Toxicity of 3-Aminobenzamide to Chinese Hamster Cells Containing 5-Hydroxymethyluracil in Their DNA

Robert J. Boorstein, Dan D. Levy, and George W. Teebor

Departments of Pathology [R. J. B., G. W. T.] and Environmental Medicine [D. D. L.], New York University Medical Center, New York, New York 10016

ABSTRACT

V79 cells incorporated 5-hydroxymethyl-2'-deoxyuridine (HmdUrd) into their DNA linearly over a wide range of concentrations and time. Cells grew normally when 0.03% of thymidine residues were replaced with HmdUrd. At this level of substitution, 5-hydroxymethyluracil (HmUra) was removed from DNA at a rate of 30-40%/24 h. Concentrations of HmdUrd in the growth medium which produced higher levels of substitution reduced survival and caused cells to delay their transit through S phase. However, the treatment of HmdUrd-containing cells with 3-aminobenzamide caused extensive cell death. At levels of HmdUrd substitution compatible with near 90% survival, the addition of 3-aminobenzamide, an inhibitor of poly(adenosine diphosphoribose) synthesis, killed over 90% of the cells. This toxicity was not due to inhibition of the removal of HmUra from DNA. Cells killed by this combination of agents were arrested in the G2 phase of the cell cycle. We conclude that the toxicity of HmdUrd resulted primarily from the repair of the HmUra residue in DNA and not from any intrinsic toxicity of the HmUra residue itself. We also conclude that the cytotoxicity of 3-aminobenzamide resulted from interference with the completion of DNA repair following base (HmUra) excision. Since HmUra is also formed in DNA through the action of ionizing radiation, it may be among the components of radiation-induced DNA damage which sensitizes cells to 3-aminobenzamide.

INTRODUCTION

The chemically reactive oxidative species generated by ionizing radiation and activated leukocytes modify the bases of DNA (1-3). Among these modified bases is HmUra which is formed from thymine (4-7). HmUra is removed from DNA in vitro through the action of HmUra-DNA glycosylase which was first identified and partially purified from murine cells (8). Subsequently, HmUra-DNA glycosylase activity was measured in hamster and human cells and in various murine tissues (9). The repairability of HmUra suggests that it is deleterious to cells but, as yet, the nature of its effects on cell function are uncertain. HmUra can also be introduced into cellular DNA as a result of the incorporation of the nucleoside HmdUrd (10-12). HmdUrd has been reported to be toxic to cells in culture and to animals (11, 13, 14) but the mechanism of this toxicity is unknown. No specific inhibitory effect on DNA biosynthesis has been reported.

To study the effects of HmUra in DNA on cell growth, cells were exposed to increasing concentrations of HmdUrd in their growth medium to determine whether toxicity increased as a function of the number of HmUra residues in DNA. Since the HmUra residue could be radiolabeled, its disappearance from DNA could be followed as a measure of in vivo HmUra-DNA glycosylase-mediated repair.

3AB, an inhibitor of the synthesis of poly(ADP-ribose) (15), potentiates the toxicity of ionizing radiation (16-20). This potentiation of toxicity has been attributed to interference with DNA repair processes which require synthesis of poly(ADP-ribose) (21-24). The repairable DNA modification produced by ionizing radiation which sensitizes cells to 3AB has not yet been conclusively identified. Therefore, effects of 3AB on cells treated with HmdUrd were studied to determine whether the potentiation of radiation toxicity by 3AB might, at least in part, result from interference with the repair of radiogenically formed HmUra.

MATERIALS AND METHODS

5-HmUra purification. [6-3H]HmUra (9 Ci/mmol) was purchased from Moravek Biochemicals. Immediately prior to use, it was purified by HPLC on a 5-μm octadecylsilane C8 column (10 mm x 25 cm) using water as eluant. Radioactivity was detected by adding 10 ml of Redi-solv HP scintillation fluid to aliquots of 1-min (2-ml) fractions. Radioactive material eluting with the retention time of authentic HmUra (Sigma) was evaporated to dryness and redissolved in water. This HmUra was at least 99.6% pure with no detectable contaminating dUrd (less than 0.1%) when analyzed by HPLC using the above conditions.

