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BIOCHEMICAL PHARMACOLOGY AND TOXICOLOGY OF 8-AZAADENOSINE ALONE AND IN COMBINATION WITH 2′-DEOXYCOFORMYCIN (PENTOSTATIN)*

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Abstract—The toxicology and metabolism of 8-azaadenosine (8-azaAdo) were examined both as a single agent and in combination with the adenosine deaminase inhibitor, 2′-deoxycoformycin (dCF). The LD_{10} (mice) for 8-azaAdo alone on a once daily for 5 days (q.d. × 5) schedule was 30 mg·kg^{-1}·day^{-1}. When the animals were pretreated with 0.1 mg·kg^{-1}·day^{-1} of dCF, the LD_{10} dose was reduced to 10 mg·kg^{-1}·day^{-1} × 5. The major organ toxicity seen was hepatic. Bone marrow cellularity was only slightly altered at the LD_{10} dose. 8-AzaAdo nucleotides were detected in the livers of treated mice as determined by high performance liquid chromatography. Further, after 2 hr of incubation, isolated rat hepatocytes accumulated 8-azaATP to levels of 2.2 μmoles/g of cells with 8-azaAdo (1 mM) alone and to 4.3 μmoles/g of cells when 8-azaAdo was used in combination with dCF (1 μg/ml). ATP levels decreased to below the limits of detection after 2 hr in cells treated with the combination. The replacement of cellular ATP by 8-azaATP may provide an explanation for the hepatotoxicity observed in the murine toxicology studies.

The development of the specific adenosine deaminase (ADA) inhibitor 2′-deoxycoformycin (dCF) has renewed interest in a number of adenosine analogs, such as 8-azaadenosine, formycin and 2,6-diaminopurine ribonucleoside, which are rapidly deaminated by this enzyme [1]. One of these analogs, 8-azaadenosine (8-azaAdo), is subject to a complex series of metabolic reactions that are believed involved in the mechanism of cytotoxicity [2]. These reactions are summarized in Fig. 1. Briefly, 8-azaAdo may be phosphorylated by adenosine kinase to 8-azaadenosine-5′-monophosphate (8-azaAMP) and then converted to 8-azaadenosine-5′-diphosphate (8-azaADP) and 8-azaadenosine-5′-triphosphate (8-azaATP) by the sequential actions of adenylate kinase and nucleoside diphosphokinase (NDP kinase). As 8-azaATP, 8-azaAdo can be incorporated into polynucleotides [2,3]. In addition, 8-azaAdo can be incorporated into polynucleotides as 8-azaguanine after conversion to 8-azaguanosine-5′-triphosphate (8-azaGTP) [2]. The formation of 8-azaGTP from 8-azaAdo has been proposed to take place via the intermediate synthesis of 8-azainosine-5′-monophosphate (8-azaIMP). The synthesis of 8-azaIMP can occur directly via the action of adenosine kinase on 8-azainosine (8-azaIno) [3, 4], a compound produced by the action of ADA on 8-azaAdo, or indirectly via the sequential action of the enzymes ADA, purine nucleoside phosphorylase (PNP), and hypoxanthine-guanine phosphoribosyltransferase (HGPRT) on 8-azaAdo [2]. A third possible route of 8-azaIMP formation is through deamination of 8-azaAMP by adenylate deaminase. 8-AzaGTP is formed from 8-azaIMP in a sequence of reactions catalyzed by the enzymes IMP dehydrogenase, guanylate synthetase, GMP kinase and NDP kinase [2]. In both human epidermoid carcinoma (H.Ep.2) and murine adenocarcinoma 755 (Ca755) cells, 8-azaAdo is incorporated about equally as 8-azaadenine and 8-azaguanine [2].

As noted above, dCF, a tight-binding inhibitor of ADA [5, 6], prevents the deamination of 8-azaAdo. When human erythrocytes, pretreated with dCF to inactivate the ADA, are incubated with 8-azaAdo, large amounts of 8-azaadenine-containing nucleotides accumulate. In the absence of dCF, little analog nucleotide formation is seen [5].

