Biochemical Pharmacology, Vol. 28, pp. 607–612

0006-2952/79/0301-0607 $02.00/0

CYTOTOXICITY OF N^6-SUBSTITUTED ADENOSINE ANALOGS TO CULTURED TROPHOBLASTIC TUMOR CELLS*

RONALD W. TREWYN† and SYLVIA J. KERR
Department of Biochemistry, University of Colorado Medical Center, Denver, CO 80262, U.S.A.

(*Received* 23 *December* 1977; *accepted* 24 *July* 1978)

Abstract—Cultured trophoblastic tumor cells were found to have an elevated adenosine kinase activity relative to levels in normal trophoblasts and those derived from a hydatidiform mole. The effect of N^6-substituted adenosine analogs on the various cell lines was investigated to determine whether the differential enzyme levels might, in turn, result in differential sensitivity of the cell lines to the compounds. When cells were exposed to appropriate concentrations of N^6-methyladenosine or N^6,N^6-dimethyladenosine, it was found that the nucleosides were only cytostatic to non-malignant trophoblastic cells derived from placenta or hydatidiform mole, and cell lines of non-trophoblastic origin. However, the same concentrations of the nucleosides were cytotoxic to the malignant trophoblastic cell lines. The primary effect of exposure of cells to N^6,N^6-dimethyladenosine appeared to be inhibition of DNA synthesis. A third adenosine analog, N^6-(Δ^2-isopentenyl)adenosine, was toxic to almost all cell lines regardless of origin.

Numerous N^6-substituted adenosine analogs are substrates for adenosine kinase (ATP:adenosine 5′-phosphotransferase, EC 2.7.1.20) *in vitro* [1, 2], and based on their conversion to nucleoside monophosphates, are presumed to be substrates for the enzyme *in vivo* as well [3, 4]. N^6-methyladenosine (6-meAdo) and N^6,N^6-dimethyladenosine (6-me$_2$Ado) are excellent substrates for adenosine kinase isolated from cultured human tumor cells, while compounds with larger substitutions on the amino moiety are not substrates *in vitro* [2]. However, N^6-(Δ^2-isopentenyl) adenosine (6-ipAdo) is a substrate *in vitro* for the enzyme from cultured mouse sarcoma cells [1], and this analog does appear to be phosphorylated by a variety of cultured mammalian cells, including those of human origin [5].

Many of the N^6-substituted adenosine analogs are cytotoxic to cultured mammalian cells [6]. The cytotoxicity appears to be a consequence of their phosphorylation by adenosine kinase [7], and often the subsequent inhibition of purine synthesis *de novo* by the phosphorylated derivatives [8]. Adenosine itself can be toxic to human cells at levels exceeding 35 μM [9]. However, in that instance, the source of the toxicity has been attributed to the inhibition of pyrimidine biosynthesis [9] or to the wasting of rRNA [10], and thus, is distinct from the mode of action of the N^6-substituted adenosine derivatives.

A direct relationship has been reported between the relative cytotoxicity of 6-ipAdo and levels of adenosine kinase activity in target cells [5]. This variability in distribution of adenosine kinase activity with cell type might then be exploited to differentially kill those cell types which synthesize relatively high levels of the enzyme.

Studies in our laboratory with cultured human trophoblastic tumor cells revealed that these cell lines have elevated levels of adenosine kinase activity when compared to levels observed with normal trophoblastic cells. This biochemical difference made possible a comparative study of the sensitivity of normal and neoplastic trophoblastic cells to N^6-substituted adenosine analogs, which is the subject of this report.

MATERIALS AND METHODS

Cells and culture conditions. BeWo [11], Reid, and JEG-3 [12] choriocarcinoma cell lines were obtained from Dr. Charles August, Department of Pediatrics, University of Colorado Medical Center. HyDat, a trophoblastic cell line derived from a hydatidiform mole, and the C_2JAr choriocarcinoma cell line were obtained from Dr. John Brewer, Department of Obstetrics and Gynecology, Northwestern University, Chicago. Human embryonic lung (HEL) fibroblasts, passage 11–19, were obtained from Dr. Kenneth McIntosh, Department of Pediatrics, University of Colorado Medical Center. BeWo-R6 cells (resistant to 6-meAdo) were obtained by continuous treatment of BeWo cells with 100 μM 6-meAdo. There was an initial killing of approximately 80–90 per cent of the culture, followed by a prolonged period when the nucleoside was cytostatic. After a few weeks, clones resistant to 6-meAdo began to grow out. These cells continue to secrete human chorionic gonadotropin (hCG), and are indistinguishable morphologically from sensitive BeWo cells. All of the above cells were grown in monolayer in RPMI 1640 medium containing 10% fetal calf serum. BeWo-R6 medium was supplemented with 100 μM 6-meAdo.

