Induction of Plasminogen Activator by Alkylating Agents in a Repair Defective Human Glioblastoma Cell Strain

Branko Brdar
Central Institute for Tumors and Allied Diseases, Ilica 197, 41000 Zagreb, Yugoslavia

ABSTRACT

Alkylating agents, mechlorethamine and N-methyl-N'-nitro-N-nitrosoguanidine, induce the production of plasminogen activator in U-87MG cells, an alkylation DNA repair deficient (Mer−) human glioblastoma strain. Enzyme induction was observed, however, in U-178MG and SH-101 cells, alkylrepair proficient (Mer+) glioblastoma strains, or in HeLa cells, which reactivated and supported well the growth of alkylated damaged adenovirus 3. In the alkylrepair defective U-87MG strain, enhanced production of plasminogen activator occurred in a narrow concentration range of treatment with either alkylating agent, causing a 20 to 50% inhibition of [3H]thymidine incorporation. Maximum plasminogen activator induction was observed between 32 and 48 h after alkylrepair treatment and the levels of enzyme produced were 5 to 10 times those of untreated control levels. This alkylrepair dependent enzyme induction required protein synthesis for it did not occur in the presence of cycloheximide. It was hence concluded that plasminogen activator induction in alkylrepair deficient human cells is caused by unrepaired DNA damage and that it may represent an eukaryotic SOS-like function. In addition, plasminogen activator induction may be useful as a sensitive assay for the identification of alkylrepair deficient human tumors for which the susceptibility to alkylrepair chemotherapy should be expected to increase.

INTRODUCTION

As recently reported, physical and chemical agents which damage DNA induce PA2 synthesis in a variety of embryonic cells (1) (alkylating agents, UV) and in skin fibroblasts of XP (2) (UV); PA is a specific serine protease closely associated with DNA repair which may be classified as an eukaryotic SOS-like function. Several SOS-like (4) activities of cycloheximide. It was hence concluded that plasminogen activator induction required protein synthesis for it did not occur in the presence of cycloheximide. It was hence concluded that plasminogen activator induction in alkylrepair deficient human cells is caused by unrepaired DNA damage and that it may represent an eukaryotic SOS-like function. In addition, plasminogen activator induction may be useful as a sensitive assay for the identification of alkylrepair deficient human tumors for which the susceptibility to alkylrepair chemotherapy should be expected to increase.

INTRODUCTION

As recently reported, physical and chemical agents which damage DNA induce PA2 synthesis in a variety of embryonic cells (1) (alkylating agents, UV) and in skin fibroblasts of XP (2) (UV); PA is a specific serine protease closely associated with DNA repair which may be classified as an eukaryotic SOS-like function. Several SOS-like (4) activities

RESULTS AND DISCUSSION

The effect of HN2 and MNNG on PA production by two human glioblastoma cell strains, U-87MG and U-178MG, was examined. U-87MG cells, unlike U-178MG cells, support the growth of HN2 damaged DNA viruses (adenovirus 3) poorly (Fig. 1). At 10 mM HN2 the residual repair capacity of U-87MG cells was 30% while that of U-178MG cells was 70%. Such DNA repair deficiency of the U-87MG strain in reactivating alkylated damaged virus was previously designated as the “Mer−” phenotype (MNNG damage repair minus) (6). It suggests the diminished ability of U-87MG cells, as compared to the U-178MG cells, to recombine alkylated DNA.
Fig. 1. Host cell reactivation of HN2 treated adenovirus 3 by human tumor cell strains. Purified human adenovirus 3 was diluted 1:100 in 0.3 M Tris, pH 8.1, and treated with the indicated concentrations of HN2 by incubation at 37°C for 30 min. The virus plaque forming ability was then determined on confluent monolayers of the cell strains indicated and expressed as percentage of survival of untreated virus. Each point is the average of four values. (O) U-87MG; (A) U-178MG; (•) SH-101; (■) HeLa.

Fig. 2. Effect of HN2 or MNNG on intra- and extracellular PA production in human glioblastoma cell strains. U-87MG (Mer') and U-178MG (Mer') strains were seeded at 2 x 10⁵ cells/100-mm Petri dish. After 24 h of incubation indicated concentrations of HN2 or MNNG were added to some cultures, other serving as controls. The cultures were incubated for 2 h, washed twice with Dulbecco’s modified Eagle’s medium, and then incubated for a further 46 h with drug free Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum or 5% plasminogen-depleted fetal bovine serum. Monolayers and conditioned medium were collected, cell lysates were prepared with 0.3% Triton in 0.1 M Tris-HCl (pH 8.1), and protein was determined and assayed for intra- and extracellular PA. (O) intracellular PA, U-87MG cells; (■) extracellular PA, U-87MG cells; (□) intracellular PA, U-178MG cells; (△) extracellular PA, U-178MG cells.