The growth inhibitory effects against human lung, colon and pancreatic carcinoma cells grown in culture caused by 8-azaAdo are markedly potentiated by pretreatment of the cultured cells with dCF [7, 8]. The combination of 8-azaAdo and dCF has activity against a human lung xenograft tumor growing in immunosuppressed mice [7]. Based on these results, the toxicology of 8-azaAdo alone and in combination with dCF was examined in preparation for testing

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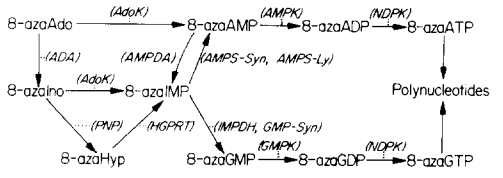

Fig. 1. Reactions of 8-azaadenosine metabolism. Abbreviations in parentheses are those of the enzymes which catalyze the various reactions. The abbreviations used in this figure are: 8-azaAdo, 8-azaadenosine; 8-azaAMP, 8-azaADP and 8-azaATP, 8-azaadenosine-5′-mono-, di- and triphosphate, respectively; 8-azaIno, 8-azainosine; 8-azaHyp, 8-azahypoxanthine; 8-azaIMP, 8-azainosine-5′-monophosphate; 8-azaGMP, 8-azaGDP and 8-azaGTP, 8-azaguanosine-5′-mono-, di- and triphosphate, respectively; AdoK, adenosine kinase, EC 2.7.1.20; AMPK, adenylate kinase, EC 2.7.4.3 NDPK, nucleoside diphosphokinase, EC 2.7.4.6; ADA, adenosine deaminase, EC 3.5.4.4; PNP, purine nucleoside phosphorylase, EC 2.4.2.1; HGPRT, hypoxanthine-guanine phosphoribosyltransferase, EC 2.4.2.8; AMPS-Syn, adenylosuccinate synthetase, EC 6.3.4.4; AMPS-Ly, adenylosuccinate lyase, EC 4.3.2.2; IMPDH, IMP dehydrogenase, EC 1.2.1.14; GMP-Syn, GMP synthetase, EC 6.3.5.2; AMPDA, adenylate deaminase, EC 3.5.4.6; and GMPK, guanylate kinase, EC 2.7.4.8.

this drug on human tumors grown as xenografts in athymic mice.

MATERIALS AND METHODS

Materials: 8-Azaadenosine was synthesized by the method of Elliott and Montgomery [9] and was dissolved in saline at a concentration of 2 mg/ml for toxicology studies. The dCF was obtained from Dr. John Douros (Drug Development Branch, National Cancer Institute, Bethesda, MD) and was dissolved in saline at a concentration of 0.01 mg/ml prior to being used.

Mice used in this study were male and female outbred Swiss heterozygous for the nude gene (nu/+), bred at the Roger Williams Cancer Center Animal Care Facility. The animals were used when they were between 8 and 16 weeks of age and weighed 20–35 g. Animals were fed and watered *ad lib.* during these studies.

Rats (CD_1 albino; 250–300 g), from which hepatocytes were isolated, were purchased from the Charles River Breeding Laboratories (Wilmington, MA). These animals were kept in the Animal Care Facility at Brown University and were fed and watered *ad lib.*

Determination of dCF dose: Several doses of dCF both alone and in combination with 8-azaAdo were used in preliminary experiments in order to determine the lowest dose of dCF which caused inhibition of mouse erythrocyte ADA activity (data not shown). The doses of dCF ranged from 0.1 to 10 mg/kg. At a dCF dose of 10 mg/kg, weight loss, tremors and hemoglobinuria were noted. Thus, in these experiments, we used a dose of dCF (0.1 mg/kg) that caused complete inhibition of erythrocytic ADA activity (determined by the method of Chassin *et al.* [10]), but that was not associated with toxicity when given on a once daily for 5 days (q.d. × 5) schedule (data not shown). A 1-hr interval between dCF and 8-azaAdo administrations was used to ensure complete inhibition of erythrocytic ADA activity. As had been expected, the combination of 8-azaAdo with dCF proved more toxic than 8-azaAdo alone, with the LD_{10} decreasing 3-fold from 30 to 10 mg/kg on a q.d. × 5 schedule.

In vivo *toxicological studies*. Lethal dose for 10% of the animals (LD_{10}) and the lethal dose for 50% of the animals (LD_{50}) were determined by treating mice on a q.d. × 5 schedule. Twelve animals were treated per group and all injections were given intraperitoneally (i.p.). When 8-azaAdo was used in combination with dCF, the dCF, at a dose of 0.1 mg/kg, was given 1 hr prior to the 8-azaAdo. All animals were weighed on day 6 and on day 14; blood cell counts and blood chemistries were determined on days 6 and 14. Cages were checked twice daily for deaths. A complete autopsy was done on at least one animal per group on day 6.