HeLa cells and D98/AH 2 cells (human sternal marrow cells of the Detroit-98 line; resistant to 8-azahypoxanthine) were purchased from American Type Culture Collection, Rockville, MD, and were cultured in Eagle's Minimum Essential Medium containing 10% fetal calf serum.

Human chorionic tissue from 16-week and 19-week

* This work was supported in part by Contract CM-12186 and Grants CA-05441 and CA-12742 awarded by the National Cancer Institute, DHEW.

† A Postdoctoral Fellowship Awardee of the National Cancer Institute.

aborted placentas was obtained through the courtesy of Dr. Watson Bowes, Department of Obstetrics and Gynecology, University of Colorado Medical Center. Chorionic villi were dissected and minced as described by Pattillo *et al.* [13], and those from the 19-week placenta were distributed into 35 mm Cluster dishes (Falcon Plastics, Oxnard, CA) in approximately equal amounts. The minced villi were maintained in RPMI 1640 medium containing 20% fetal calf serum plus Garamycin (200 μg/ml) and Fungizone (5 μg/ml).

hCG Determination. 6-meAdo was added to the culture medium to yield a final concentration of 100 μM. BeWo and Reid cells were treated 1 day after plating when the cultures were fed with fresh medium. The nucleoside was added to the chorionic villi 4 days after the initial plating when all cultures had been established as positive for hCG production. The medium was changed at 2–4-day intervals, and after 6–12 days, hCG was assayed in the spent medium using the Direct Agglutination Pregnancy Test. The sensitivity of this test permits determination of hCG concentrations in excess of 2 I.U./ml. The concentration of hCG in each sample was estimated by making serial dilutions in isotonic saline. The concentration reported is based on the highest dilution giving an unequivocally positive result. Duplicate cultures were tested in all cases. Good correlation was observed between viable cell number and hCG concentration in the culture medium when the above methods were followed.

Preparation of cell extracts. Cell monolayers were harvested and washed with 0.9% NaCl as described previously [14]. Chorionic villi from the 16-week placenta, dissected from the chorionic tissue as described above, were also washed with 0.9% NaCl. Cells and villi were resuspended in 2–4 vol. (v/v) of hypotonic buffer [10 mM Tris–HCl (pH 7.4), 10 mM NaCl, 1.5 mM $MgCl_2$, and 1.0 mM dithiothreitol], and homogenized with a Tekmar Tissumizer. The homogenates were centrifuged at 30.000 *g*, and the supernatant fractions were assayed for various enzyme activities.

Enzyme assays. The spectrophotometric assay for adenosine deaminase described by Kalckar [15] was used. The substrate, adenosine, was present at 100 μM, and its conversion to inosine was monitored at 265 nm. The cell extract was added at a concentration of 20–80 μg protein/ml.

The nucleoside phosphorylase assay was a modification of the method of Kim *et al.* [16] using the coupled xanthine oxidase method [15]. The reaction mixture contained 0.1 M phosphate buffer (pH 7.2), 200 μM inosine, 100 units xanthine oxidase, and enzyme extract (20–80 μg protein/ml), in a total volume of 1 ml. The reaction was started by adding the enzyme extract, and the synthesis of uric acid was monitored spectrophotometrically at 293 nm.

Adenosine kinase was assayed using the method of Chan *et al.* [17], but with 100 μM [2-^{3}H]adenosine (50 mCi/m-mole). In addition, deoxycoformycin was included at a concentration of 1 μM which totally inhibited adenosine deaminase without affecting kinase activity. The rate of the reaction was linear for over 20 min and was not changed by increasing the substrate concentration. The product, [^{3}H]-AMP, was precipitated with 1 ml of 0.1 M lanthanum chloride [18] after a 10-min reaction time. Blanks to which ATP was added at the end of the 10-min reaction were run in all cases. After 30 min on ice, the precipitates were collected on glass fiber filters, and washed with 50 ml of cold water. The dried filters were counted by liquid scintillation.