Cell culture. Stocks of Chinese hamster V79 cells were grown in Ham's F-12 medium supplemented with 5% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were maintained in 75-cm² tissue culture flasks in a 5% CO² saturated incubator at 37°C. Cells were trypsinized every 3-4 days and divided at a ratio of 1:10 or 1:20 into fresh flasks. Fresh cultures were started from frozen stocks every 4-6 weeks.

Growth Curves. Cells were plated at concentrations of 5 x 10⁴/35-mm dish in complete DME unless otherwise indicated 24 h prior to the start of an experiment. At zero time, different concentrations of HmdUrd were added, and the cells were incubated for 24 h. The cells were washed twice with complete DME and then grown in the presence or absence of 3AB (Pfalz and Bauer). Cells were counted at 24-h intervals using a Model Zx Coulter Counter. Cells were prepared for counting by trypsinization followed by dilution in Isoton (Coulter).

Colony Formation Assays. Cells were plated in complete DME at densities of 5 x 10⁴/35-mm dish. HmdUrd was added 24 h after plating, and the cells were incubated for 24 h. The cells were then washed twice with trypsin-EDTA ( Gibco) and incubated for 2 min in 1 ml trypsin-EDTA. They were then diluted and plated in triplicate at concentrations of both 100 cells/60-mm dish and 1000 cells/60-mm dish in complete DME. 3AB was then added to the cells, which were then incubated for 24 h. The cells were then washed and refed with complete DME. After 10 days, the cells were fixed in a solution of 10% formalin in normal saline and stained with crystal violet. Colonies of greater than 30 cells were counted. Control plating efficiencies ranged from 90 to 100% when 100 cells were plated. The number of surviving colonies in drug treated plates was expressed as a percentage of colonies in untreated controls. For conditions in which survival was less than 15% on the dishes plated at 100 cells/plate, survival was determined from the dishes plated at 1000 cells/plate.

Received 1/5/87; revised 5/21/87; accepted 5/27/87.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Grants ES 03847 (G. W. T. and R. J. B.) and CA 16669 (G. W. T.) from the NIH and by Postdoctoral Fellowship PP 2893 (R. J. B.) from the American Cancer Society. Preliminary accounts of this work were presented at the 35th Annual Meeting of the Radiation Research Society, Atlanta, GA, February 1987, and at the Second International Conference on Anticarcinogenesis and Radiation Protection, Gaithersburg, MD, March 1987.

To whom requests for reprints should be addressed, at Department of Pathology, Room 605 MSB, NYU School of Medicine, 550 First Avenue, New York, NY 10016.

The abbreviations used are: HmUra, 5-hydroxymethyluracil; HmdUrd, 5-hydroxymethyl-2'-deoxyuridine; 3AB, 3-aminobenzamide; HPLC, high-performance liquid chromatography; complete DME, Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin; TCA, trichloroacetic acid; dThd, deoxythymidine; dUrd, deoxyuridine; dCyd, deoxycytidine.

4372
Flow Microfluorometry. Cells were grown at the same density as for growth curves but in 60-mm dishes (10^6 cells/dish). At the times indicated, cells were prepared for fluorometric analysis by washing and suspending in a 50-μg/ml solution of propidium iodide in 0.1% sodium citrate (25). They were then analyzed on a Model 50H Cytofluorograf equipped with a Model 2150 computer (Ortho Diagnostics). Flow microfluorometry patterns were plotted on arbitrary scales of cell number versus fluorescence (DNA content). Histograms were obtained to display 1000 cells in the channel containing the largest fraction of cells. Relative G1, S, and G2-M populations expressed as percentages of the total were determined using the Quickfit computer program (Ortho Diagnostics).

Measurement of [3H]HmdUrd Incorporation. Cells (5 × 10^6) were plated in DME in duplicate in parallel with dishes for cell growth and colony formation assays. After 24 h, medium was removed, and 1 μCi of [3H]HmdUrd in 1 ml of medium was added. Additional nonradioactive HmdUrd was added to make final concentrations of HmdUrd between 0.1 and 100 μM. After 24 h, cells were washed three times with 1 ml of Hanks' balanced salt solution (Gibco) (4°C), once with 1 ml of 10% TCA, and then left in 1 ml of 10% TCA for 15 min at 4°C. The TCA was removed and 1 ml of 0.2 N NaOH was added to each dish. After 30 min at 37°C, 0.5-ml aliquots were counted in 10 ml of scintillation fluid in a liquid scintillation counter. HmdUrd incorporation per dish was determined by dividing the [3H]HmdUrd incorporated per dish by the specific activity of the [3H]HmdUrd in the growth medium.