8-AzaAdo either alone or after pretreatment with dCF was also administered on a once per week (q.w.) schedule for 4 or 6 weeks. The doses were 100 mg/kg, 150 mg/kg, 225 mg/kg, and 300 mg/kg when 8-azaAdo was given alone; when dCF was given at a dose of 0.1 mg/kg 1 hr prior to 8-azaAdo the doses were 75 mg/kg, 100 mg/kg, 150 mg/kg and 300 mg/kg.

Peripheral leukocyte counts were determined using a Becton Dickinson white blood cell counting kit. Glucose, blood urea nitrogen (BUN), creatinine, serum glutamic oxaloacetic transaminase (SGOT) and bilirubin tests were performed in the Clinical Chemistry Laboratory at Roger Williams General Hospital.

Autopsies were performed after cervical dislocation. All organs including brain were examined both grossly and microscopically. Slides were prepared after fixation in 10% glutaraldehyde and formalin and were stained with hematoxylin and eosin. Selected livers were quick frozen and stained with periodic acid Schiff (PAS) and with Sudan III fat stain for glycogen and fat.

In vitro *incubation of isolated hepatocytes with 8-azaAdo and dCF.* Rat hepatocytes were isolated using the two-step collagenase perfusion method of Seglen [11]. The perfusion buffer was Krebs–Ringer bicarbonate (pH 7.45) gassed with 95% O_2:5% CO_2. Metabolic studies were performed in incubation medium at pH 7.4 that contained 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) (50 mM), NaCl (95 mM), KCl (45 mM), $CaCl_2 \cdot 2H_2O$ (2.5 mM), KH_2PO_4 (1.2 mM), $MgSO_4 \cdot 7H_2O$ (1.2 mM) and glucose (5 mM). A shaking water bath at 37° with an atmosphere of 95% O_2:5% CO_2 was used for these incubations. Hepatocytes (2–3%, v/v) were preincubated with dCF (1 μg/ml) for 20 min (when used) before addition of 8-azaAdo (1 mM final concentration). At appropriate time points (up to 2 hr) after addition of 8-azaAdo, aliquots of the reaction mixture were removed; and the cells were extracted with cold perchloric acid (PCA; 4% final concentration). After centrifugation (3600 *g*) the acid-soluble extract was neutralized with KOH and centrifuged to remove

Table 1. Toxicity of 8-azaadenosine (8-azaAdo) alone and in combination with 2′-deoxycoformycin (dCF) to athymic mice*

	LD_{10} ($mg \cdot kg^{-1} \cdot day^{-1}$)	LD_{50}
8-azaAdo alone	30	50
8-azaAdo plus dCF	10	17

* Mice were treated once per day for five successive days by i.p. injection. When dCF was used in combination with 8-azaAdo, the dCF, at a dose of 0.1 mg/kg, was administered 1 hr prior to the 8-azaAdo. Twelve mice were treated per group. The values listed above were obtained from a log-probit mortality plot.

$KClO_4$ (3600 *g*), and the supernatant solution was frozen until analyzed. All extraction and neutralization procedures were performed at 4°. Nucleotide profiles were determined by anion-exchange high performance liquid chromatography (HPLC) as described previously [12]. HPLC effluents were monitored at two wavelengths, 254 nm to visualize natural nucleotides and 278 nm for 8-azaAdo compounds.

Accumulation of 8-azaAdo nucleotides in mouse liver in vivo. Mice were injected on a q.d. × 5 schedule with dCF (0.1 mg/kg) or saline 1 hr prior to injection of 8-azaAdo (8.4 mg/kg or 31 mg/kg respectively). Mice were killed by cervical dislocation at 15, 30 and 45 min after the fifth injection of 8-azaAdo, and immediately thereafter laparotomy was performed. Livers were freeze-clamped *in situ* using stainless-steel clamps which had been immersed previously in a dry ice–ethanol bath. Portions of the frozen livers were placed in chilled (4°) Dounce homogenizers containing 4% PCA. Following homogenization, the extracts were centrifuged (3600 *g*), and the supernatant fraction was neutralized with KOH. $KClO_4$ was removed by centrifugation (3600 *g*), and the supernatant solution was frozen until analyzed by HPLC. All centrifugation steps were performed at 4°.