Units of enzyme activity are all expressed in nmoles/min.

Growth inhibition studies. Dose–response curves for Reid and HEL cells treated with 6-meAdo, 6-me_2Ado, and 6-ipAdo were established for cultures plated at 2×10^5 cells/25 cm^2 culture flask. Duplicate cultures were treated with 0.1, 1.0, 10, 100 and 1000 μM of each of the nucleosides the day after plating and compared with duplicate untreated control cultures. After treatment for 3 days, all cultures were fed with fresh growth medium which was supplemented with the appropriate concentration of nucleoside for test cultures. After an additional 3 days, cultures were trypsinized and counted with a hemacytometer.

DNA determinations were done by the diphenylamine method [19]. Cells were plated at 2×10^6 cells/150 cm^2 culture flask. The following day some cultures were treated with 10 μM 6-me_2Ado, and others were harvested to determine the initial DNA content. Control and treated cultures were fed at 3–4 day intervals, and after treatment for 10 days, cultures were harvested to determine the final DNA content.

Macromolecular synthesis in cultured cells. The effects of 10 μM 6-me_2Ado on macromolecular synthesis in various cell types were determined using the scintillation vial cell culture technique of Ball *et al.* [20]. The culture media were supplemented with 10 mM sodium formate, 5 μM uridine, and 5 μM thymidine. 6-me_2Ado was added to a final concentration of 10 μM 18–20 hr after establishing the cultures and 4 hr before terminating the experiment. Control and treated cells were pulse-labeled for 90 min with 2 μCi [methyl-^{3}H]thymidine, 1 μCi [5-^{3}H]uridine, or 1 μCi L-[methyl-^{14}C]methionine to measure DNA, RNA, and protein syntheses respectively. Results were corrected for changes in the size of soluble pools, but only in the case of [^{3}H]uridine was there ever a decreased pool size of more than 10 per cent following treatment for 4 hr with 10 μM 6-me_2Ado. The magnitude of this decrease in the trophoblastic cells was never as great as the decreased incorporation into nucleic acids.

Chemicals and reagents. 6-Methylaminopurine-9-ribofuranoside (6-meAdo) and 6-(γ,γ-dimethylallylamino)purine riboside (6-ipAdo) were purchased from Sigma Chemical Co., St. Louis, MO, and N^6,N^6-dimethyladenosine (6-me_2Ado) was purchased from P-L Biochemicals, Inc., Milwaukee, WI. The Direct Agglutination Pregnancy Test was obtained from Wampole Laboratories, Stamford, CT, and xanthine oxidase was obtained from Boehringer Mannheim, San Francisco, CA.

Growth media and serum were purchased from Grand Island Biological Co., Grand Island, NY. Garamycin (gentamicin sulfate) was obtained from Schering Pharmaceutical Corp., Kenilworth, NJ, and Fungizone (amphotericin B) was obtained from E. R. Squibb & Sons, Inc., Princeton, NJ. L-[Methyl-^{14}C]methionine (54 mCi/m-mole) and [2-^{3}H]adenosine (12 Ci/m-mole) were purchased from New England Nuclear, Boston, MA. [5-^{3}H]uridine (5 Ci/m-mole) and [methyl-^{3}H]thymidine (5 Ci/m-mole) were obtained from Amersham/Searle Corp., Arlington Heights, IL.

Deoxycoformycin was the gift of Dr. S. S. Cohen, Department of Microbiology, University of Colorado Medical Center.

RESULTS

Exposure of BeWo cells to 100 μM 6-meAdo was observed to cause extensive cell killing. During the first 3–4 days of treatment, the treated and untreated cultures appeared identical. There was then an 80–90 per cent decrease in cell density in the cultures containing the nucleoside. After the first 10–12 days of treatment, the cell number did not change significantly for many weeks, but the surviving exposed cells were still capable of renewed proliferation if the nucleoside was removed. In addition, cells resistant to the 6-meAdo eventually were obtained if the nucleoside was not removed.

A comparison of the levels of some enzymes involved in nucleoside metabolism in human cell lines was carried out to investigate the biochemical basis for the sensitivity to 6-meAdo. These enzymes were adenosine kinase, which could activate the nucleoside by phosphorylation, nucleoside phosphorylase, which might inactivate the nucleoside by converting it to the free base [5], and adenosine deaminase. This last enzyme has been shown to be inhibited by 6-meAdo and to a lesser extent by 6-me$_2$Ado [21, 22]. Inhibitors of the deaminase might induce adenosine toxicity, since the concentration of the adenosine could then build up to toxic levels.