Fig. 3. Kinetics of PA production in HN2-treated human glioblastoma cell strains; effect of cycloheximide. U-87MG and U-178MG cells were cultured and treated with 10 μM HN2 as in Fig. 2. Following exposure to HN2 all cultures were incubated further in fresh medium, one group receiving cycloheximide (2 μg/ml) and a second group serving as control. Dishes were removed at the indicated times; cells were collected, lysed, and analyzed for intracellular PA. (O) HN2, U-87MG; (□) control, U-87MG; (△) HN2, cycloheximide, U-87MG; (■) HN2, U-178MG; (△) control, U-178MG; (□) HN2, cycloheximide, U-178MG.

Fig. 4. Drug-induced PA synthesis in U-87MG cell strains. Cells were cultured in drug free Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum or 5% plasminogen-depleted fetal bovine serum. Monolayers and conditioned medium were collected, cell lysates were prepared with 0.3% Triton in 0.1 M Tris-HCl (pH 8.1), and protein was determined and assayed for PA. Treatments: (O) HN2, U-87MG; (□) control, U-87MG; (△) HN2, cycloheximide, U-87MG; (■) HN2, U-178MG; (△) control, U-178MG; (□) HN2, cycloheximide, U-178MG.

HN2 may be due to its inhibitory effect on DNA synthesis and/ or to inactivation of PA coding genes. Indeed, a 4-h exposure of U-87MG or U-178MG cells to 12.5 μM HN2 caused a suppression of DNA synthesis by 67 and 72%, respectively (not shown). Under these experimental conditions the cells remained attached in the monolayer for at least 48 h, although HN2 partly inhibited cell division and total protein synthesis.

The kinetics of PA production by HN2 (10 μM) treated U-87MG (Mer') and U-178MG (Mer') strains is given in Fig. 3. The level of PA activity in drug treated U-87MG cells did not significantly change during 24 h and then rose at 32 and 48 h to values at least 5 times as high as those in the untreated control. In the same time the U-178MG strain did not produce elevated levels of PA. As compared to U-87MG cells, PA induction occurred neither in the SH-101 glioblastoma cell strain nor in HeLa cells (Fig. 4), both being alkylation repair competent strains (Fig. 1). PA synthesis, however, was enhanced in chicken embryo fibroblasts, following exposure to between 10 and 50 μM HN2 (Fig. 4). This matches the observation that PA production is inducible in cultured embryonic cells of different origin after UV irradiation or alkylation treatment (1). We have no explanation why alkylation repair deficient U-87MG strain and embryonic cells responded alike to alkylation treatment by enhanced PA synthesis, but it is of interest to note that maximum enzyme induction in U-87MG occurred at lower HN2 concentrations than in chicken embryo fibroblasts.

The enhanced PA production by HN2 treated U-87MG cells required protein synthesis for it did not occur in the presence of cycloheximide (Fig. 3). This indicates that alkylation mediated induction of PA is genetically regulated involving gene translation. The expression of several other SOS-like functions in mammalian cells also requires protein synthesis. These are induced viral reactivation (7, 8), induction of virus mutagenesis by UV, and enhanced postreplication repair (10, 21).

PA synthesis is enhanced between 32 and 48 h after exposure to U-178MG cells (Mer'), to repair DNA lesions caused by MNNG involving O6-mGua residues (19). DNA lesions caused by HN2 also include methylated DNA bases (20).

As shown in Fig. 2, measurable induction of both intra- and extracellular PA production occurred only in U-87MG cells following a 48-h exposure to a 2.5 to 10 μM concentration range of both HN2 and MNNG and resulted in a more than 5-fold increase of PA control levels. The U-178MG strain produced slightly elevated levels of PA following treatment with either HN2 or MNNG rising to a maximum at alkylation fluences 4 times higher than those causing maximal PA induction in U-87MG cells. This matches the observation (2) that the UV fluences required for PA induction in a variety of XP complementation groups increase as a function of their residual repair capacity. The decline in PA production above 10 μM HN2 may be due to its inhibitory effect on DNA synthesis and/or to inactivation of PA coding genes. Indeed, a 4-h exposure of U-87MG or U-178MG cells to 12.5 μM HN2 caused a suppression of DNA synthesis by 67 and 72%, respectively (not shown). Under these experimental conditions the cells remained attached in the monolayer for at least 48 h, although HN2 partly inhibited cell division and total protein synthesis.