RESULTS

The toxicity of 8-azaAdo was potentiated about 3-fold by pretreatment with dCF (Table 1). 8-AzaAdo as a single agent on a q.d. × 5 schedule had a LD_{50} dose of 50 $mg \cdot kg^{-1} \cdot day^{-1}$ and an LD_{10} dose of 30 $mg \cdot kg^{-1} \cdot day^{-1}$. When dCF was given at a dose of 0.1 $mg \cdot kg^{-1} \cdot day^{-1}$ 1 hr prior to 8-azaAdo, the LD_{50} dose was 17 $mg \cdot kg^{-1} \cdot day^{-1}$ × 5 days and the LD_{10} dose was 10 $mg \cdot kg^{-1} \cdot day^{-1}$ × 5 days.

All mice treated with doses of 8-azaAdo of 26 $mg \cdot kg^{-1} day^{-1}$ × 5, or greater, lost weight by day 6, and this weight loss was only partially restored by day 14. As would be expected, weight loss was more severe in the animals treated with the higher doses. All treatment groups had begun to regain weight by day 14.

When 8-azaAdo was given on a q.w. schedule either alone or after pretreatment with 0.1 mg/kg dCF, much larger doses were tolerated than on a q.d. × 5 schedule. Weight losses occurred in all treatment groups except one (8-azaAdo, 100 $mg \cdot kg^{-1} \cdot week^{-1}$ × 6). Total leukocyte suppression was mild and was not progressive.

Total leukocyte counts were determined on two animals per group on day 6 and on two additional animals per group on day 14, and the counts on each day were averaged. Animals treated with 8-azaAdo alone did not show leukopenia on day 6 at doses lower than the LD_{10}. When dCF pretreatment was given, total leukocyte suppression was seen only at doses greater than the LD_{10} and, in the surviving animals, the total leukocyte count was restored to pretreatment values by day 14. Lymphopenia was not observed with dCF alone at a dose of 0.1 mg/kg.

Several abnormalities were observed in the blood chemistries determined on day 6 (Table 2). Marked hypoglycemia (blood glucose less than 50 mg/dl) was observed in some animals treated with doses of 8-azaAdo at the LD_{10} value or greater. Increases in SGOT consistent with acute hepatocellular damage were seen in animals treated with the toxic doses of

Table 2. Clinical chemistry results from athymic mice treated with 8-azaadenosine (8-azaAdo) alone or in combination with 2′-deoxycoformycin*

8-azaAdo dose (mg/kg/day)	dCF dose (mg/kg/day)	Glucose (mg/dl)	SGOT (IU)	Bilirubin (mg/dl)	BUN/Creatinine (mg/dl)/(mg/dl)
0	0	135	59	0.2	19/0.3
20	0	132	57	0.5	21/0.4
26	0	43	671	1.2	41/0.5
33.8	0	34	270	0.2	41/0.6
44	0	108	268		
0	0.1	225	61	0.1	26/0.3
10.3	0.1	127	56	0	23/0.5
13.5	0.1	77	96	0.2	14/0.4
17.5	0.1	111	53	0.1	26/0.4
22.8	0.1	68	86	0	15/0.4

* Drugs were administered to mice by i.p. injection once per day for five successive days. When dCF was administered in combination with 8-azaAdo, the dCF was given 1 hr prior to the 8-azaAdo. Twelve mice were treated per group. Chemistry values were determined on two mice from each group on day 6. The values listed above were averages from these two animals. Normal values for the parameters listed above (with ranges) were: glucose, 209 mg/dl (123–280); SGOT, 52 IU (41–61); bilirubin, 0.4 mg/dl (0.1–0.7); BUN, 29 mg/dl (24–32); and creatinine, 0.3 mg/dl (0–0.5).