The specific activities of the three enzymes in BeWo cells and in the 6-meAdo resistant BeWo-R6 cells are shown in Table 1. Adenosine kinase activity was the only one changed significantly in BeWo-R6 cells compared to the parental BeWo cells. The addition of 6-meAdo to BeWo cultures 18 hr prior to harvesting for enzyme assays did not decrease adenosine kinase specific activity, indicating that 6-meAdo or its metabolites in BeWo-R6 crude cell extracts were not responsible for the lower enzyme activity. The results also suggest that cells with lower adenosine kinase appeared by natural selection after prolonged exposure to the methylated nucleoside.

Table 1 also shows the levels of adenosine kinase, adenosine deaminase, and nucleoside phosphorylase in a number of other trophoblastic tumor cell lines, in a cell line, derived from a hydatidiform mole, which produces a non-invasive chorionic neoplasm with a significant probability of malignant progression into frank chorio-carcinoma, and in normal chorionic tissue. All the trophoblastic cell lines of tumor origin have higher levels of adenosine kinase activity than are contained in normal trophoblasts from placenta or in the trophoblastic line derived from the non-malignant hydatidiform mole. The levels of the two degradative enzymes do not exhibit any uniform pattern.

The adenosine kinase results suggested that normal trophoblasts from chorionic villi should be less sensitive to 6-meAdo than trophoblastic tumor cells. This was tested using *in vitro* culture techniques. Normal trophoblasts, although they do not divide, continue to produce hCG for up to 3 weeks in culture [13]. Thus, even though drug-induced effects on cell division cannot be measured, monitoring the secretion of hCG is a means of assessing the effect of drug exposure in a mixed cell population. As shown in Table 2, there was a reduction of hCG secretion after exposure of chorionic villi for 8 days to 100 μM 6-meAdo. The tumor derived (BeWo and Reid) lines were more sensitive, as evidenced by greater inhibition of hCG secretion after only 6 days of exposure to the compound. In turn, secretion of hCG by the resistant line, BeWo-R6, was the least affected by exposure to 6-meAdo.

In Figure 1, the relations between concentration of nucleosides and extent of inhibition of hCG secretion by BeWo and BeWo-R6 cells are presented. The BeWo-R6 cells were not only more resistant to 6-meAdo, but also to 6-me$_2$Ado and 6-ipAdo. When the two lines were exposed to 6-me$_2$Ado, a 30- to 50-fold difference in sensitivity between BeWo and BeWo-R6 cells was observed.

The effects of the three N^6-substituted analogs on the growth of trophoblastic tumor cells and non-trophoblastic cells were examined to study analog specificities. Dose–response curves for Reid and HEL cells treated for 6 days with 6-meAdo and 6-ipAdo are shown in Fig. 2. At relatively high concentrations, 6-meAdo was more toxic to Reid cells than to HEL cells, and the magnitude of this difference could be increased by treating for a longer period. It is apparent that 6-ipAdo was more toxic than 6-meAdo to both cell lines, but at low concentrations this agent was actually more cytotoxic to normal HEL fibroblasts than to Reid choriocarcinoma cells.

Table 1. Enzyme activities in human trophoblastic cells

Cells	Origin	Enzyme activity* (nmoles/min/mg protein) Adenosine kinase	Adenosine deaminase	Nucleoside phosphorylase
BeWo	Choriocarcinoma	0.61 ± 0.02	17.0 ± 2.6	21.3 ± 0.7
BeWo-R6	Choriocarcinoma	0.23 ± 0.03†	21.0 ± 1.7	24.7 ± 2.2
Reid	Choriocarcinoma	0.83 ± 0.08	5.3 ± 3.8	75.0 ± 4.6
C_2JAr	Choriocarcinoma	0.47 ± 0.06	20.7 ± 2.7	48.7 ± 12.8
JEG-3	Choriocarcinoma	0.51 ± 0.08	4.0 ± 1.2	94.7 ± 0.7
HyDat	Hydatidiform mole	0.25 ± 0.02	14.3 ± 3.7	48.3 ± 16.2
Chorionic villi	Placenta	0.14 ± 0.02	1.0 ± 1.0	47.5 ± 1.5

* Mean ± S. E. for three experiments except with chorionic villi where only two experiments were carried out.