The amount of HmdUrd incorporated per cell was determined by dividing the HmdUrd incorporation per dish by the number of cells in additional dishes treated equivalently with HmdUrd for 24 h. These additional dishes for cell number determination were treated identically to those used for HmdUrd incorporation except that [3H]HmdUrd was replaced with an equivalent amount of nonradioactive HmdUrd. To calculate the level of HmUra substitution relative to thymine, it was assumed that V79 cells contain 6 pg DNA/cell, 30% of which is dThd (26).

In these experiments, less than 1% of radioactivity added to the medium was incorporated into DNA. As a control of the ability of cells to incorporate exogenous nucleoside, parallel cultures were incubated with final concentrations of 0.2 to 0.8 μM [2-14C]dThd (50 mCi/mmol), essentially all of which was incorporated into DNA during a 24-h labeling period. Our results are consistent with previous results of Kahilainen et al. (11) who showed that human leukemia cells incorporated dThd into DNA in preference to HmdUrd by a factor of 42.

Measurement of [3H]HmdUrd Incorporation and Repair. Cells (2 × 10^6) were plated per 35-mm dish. After 24 h, the cells were exposed to 0.01 μCi [2-14C]dThd/2 ml medium to uniformly prelabel them. They were then washed with complete DME and [3H]HmdUrd was added in fresh medium. After 24 h, the HmdUrd was removed, and the cells were washed twice with complete medium. They were then reincubated in complete medium in the presence of 3AB or equal volumes of phosphate-buffered saline (control). Amounts of TCA-precipitable H and 14C radioactivity were determined at indicated times as above. The loss of 14C radioactivity (HmUra) relative to 14C radioactivity (thymine) was considered to be the result of cell death while the loss of H radioactivity relative to 14C radioactivity was considered to result from specific repair.

Definitive Identification of Incorporated Nucleosides. Cells (2 × 10^6) were plated in 150-cm² flasks in DME. After 24 h, one-half of the flask was treated with 0.1 μCi [2-14C]dThd to prelabel the cellular DNA. After an additional 24 h, the cells were washed and labeled with 10^{-5} M [3H]HmdUrd (9 Ci/mmol). After 24 h, the cells were rinsed with 4 ml trypsin-EDTA per flask and incubated for 2 min in 4 ml trypsin-EDTA. The cells were trituated to break up clumps, and 6 ml of complete medium were added to neutralize the trypsin. The cells were centrifuged and then washed three times with Hanks's balanced salt solution. The DNA was extracted and enzymatically digested to 2'-deoxynucleosides (7). Authentic dUrd, HmdUrd, and dThd were added as UV-absorbing markers. HPLC analysis was then performed on a 5-μm Ultrasphere analytical octadecylsine C18 column (4.6 mm x 25 cm) using 0.1 M ammonium formate, pH 6.5, as an initial eluant at a flow rate of 1 ml/min. After 40 min, a gradient to 100% acetonitrile was begun to elute dThd. Radioactivity was measured with a Radiomatic Beta IC radioactivity flow detector. The ratio of scintillant (Flo-Scint 2; Radiomatic) to eluant was 4:1.

RESULTS

Incorporation and Toxicity of HmdUrd. V79 cells grown for 24 h in medium containing [3H]HmdUrd incorporated 3H into TCA-precipitable material over a wide range of concentrations (Table 1). This incorporation was nearly linear, falling off at high concentrations which produced up to 10–20% substitution of thymine in DNA by HmUra. Cell survival as measured by colony formation assays (Table 1) was not inhibited at levels of substitution of 1 HmUra per 3600 thymine residues, indicating that hamster cells can tolerate large amounts of HmUra in their DNA.