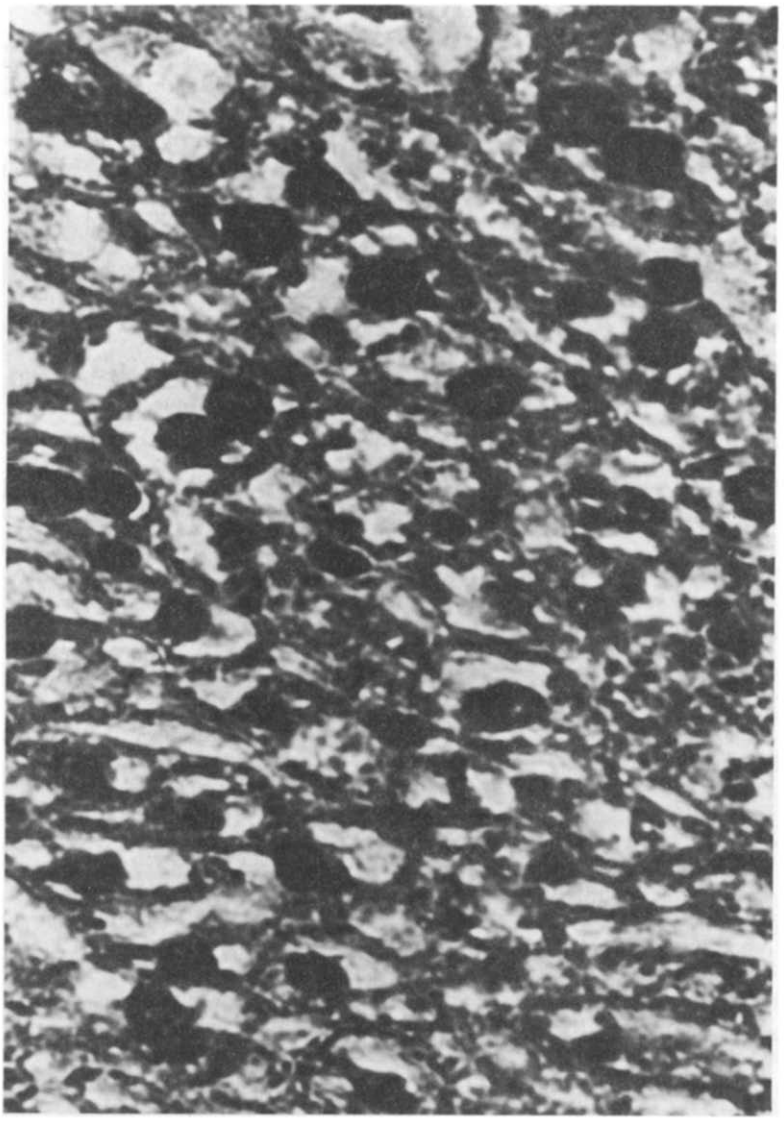

Fig. 2. Histologic section of liver from a mouse treated with 8-azaadenosine (8-azaAdo). 8-AzaAdo was administered i.p. on a q.d. × 5 schedule at a dose of 33.8 mg·kg^{-1}·day^{-1}. Histology was performed on day 6. Magnification, 400 ×.

8-azaAdo. Mild elevations of bilirubin were noted in the animals treated with 20 and 26 mg/kg of 8-azaAdo alone. Alterations in serum creatinine levels were not seen; thus, the increases in blood urea nitrogen (BUN) noted in the two highest 8-azaAdo treatment groups probably reflect dehydration.

Gross post-mortem examinations were performed on day 6 on animals treated with 8-azaAdo alone at doses of 20 mg·kg^{-1}·day^{-1} × 5 or greater, or with greater than 10 mg·kg^{-1}·day^{-1} × 5 of 8-azaAdo in combination with dCF. These animals had pale swollen and smooth livers, whereas animals killed on day 14 (9 days after the end of treatment) had normal livers on macroscopic examination. The remainder of the gross post-mortem examination was within normal limits.

Histologic examinations were performed after hematoxylin and eosin staining on liver, heart, lungs, small and large intestine, esophagus, stomach, pancreas, kidney, bone marrow, spleen, thymus and brain. The most notable changes were seen in the liver which showed swollen, vacuolated cells, some cytomegaly and some areas of early regeneration (see Figs. 2 and 3). PAS and Sudan III fat stains indicated that the vacuoles contained fat. Microscopic examination of the bone marrow correlated well with the peripheral leukocyte counts. Marked marrow hypoplasia was seen only in the animals treated with 33.8 mg·kg^{-1}·day^{-1} × 5 of 8-azaAdo alone, and slight to moderate depressions of both erythrocytic and granulocytic precursors were seen with animals treated with doses of 8-azaAdo of 17.5 and 22.8 mg·kg^{-1}·day^{-1} × 5 after pretreatment with dCF. Examination of the gastrointestinal tract showed cystic changes in the mucus-secreting glands of the stomach in animals treated with the higher doses of 8-azaAdo. Mild to moderate peritonitis occurred in most animals, probably secondary to a local irritative effect of 8-azaAdo.