† Significantly different from the enzyme activity in parental BeWo cells, $P \leqslant 0.05$.

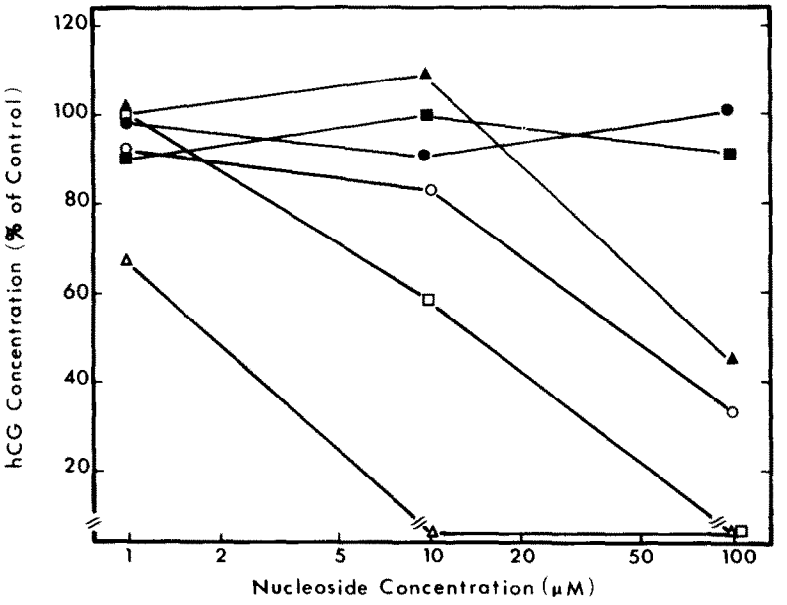

Fig. 1. Sensitive and resistant BeWo Cells treated with 6-meAdo, 6-me_2Ado, and 6-ipAdo. BeWo cells were treated for 12 days with 6-meAdo (○), 6-me_2Ado (△), and 6-ipAdo ( ), and BeWo-R6 cells were treated for 12 days with 6-meAdo (●), 6-me_2Ado (▲), and 6-ipAdo (■). Cells were plated at 4×10^5 cells/25 cm^2 culture flask. Cultures were fed 1 day after plating, and the appropriate nucleoside concentrations were added at that time. The cultures were then fed at 3-day intervals, and after 12 days of treatment, hCG was assayed in the medium as described in Materials and Methods.

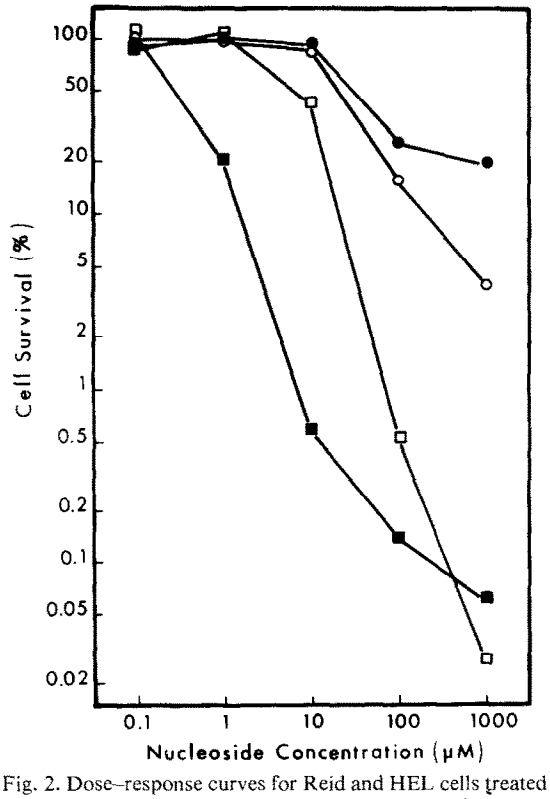

Fig. 2. Dose–response curves for Reid and HEL cells treated with 6-meAdo and 6-ipAdo. Reid cells were treated for 6 days with 6-meAdo (○) and 6-ipAdo ( ), and HEL cells were treated for 6 days with 6-meAdo (●) and 6-ipAdo (■). Cells were plated at 2×10^5 cells/25 cm^2 culture flask. Cultures were fed 1 day after plating, and the appropriate nucleoside concentrations were added at that time. The cultures were fed again after 3 days, and on day 6 duplicate cultures were trypsinized and cells were counted with a hemacytometer.