Further evidence that the cells tolerated high levels of substitution with HmdUrd was provided by flow microfluorometric analysis. Cells treated with 0.1–0.4 μM HmdUrd showed no significant change in cell cycle distribution from untreated control cells. However, cells treated with higher doses (1 μM) showed a marked increase in the percentage of cells in late S phase (Fig. 1).

To confirm that the radioactive material incorporated into TCA-precipitable material was exclusively [3H]HmdUrd in DNA, cells were labeled with [3H]HmdUrd. The cellular DNA was extracted and digested to 2'-deoxynucleosides. When this digest was analyzed by HPLC, the only peak of 3H-containing material coeluted with authentic UV marker HmdUrd (Fig. 2). No detectable radioactive material coeluted with dCyd, dUrd, or dThd. The UV-absorbing material at the front represents absorption by acetone salts (7). To confirm

<table>
<thead>
<tr>
<th>Table 1 Incorporation and toxicity of HmdUrd</th>
</tr>
</thead>
<tbody>
<tr>
<td>HmdUrd (μM)</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 1. Cytofluorometric analysis of V79 cells treated with HmdUrd. Cells were not treated (---) or were treated for 24 h with 0.1 μM HmdUrd (-----), 0.4 μM HmdUrd (-----), or 1 μM HmdUrd (-----), and analyzed as described in "Materials and Methods." Ordinate, relative cell number; abscissa, DNA content where the initial major peak indicates cells with a G1 DNA content. Data are from a representative determination.
that the extracted DNA was representative of total DNA, cells were prelabeled with [2-14C]dThd prior to labeling with [3H]-HmdUrd. The [14C]/dThd/[3H]HmdUrd ratio in DNA as determined by HPLC was the same as the 14C/3H ratio in TCA-precipitable material.

Removal of HmdUrd from DNA. To measure the overall removal rate of HmUra from DNA, V79 cells were treated with nontoxic concentrations of [3H]HmdUrd. To distinguish repair from alternate causes for the loss of 3H-containing material from the DNA of HmdUrd-treated cells such as cell death, the cellular DNA was prelabeled with [14C]dThd. During the 2 days following the removal of HmdUrd from the growth medium, 30–40% of the [3H]HmUra was removed per day (Fig. 3). In these experiments, the amount of 14C declined less than 10% during the course of the experiment. Control cells pretreated with [14C]dThd but not treated with [3H]HmdUrd lost a similar amount of TCA-precipitable 14C during the same time period. Therefore, we concluded that the loss of 3H from these cells reflected the specific removal of [3H]HmUra from [3H]HmdUrd-containing DNA.

Effects of 3AB on Toxicity and Repair of HmdUrd. 3AB substantially enhanced the lethality of HmdUrd in V79 cells as assayed by colony formation (Fig. 4). Doses of HmdUrd which were minimally toxic (0.1–0.4 μM) were made up to 95% toxic by subsequent treatment of the cells with 4 mM 3AB. By itself 4 mM 3AB was nontoxic and is known to have relatively little effect on other cellular functions (27, 28). A lower concentration of 3AB (1 mM) also increased cell killing of HmdUrd-treated cells.

Similar effects were seen when cell growth following HmdUrd treatment was examined. 3AB had a marked inhibitory effect on the proliferative ability of cells pretreated with 0.1–1.0 μM HmdUrd (Fig. 5). The same concentrations of 3AB had no effect on the growth of untreated cells. Therefore, V79 cells could tolerate significant levels of HmdUrd in their DNA, they were unable to tolerate such levels of substitution when subsequently grown in the presence of 3AB.

To determine whether 3AB affected the initial step of removal of HmUra from DNA, V79 cells, which were prelabeled with [14C]dThd, were treated with indicated concentrations of [3H]HmdUrd for 24 h as described in “Materials and Methods.” The HmdUrd was removed, and the cells were incubated for an additional 24 h in the presence or absence of 4 mM 3AB. The loss of 3H radioactivity relative to 14C radioactivity during this interval was determined and expressed as the percentage of HmdUrd.
TOXICITY OF HmdUrd

Fig. 6. Cytofluorometric analysis of V79 cells treated with HmdUrd and 3AB. Cells were untreated (---) or were treated for 24 h with 0.1 mM HmdUrd (-----) or 0.4 mM HmdUrd (----). The cells were washed and 0 mM 3AB (A), 1 mM 3AB (B), or 4 mM 3AB (C) was added as in Fig. 5. After an additional 24 h, the cells were analyzed for cell cycle distribution as described in “Materials and Methods.” Ordinate, relative cell number; abscissa, DNA content where the initial major peak indicates cells with a G1 DNA content. Data are from a representative determination.