In an attempt to explain biochemically the hepato-

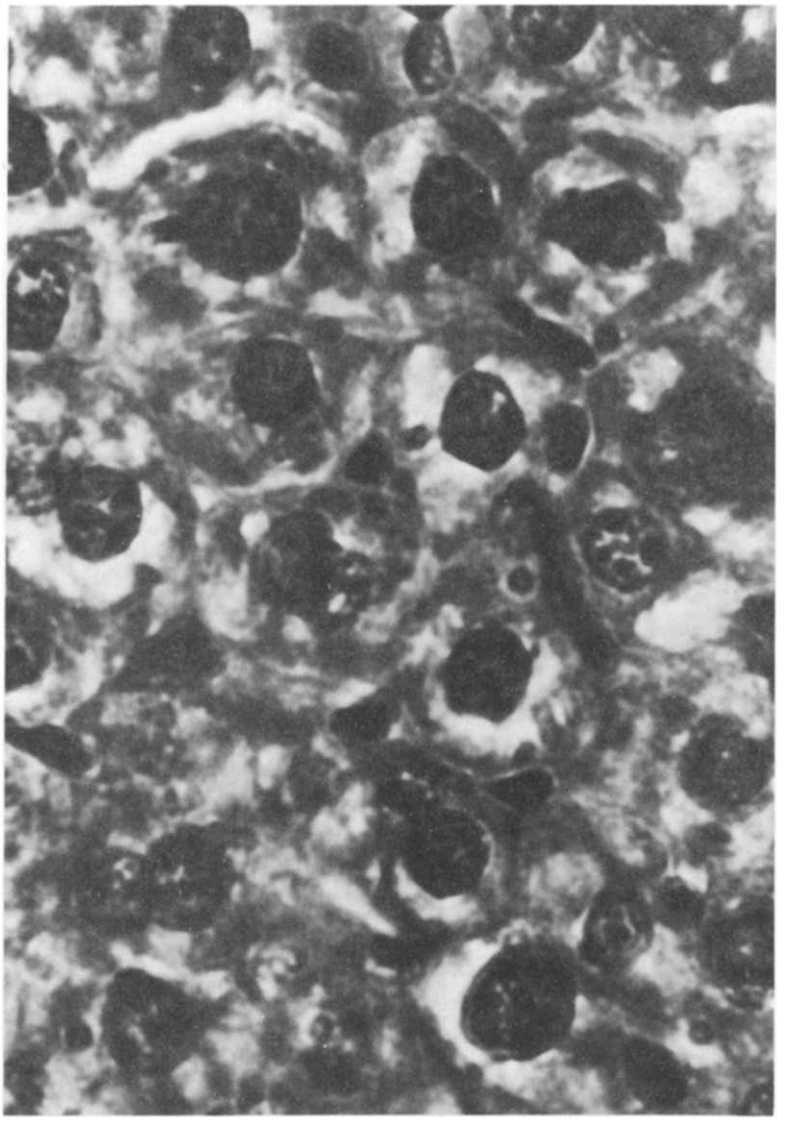

Fig. 3. Histologic section of liver from a mouse treated with the combination of 8-azaadenosine (8-azaAdo) plus 2′-deoxycoformycin (dCF). 8-AzaAdo was administered i.p. on a q.d. × 5 schedule at a dose of 13.5 mg·kg^{-1}·day^{-1} 1 hr after i.p. administration of dCF (0.1 mg·kg^{-1}·day^{-1}). Histology was performed on day 6. Magnification, 1000 ×.

toxicity observed in animals treated with the combination of 8-azaAdo plus dCF, isolated rat hepatocytes were incubated *in vitro* with 8-azaAdo (1 mM) alone or in combination with dCF (1 μg/ml). After extraction, aliquots of the acid-soluble material were subjected to anion-exchange HPLC to elucidate the nucleotide profiles of these cells. Figure 4 shows HPLC profiles of the control and 8-azaAdo plus dCF samples taken after 2 hr of incubation. It is apparent from this figure that, as 8-azaAdo nucleotides accumulate in these cells, the cellular ATP levels fall. In fact, in the cells treated with the combination of 8-azaAdo plus dCF, ATP levels decreased to below the limits of detection in 2 hr. The levels of 8-azaATP which accumulated were 2.2 and 4.3 μmoles/g cells when 8-azaAdo was used alone and in combination with dCF respectively.