6-me_2Ado was also tested with Reid and HEL cells; these results are presented in Fig. 3. This agent was very cytotoxic to Reid cells, but not to HEL cells. The initial cell density at which cultures were plated was 7 per cent of the final untreated cell density. HEL cells exposed to high concentrations of 6-me_2Ado maintained their original cell density, indicating that the compound was only cytostatic. HEL cells treated for 6 days with 1 mM 6-me_2Ado were still viable, and resumed proliferation when 6-me_2Ado was removed from the growth medium. The effects of the compound on the growth of Reid cells were also reversible for up to 6 days with cells exposed to 0.1 or 1.0 μM 6-me_2Ado. At 100 and 1000 μM, the effects were irreversible at 6 days. The effects of the compound on either cell line were not overcome by the addition of 100 μM hypoxanthine and/or cytidine or uridine, indicating that its action was not analogous to that of high levels of adenosine [8, 9].

The differential toxicity of 6-me_2Ado was investigated further by examining its effects on a variety of cell types. The DNA contents of treated and untreated cultures, shown in Table 3, provide a measure of the selective cytotoxicity of 6-me_2Ado to choriocarcinoma

Table 2. Effect of 100 μM 6-meAdo on hCG secretion by trophoblastic cells in culture*

Cells	Days treated	Units hCG/ml Control	6-meAdo
BeWo	6	13 ± 1	3 ± 1
BeWo-R6	6	12 ± 2	9 ± 1
Reid	6	19 ± 1	< 2
Chorionic villi	8	4 ± 0	2 ± 0

* Duplicate cultures were treated with 100 μM 6-meAdo as described in Materials and Methods.

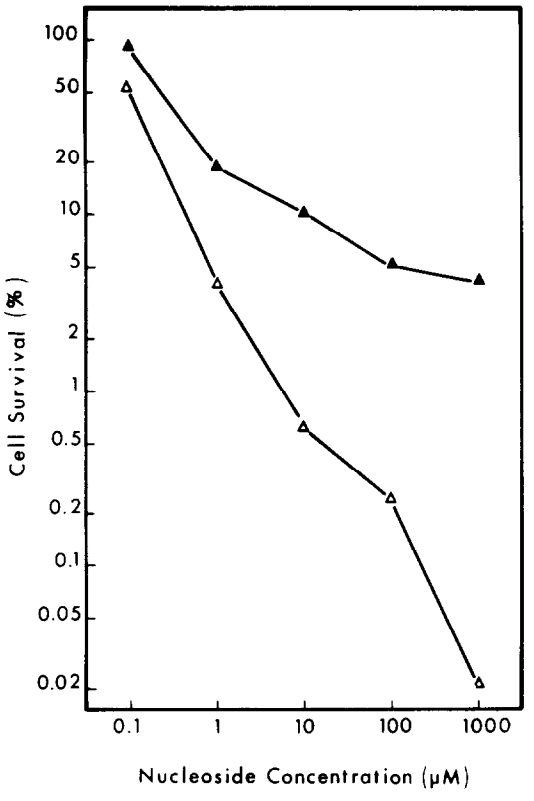

Fig. 3. Dose–response curves for Reid and HEL cells treated with 6-me$_2$Ado. Reid cells (△) and HEL cells (▲) were treated for 6 days with 6-me$_2$Ado. Conditions were as described in Fig. 2.

cells. HyDat, a trophoblastic line derived fom a hydatidiform mole, was not killed by 10 μM 6-me$_2$Ado but cell division was arrested. With non-trophoblastic cells, 6-me$_2$Ado was either cytostatic or ineffective.

The early effects of 10 μM 6-me$_2$Ado on the syntheses of DNA, RNA and protein in trophoblastic and non-trophoblastic cell lines were measured in pulse-label experiments. The results are presented in Table 4. After 4 hr of exposure, the most pronounced effect seen with trophoblastic cells was the inhibition of

Table 3. Effect of 10 μM 6-me$_2$Ado on human cells in culture

	DNA* (μg/culture)		
	Control		6-me$_2$Ado
Cells	Initial	Final	treated
BeWo	69	1060	ND†
Reid	42	1034	ND†
C_2JAr	48	592	ND†
JEG-3	69	1444	ND†
HyDat	59	948	109
HEL	30	257	83
D98/AH-2	26	1100	956
HeLa	63	937	145

* Results are presented as μg DNA/150 cm^2 culture flask before and after treatment for 10 days with 10 μM 6-me$_2$Ado.

