR and IPAR. After hydrolysis with phosphodiesterase, moreover, each peak (ADP, ATP) was resolved into the two radioactive spots corresponding to AMP and IPAMP. In studies of patients treated with IPAR, the hydrated form of IPAR has been found in urine in low concentrations, about 1–2 per cent of the total dose.[13] Thus, it is also possible that some conversion to the hydrated or reduced forms occurs in rat liver and that the methods of separation used may not be sufficiently discriminating for their identification.

TABLE 1. R_f VALUES FOR PURINE BASES, RIBONUCLEOSIDES, RIBONUCLEOTIDES AND MODIFIED NUCLEOSIDES ON SILICA GEL PLATES IN WATER

Compounds	R_f (pure)	R_f* Alkaline phosphatase	R_f* Phosphodiesterase
A	0·52		
AR	0·77	0·75	
IPA	0·11		
IPAR	0·27	0·30	
IPAMP	0·50		0·52
AMP	0·87		0·84
ADP	0·87		
ATP	0·87		

* R_f values for the enzymatically digested samples of mono-, di- and triphosphates of Fig. 2 were determined by u.v. absorption and by ^{14}C-radioactivity measurement.

The data on conversion of labeled IPAR to its ribonucleoside mono-, di- and triphosphates, as well as IPAR dealkylation and subsequent phosphorylation to adenosine ribonucleotides, are summarized in Table 2. Each ribonucleotide component represents pooled radioactive fractions eluted from the DE-52 column shown in Fig. 2. Per cent distribution of ^{14}C label into AR and IPAR was determined after subjecting pooled fractions to alkaline phosphatase treatment and subsequent separation and identification of the labeled nucleosides on Silica gel plates. The ribonucleosides thus obtained were identified by the correspondence of R_f values (see Table 1) and 280/260 absorbance ratios with pure AR (0·15) and IPAR (0·72). Results in Table 2 also show that the molar ratios of IPAR/AR were 3:1 in the monophosphate, 1:3 in the diphosphate and 1:1 in the triphosphates.

Results obtained in these studies indicate that in rat liver the drug is phosphorylated to its ribonucleoside mono-, di- and triphosphates. The results also show that IPAR or its ribonucleotides are *N*-dealkylated, resulting in the formation of adenosine and its ribonucleosides AMP, ADP and ATP. Hall *et al.*[14] isolated an enzyme, adenosine aminohydrase, that catalyzes the conversion of AR and IPAR to inosine. The rate of catalysis was shown to be both tissue and species dependent.[14–17] It is not clear at this time, however, whether dealkylation in rat liver occurs at the nucleoside or nucleotide levels of IPAR or whether the nucleotides are reduced to the deoxyribose forms. Our studies indicate that the metabolic onversions of IPAR in rat liver are also different from those observed in cellular systems such as L1210, S180, TA3

TABLE 2. INCORPORATION OF IPAR-8-^{14}C INTO RIBONUCLEOTIDE POOLS FROM ACID-SOLUBLE FRACTION OF RAT LIVER*

Components	Radioactivity (cpm/g liver)	Amount (pmoles/g liver)
Monophosphates		
AR	2200	27
IPAR	5900	71
Diphosphates		
AR	7550	91
IPAR	3250	39
Triphosphates		
AR	11,000	132
IPAR	9000	110
Total recovery (%)†	89	

* At 4 hr after an i.v. administration of 0·3 μmole IPAR-8-^{14}C (50 μCi/μmole) liver was removed and acid-soluble fraction was prepared as described in Methods.

† Represents radioactive nucleotides recovered as a per cent of the total radioactivity applied to the DE-52 column.

and canine kidney in which IPAR was phosphorylated to 5′-monophosphate only and breakdown of IPAR to adenosine was not detected.[6–8]

This study documents, for the first time, the phosphorylation of IPAR to the nucleoside mono-, di- and triphosphates in treated animals.

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