To verify that 8-azaAdo nucleotides accumulate in the livers of animals treated with the combination of 8-azaAdo plus dCF, animals were killed at appropriate times after 8-azaAdo injection, livers were freeze-clamped, and portions were excised and extracted with perchloric acid. When the nucleotide profiles of the tissue were determined by HPLC, 8-azaAdo nucleotides were detected. The maximal amount of analog nucleotides which accumulated was approximately 0.3 μmole/g of tissue (data not shown).

DISCUSSION

Purine nucleoside analogs are of interest as antiviral, immunosuppressive and antineoplastic agents.

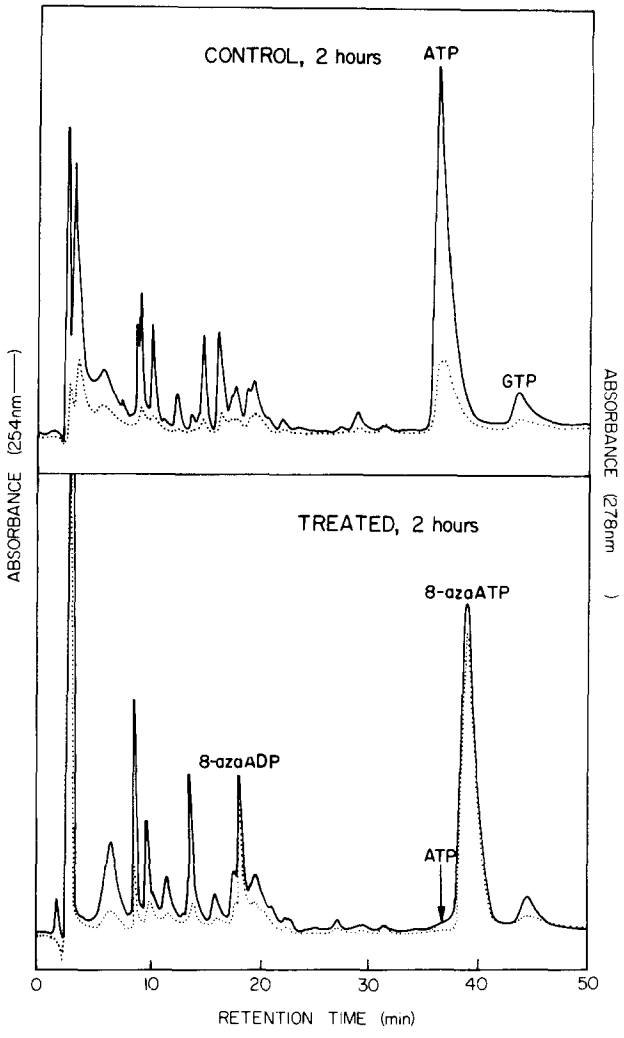

Fig. 4. Nucleotide profiles of isolated rat hepatocytes incubated *in vitro* for 2 hr in the absence (upper frame) or presence (lower frame) of 8-azaadenosine (1 mM) and 2′-deoxycoformycin (1 μg/ml). The procedures for cell isolation, incubation and extraction and for high performance liquid chromatography (HPLC) are outlined in Materials and Methods. HPLC column effluents were monitored at two wavelengths, 254 nm (solid lines) to detect natural nucleotides and 278 nm (dotted lines) to detect 8-azaadenosine nucleotides.

One of these analogs, 8-azaAdo, is anabolized to 8-azaATP in a sequence of reactions beginning with the formation of the 5′-monophosphate; this initial reaction is catalyzed by adenosine kinase [2] As 8-azaATP, this analog can be incorporated into RNA [2, 3]. Furthermore, 8-azaGTP can be formed from 8-azaAdo, and this nucleotide can also be incorporated into polynucleotides [2]. High intracellular levels of 8-azaATP and 8-azaADP can decrease the formation of 5-phosphoribosyl-1-pyrophosphate.* 8-AzaAdo is an excellent substrate for human erythrocytic ADA and can be rapidly converted to 8-azainosine [1]; this, in turn, may be further catabolized to 8-azahypoxanthine or phosphorylated by adenosine kinase to 8-azainosine-5′-monophosphate (8-azaIMP) [3, 4]. Further metabolism results in the formation of both 8-azaATP and 8-azaGTP. Thus, 8-azaAdo might act differently at the biochemical level when given alone than when administered in combination with an ADA inhibitor.