† None detected by the diphenylamine assay which has a lower limit of detection of approximately 2 μg.

Table 4. Effects on cellular macromolecular synthesis of exposure to 10 μM 6-me$_2$Ado for 4 hr

	Rate of synthesis* (% of control)		
Cells	DNA	RNA	Protein
BeWo	42.7 ± 1.4†‡	89.0 ± 5.7	82.0 ± 8.5
Reid	50.0 ± 2.0†‡	89.0 ± 7.5	77.7 ± 1.8
C_2JAr	45.0 ± 6.6†	69.0 ± 0.6	60.3 ± 3.8
JEG-3	44.7 ± 2.0†	62.3 ± 4.3	70.3 ± 15.5
HyDat	45.0 ± 1.2†‡	91.3 ± 7.7	85.3 ± 8.7
HEL	71.7 ± 3.3	59.0 ± 6.1	81.7 ± 5.4
D98/AH-2	87.3 ± 8.2	109 ± 7	112 ± 2
HeLa	132 ± 24	119 ± 8	105 ± 3

* Mean ± S. E. for three experiments. Typical control values for the different synthetic processes in cpm/10^5 cells were: DNA, 10,000–20,000; RNA, 2,000–7,000; and protein 3,000–7,000.

† Significantly greater effect on DNA synthesis than on RNA synthesis, $P \leqslant 0.05$.

‡ Significantly greater effect on DNA synthesis than on protein synthesis, $P \leqslant 0.05$.

DNA synthesis; this parameter was not affected as greatly in non-trophoblastic cells. After 18 hr of exposure, all macromolecular syntheses were severely inhibited in the sensitive cells, with variable results in the non-trophoblastic lines.

DISCUSSION

The acquired resistance of cultured cells to the cytotoxicity of adenosine and adenosine analogs has been related to a loss of adenosine kinase activity in many cases [7, 17, 23]. Among cell lines of trophoblastic origin, malignant and non-malignant, susceptibility to the cytotoxic effects of N^6-substituted adenosine analogs could be correlated with the level of adenosine kinase activity present in the individual cell lines. Four independently derived choriocarcinoma cell lines exhibited elevated adenosine kinase activity when compared to cells derived from a non-malignant hydatidiform mole and normal chorionic tissue, and all four tumor lines were much more sensitive to the effects of 6-meAdo and 6-me$_2$Ado. In addition, a tumor-derived cell line selected for resistance to 6-meAdo displayed a significantly lowered level of adenosine kinase activity. This implicates a phosphorylated derivative as an intermediate in the cytotoxicity of these compounds [7, 9].

The nucleoside monophosphate of 6-meAdo has been isolated by high pressure liquid chromatography from extracts of Reid cells exposed to the compound, indicating that 6-meAdo is a substrate for adenosine kinase in these cells. Quantitation of phosphorylated derivatives of the methylated adenosine analogs in sensitive and resistant cell lines is underway in hopes that insight can be gained into the precise molecular mechanisms involved in the selective cytotoxicity.

Of the N^6-substituted adenosine analogs tested, 6-me$_2$Ado gave the greatest differential killing of trophoblastic tumor cells when compared to normal human fibroblasts. Even at high concentrations (up to 1 mM), the compound appeared to be only cytostatic to the fibroblasts, while it was cytotoxic to trophoblastic tumor cells at concentrations 100-fold less.

The toxicity of 6-me$_2$Ado was not reversed by exogenous purines or pyrimidines suggesting that the mode of action does not involve inhibition of *de novo* purine or pyrimidine biosynthesis as reported for adenosine [9] and certain adenosine analogs [8]. The precise mode of action by which 6-me$_2$Ado selectively kills trophoblastic tumor cells is under investigation. However, differential metabolism of the phosphorylated analogs in trophoblastic and non-trophoblastic cells may be involved, since resistant HeLa cells have adenosine kinase activity comparable to sensitive Reid cells (results not presented). Different metabolic pathways predominate in different cell types, which can lead to differential purine metabolism between varying cell types [24].