Fig. 7. Changes in G2 DNA content in V79 cells treated with HmdUrd and 3AB. V79 cells were treated with HmdUrd followed by 0 mM 3AB (•), 1 mM 3AB (○), or 4 mM 3AB (△), as in Fig. 6. Histograms of DNA content were obtained and G2 DNA content determined. Data are averaged from two experiments. The range of variation between experiments was less than 10%.

population in cells exposed to ionizing radiation (17). In our experiments, 3AB alone had no effect (Fig. 6A), but 3AB added to HmdUrd-treated cells caused a large increase in the fraction of cells in the G2 compartment (Fig. 6C). Furthermore, the increase in the number of cells in G2 (Fig. 7) correlated with the decreased colony-forming ability of cells treated in the same manner (Fig. 4). Our current experiments show that the presence of a single type of DNA modification can both sensitize cells to 3AB toxicity and cause a significant G2 arrest.

DISCUSSION

We have shown here that substitution at the level of 1 HmUra/150 thymine residues was cytotoxic and arrested cells in late S or early G2. However, HmUra in DNA at levels of substitution of 1 HmUra/3600 thymine residues was not a substantial block to replication. The growth rate, colony-forming ability, and cell cycle distribution of V79 cells treated with HmdUrd to produce this level of substitution were unchanged when compared to untreated control cells.

HmdUrd was incorporated into DNA without modification of the base moiety. It has been reported previously that HmdUrd is metabolized to dThd in humanleukemia cells (11), a result not duplicated here in V79 cells (Fig. 2). [3H]HmdUrd was purified by HPLC immediately prior to use to remove any contaminating radioactive dUrd. We suggest that contamination of radioactive HmdUrd with a small amount of radioactive dUrd, the starting material for the synthesis of HmdUrd (11, 30), would lead to significant incorporation of radioactively labeled material as dThd.

Our experiments provide the first evidence that HmUra is actively removed from cellular DNA. The similarity of the rates of in vivo repair in cells in which DNA was substituted with from 1–4 HmUra residues/106 thymines residues (Fig. 3) indicates that repair was not saturated at these levels of substitution.

The results of our experiments are consistent with, but do not prove, the hypothesis that it is the enzymatic removal of HmUra from DNA, not the presence of HmUra in DNA, which is primarily responsible for the toxicity of HmdUrd. Cells tolerated high levels of substitution, up to 1 HmUra/1000 thymine residues, and still replicated normally, removing HmUra from their DNA at a slow rate. However, toxic effects were evident at higher levels of substitution of HmUra in DNA. This might be due to the excision of HmUra residues at close proximity on opposite DNA strands, followed by apurinic/apyrimidinic endonucleolytic incision and exonucleolytic digestion, producing lesions that are essentially lethal double strand breaks. A similar mechanism of toxicity has been proposed for radiogenically formed single strand breaks that are in close proximity on complementary DNA strands (31). An additional contribution to HmdUrd toxicity may have resulted from the cyclic incorporation of HmdUrd into DNA repair patches followed by removal of HmUra by the DNA glycosylase and further excision repair. Such a mechanism is similar to that proposed for the action of methotrexate in which uracil residues in DNA are continuously being excised through the action of uracil-DNA glycosylase (32).

These experiments do not support but cannot exclude the hypothesis that HmUra toxicity results primarily from the formation of HmUra-protein cross-links (10). If the yield of such cross-links were high, it is unlikely that cells could tolerate 1 HmUra residue/1000 thymines. The fact that [3H]radioactivity was recovered only as the nucleoside HmdUrd suggests that few, if any, stable HmdUrd-amino acid products were formed. Of course, it is possible that a small number of cross-links were formed between HmUra in DNA and cellular protein, contributing to cellular toxicity. When HmUra was produced in the DNA of HeLa cells by ionizing radiation, the level of substitution was 1.2 HmUra/106 thymines/0.01 Gy (7). V79 cells
tolerated amounts of HmUra in their DNA equal to that formed by 5000 Gy. Therefore, HmUra in DNA cannot, by itself, contribute significantly to the toxic effects of ionizing radiation, since such radiation kills cells at doses 1000 times lower.