On macroscopic, histologic and biochemical examination, the liver was the organ most sensitive to the effects of 8-azaAdo either alone or in combination with dCF. The liver toxicity was further documented on microscopic examination by fatty infiltrates that were seen at all doses of 8-azaAdo above the LD_{10}. The marked hypoglycemia and the elevated SGOT reflected the hepatotoxicity. Several of the hypoglycemic animals displayed seizure activity immediately prior to death, presumably due to the low blood glucose levels. Histologic examinations of the central nervous system failed to show pathologic changes. At equitoxic doses, no qualitative or quantitative differences were detected between 8-azaAdo alone or 8-azaAdo plus dCF.

* T. M. Savarese, G. W. Crabtree and R. E. Parks, Jr., unpublished observations.

Mice treated on a once per week schedule for 4 or 6 weeks were able to tolerate much larger doses of 8-azaAdo. This may indicate that schedule dependency will be an important consideration when this drug combination is tested in xenograft systems. It is also possible that a semi-tight binding inhibitor of ADA such as erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA, K_i for human erythrocytic ADA of 2–4 nM) may have advantages over dCF [6, 13]. In contrast to dCF, where long-lasting ADA inhibition is seen in tissues such as erythrocytes, liver and heart, [10, 14, 15], by appropriate dose selection it may be possible to restrict whole body ADA inhibition by EHNA to a few hours [16], thus favoring elimination of analog nucleotides such as 8-azaATP from organs such as the liver.

In view of the reported metabolism of 8-azaAdo [2], the lack of a peak corresponding to 8-azaGTP (retention time of about 48 min) on nucleotide profiles (Fig. 4) is unexpected. The reasons for this are unclear at the present time. The possibility exists that the absence of glutamine (required for the conversion of XMP to GMP, the second of two steps in the synthesis of GMP from IMP) in the incubation medium may play a role here. Glutamine concentrations have been shown to limit this conversion [17] as well as other glutamine-requiring processes [18–22].

The fact that the ratios of natural and 8-azaadenine-containing triphosphate nucleotides to diphosphate nucleotides, e.g. 8-azaATP:8-azaADP (see Fig. 4), remain high in hepatocytes indicates that the 8-azaadenine-containing nucleotides can function effectively in many of the metabolic reactions concerned with the generation of high-energy phosphate, at least for short time periods. Further detailed studies of the behavior of 8-azaATP as a metabolic substitute for ATP may yield information on the specific biochemical lesions involved in the fatty livers.

In the studies with isolated rat hepatocytes described above, the synthesis of 8-azaATP appears to take place at the expense of ATP, i.e. with 8-azaAdo alone, as 8-azaATP is formed, ATP levels fall, and when dCF is included, the ATP pool is almost completely abolished. Similar effects were not observed in the *in vivo* mouse studies. This apparent discrepancy may be due to the high (1 mM) concentrations of 8-azaAdo used in the *in vitro* experiments relative to those used for the *in vivo* work, i.e. more analog nucleotides are synthesized on a per gram of tissue basis in the *in vitro* experiments. The fate of the ATP which is degraded in hepatocytes has not yet been elucidated. When the combination of 8-azaAdo and dCF is used, the ATP may be converted to adenosine which cannot be catabolized further since the ADA is inhibited, and some of the effects noted above may conceivably be due to the liberation of this nucleoside. When 8-azaAdo is used alone, however, any adenosine liberated would probably be catabolized further. Therefore, it is unlikely that the toxic effects reported above can be ascribed only to compounds liberated as a result of ATP catabolism. It should also be noted that the reductions in ATP pools observed in the *in vitro* experiments are specific for this nucleotide since similar reductions in the other triphosphate nucleotides (UTP, CTP, GTP) did not occur.

In summary, the combination of 8-azaAdo and dCF has proven active against human colon, pancreatic and lung cancer cell lines in culture [7, 8]. Since current clinical chemotherapy of these neoplasms is unsatisfactory, with fewer than 20% of the patients receiving clear benefit, we are extending our investigations of this promising drug combination to several human colon carcinomas grown as xenografts in athymic mice. The results reported here will be useful in determining the doses and schedule of drug treatment for our *in vivo* studies.

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