The cytotoxicity of 6-me$_2$Ado to trophoblastic tumor cells might provide a basis for chemotherapy. A number of non trophoblastic cells examined have been shown to be resistant to the effects of the compound. Although all normal cell types have not been tested for their resistance to 6-me$_2$Ado, preliminary results with animal models indicate that the nucleoside is not toxic. If trophoblastic tumors *in vivo* exhibit sensitivity to 6-me$_2$Ado similar to the tumor cells *in vitro*, the compound might be a selective chemotherapeutic agent for use in the treatment of trophoblastic neoplasia.

Acknowledgements—The authors thank Mrs. Vera Gasch for the isolation of chorionic tissue and Miss Patricia Poche for the culturing of cells. We also wish to thank Mrs. Evelyn Read for skilled technical assistance. We are grateful to Dr. John Brewer, Department of Obstetrics and Gynecology, Northwestern University, Chicago, and Dr. Charles August, Dr. Kenneth McIntosh, and Dr. Watson Bowes of this institution for their contribution of cell lines and tissues.

REFERENCES

1. A. Y. Divekar and M. T. Hakala, *Molec. Pharmac.* **7**, 663 (1971).
2. H. P. Schnebli, D. L. Hill and L. L. Bennett, *J. biol. Chem.* **242**, 1997 (1967).
3. R. E. Parks, Jr. and P. R. Brown, *Biochemistry* **12**, 3294 (1973).
4. H. T. Shigeura, S. D. Sampson and M. L. Meloni, *Archs Biochem. Biophys.* **115**, 462 (1966).
5. H. K. Slocum and M. T. Hakala, *Cancer Res.* **35**, 423 (1975).
6. M. H. Fleysher, M. T. Hakala, A. Bloch and R. H. Hall, *J. med. Chem.* **11**, 717 (1968).
7. A. Y. Divekar, M. H. Fleysher, H. K. Slocum, L. N. Kenny and M. T. Hakala, *Cancer Res.* **32**, 2530 (1972).
8. J. F. Henderson, C. M. Smith, F. F. Snyder and G. Zombor, *Ann. N.Y. Acad. Sci.* **255**, 489 (1975).
9. K. Ishii and H. Green, *J. Cell Sci.* **13**, 429 (1973).
10. J. W. Bynum and E. Volkin, *J. cell. Physiol.* **88**, 197 (1976).
11. R. A. Pattillo and G. O. Gey, *Cancer Res.* **28**, 1231 (1968).
12. P. O. Kohler and W. E. Bridson, *J. clin. Endocr. Metab.* **32**, 683 (1971).
13. R. A. Pattillo, G. O. Gey, E. Delfs and R. F. Mattingly, *Int. J. Obstet. Gynec.* **100**, 582 (1968).
14. R. Wilkinson and S. J. Kerr, *J. Virol.* **12**, 1013 (1973).
15. H. M. Kalckar, *J. biol. Chem.* **167**, 461 (1947).
16. B. K. Kim, S. Cha and R. E. Parks, Jr., *J. biol. Chem.* **243**, 1763 (1968).
17. T. S. Chan, K. Ishii, C. Long and H. Green, *J. cell. Physiol.* **81**, 315 (1973).
18. B. Bakay, M. A. Telfer and W. L. Nyhan, *Biochem. Med.* **3**, 230 (1969).
19. J. Paul, in *Cell and Tissue Culture*, p. 354. E. & S. Livingstone, Edinburgh (1970).
20. C. R. Ball, H. W. Van Den Berg and R. W. Poynter, in *Methods in Cell Biology* (Ed. D. M. Prescott), Vol. VII, p. 349. Academic Press, New York (1973).
21. R. P. Agarwal, S. M. Sagar and R. E. Parks, Jr., *Biochem. Pharmac.* **24**, 693 (1975).
22. R. W. Trewyn and S. J. Kerr, in *Onco-Developmental Gene Expression* (Eds W. H. Fishman and S. Sell), p. 101. Academic Press, New York (1976).
23. M. W. McBurney and G. F. Whitmore, *J. cell. Physiol.* **85**, 87 (1975).
24. T. S. Shenoy and A. J. Clifford, *Biochim. biophys. Acta* **411**, 133 (1975).