These experiments have also tested the hypothesis that the lethality of 3AB results from interference with the completion of excision repair of DNA base adducts which are themselves nonlethal (28). 3AB inhibits the synthesis of poly(ADP-ribose) which may be necessary for normal ligation of DNA to occur during excision repair (33, 34). HmdUrd-treated cells are a good model system in which to study the function of poly(ADP-ribose) in base excision repair because high levels of one type of repairable modified base may be introduced into cellular DNA. In addition, in contrast to alkylated purines which readily undergo ring opening or depurination, HmdUrd is relatively stable (35).

Cells treated with HmdUrd were sensitized to 3AB to a greater extent than was reported for cells exposed to alkylating agents or ionizing radiation (15, 18, 28, 34, 39–41). This is probably because much higher levels of repairable DNA lesions (HmUra residues) were achieved without the toxicity which accompanies treatment of cells by those other agents. Current models explaining 3AB action propose that poly(ADP-ribose) is synthesized in response to strand breakage mediated by repair endonucleases (28, 36, 37). The fact that HmUra was removed at a normal rate in the presence of 3AB (Table 2) supports this hypothesis. If HmUra-DNA glycosylase activity were inhibited by 3AB and the base remained on the DNA backbone, apurinic/apyrimidinic endonuclease action could not take place and the stimulus to poly(ADP-ribose) synthesis would not occur.

The results of our experiments do not support alternate models for the mechanism of 3AB toxicity which suggest that, directly or indirectly, 3AB activates nucleases which attack damaged DNA in a nonspecific fashion (29, 38). According to this model, nucleases, which are normally inhibited by poly(ADP-ribose), are activated as the result of alkylating agent damage to lysosomes. Since HmdUrd probably does not react with lysosomal proteins or lipids as do reactive alkylating agents, our data support the hypothesis that poly(ADP-ribose) is necessary for the completion of DNA base excision repair. It is still an open question whether poly(ADP-ribose) is necessary for the action of DNA ligase or whether it participates in the completion of DNA repair in another manner.

Irradiation has also been shown to lower cellular NAD concentration, stimulate poly(ADP-ribose) synthesis, and sensitize cells to 3AB (18, 39–41). However, in general, the cytotoxic effects of 3AB on irradiated cells have been found to be modest (15, 34). Most investigators have been forced to use highly toxic doses of radiation producing 90–99.9% lethality and very high concentrations of 3AB to detect significant effects (18, 39). We suggest that interference with repair of radiogenically formed HmUra may contribute to the potentiation of radiation toxicity caused by 3AB. The reason one must use high doses of radiation to observe 3AB sensitization is that the yield from ionizing irradiation of repairable DNA base modifications is relatively small (42).

In summary, the HmUra moiety in DNA is minimally toxic. Therefore, the reason for its repairability is still uncertain. The toxicity of the nucleoside HmdUrd probably results from repair of large numbers of HmUra residues in DNA. 3AB, an inhibitor of poly(ADP-ribose) synthesis, greatly enhances killing of cells containing HmdUrd without interfering with the enzymatic removal of HmUra from DNA. We conclude that this is strong evidence for the participation of poly(ADP-ribose) in base excision repair. We also suggest that repairable base modifications such as HmUra may be among those lesions formed by ionizing radiation which sensitize cells to the toxic effects of inhibitors of poly(ADP-ribose) synthesis.

ACKNOWLEDGMENTS

We thank Dr. G. Fredrickson for performing flow cytometric analysis and Dr. A. Pardee, Dr. D. Boothman, and Dr. K. Frenkel for their critical and careful reading of the manuscript.

REFERENCES


Toxicity of 3-Aminobenzamide to Chinese Hamster Cells Containing 5-Hydroxymethyluracil in Their DNA

Robert J. Boorstein, Dan D. Levy and George W. Teebor


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/16/4372