The kinetics of PA production by HN2 (10 μM) treated U-87MG (Mer') and U-178MG (Mer') strains is given in Fig. 3. The level of PA activity in drug treated U-87MG cells did not significantly change during 24 h and then rose at 32 and 48 h to values at least 5 times as high as those in the untreated control. In the same time the U-178MG strain did not produce elevated levels of PA. As compared to U-87MG cells, PA induction occurred neither in the SH-101 glioblastoma cell strain nor in HeLa cells (Fig. 4), both being alkylation repair competent strains (Fig. 1). PA synthesis, however, was enhanced in chicken embryo fibroblasts, following exposure to between 10 and 50 μM HN2 (Fig. 4). This matches the observation that PA production is inducible in cultured embryonic cells of different origin after UV irradiation or alkylation treatment (1). We have no explanation why alkylation repair deficient U-87MG strain and embryonic cells responded alike to alkylation treatment by enhanced PA synthesis, but it is of interest to note that maximum enzyme induction in U-87MG occurred at lower HN2 concentrations than in chicken embryo fibroblasts.

The enhanced PA production by HN2 treated U-87MG cells required protein synthesis for it did not occur in the presence of cycloheximide (Fig. 3). This indicates that alkylation mediated induction of PA is genetically regulated involving gene translation. The expression of several other SOS-like functions in mammalian cells also requires protein synthesis. These are induced viral reactivation (7, 8), induction of virus mutagenesis by UV, and enhanced postreplication repair (10, 21).

PA synthesis is enhanced between 32 and 48 h after exposure
Fig. 4. Effect of HN2 on intracellular PA production in alkylation repair proficient cell strains. Chicken embryo fibroblasts (CEF), SH-101, and HeLa cells were cultured and treated with HN2 as in Fig. 2. Following exposure to indicated concentrations of HN2 all cultures including controls were incubated in fresh medium for 46 h. Dishes were then removed; cells were collected, lysed, and analyzed for intracellular PA ("Materials and Methods"), except that the PA activity by chicken fibroblast lysates was determined in the presence of chicken plasminogen.

of Mer− U-87MG cells or chick embryonic fibroblasts to alkylating agents (Fig. 3). Interestingly, this appearance of PA production is concomitantly related both to the onset of DNA repair in alkylation treated Mer+ glioblastoma strains (19) and to alkylation induced viral reactivation in HeLa cells (9). In addition, enzyme induction occurred at drug concentrations which both suppressed [3H]thymidine incorporation by 30 to 50% and impaired the DNA repair functions (19). Hence, MNNG or HN2 treated Mer− cells apparently have a diminished capability to remove O2mGua residues, due apparently to a lack or depletion of O2mGua DNA methyltransferase which is normally present in Mer+ strains (19). This does not exclude some other DNA lesions produced by a variety of other chemical reactions which can result in PA induction.

It is not clear how un repaired DNA damage causes PA induction. Our experiments indicate that blockage of DNA synthesis by DNA damage is not responsible for induced PA synthesis. Thus, PA induction by HN2 occurred throughout the cell cycle of chick fibroblasts (not shown),3 suggesting that some conformational changes induced in DNA by alkylation damage, rather than suppressed DNA synthesis, may be responsible for enzyme induction.

In conclusion, our results suggest that un repaired alkylation DNA damage is causally related to PA induction which may represent an eukaryotic SOS-like function. To determine whether PA induction provides a sensitive assay for identification of DNA repair deficiencies, a number of alkylation deficient cell strains should be assayed for alkylation mediated PA induction, and this is the subject of a separate study.

ACKNOWLEDGMENTS

I am grateful to Dr. Jørgen Fogh for kindly supplying cell strains. I also thank Bosiljka Šikić and Marija Taiber for their expert technical assistance.

REFERENCES

Induction of Plasminogen Activator by Alkylating Agents in a Repair Defective Human Glioblastoma Cell Strain

Branko Brdar


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/5/2282

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.