Strahlentherapie
und Onkologie

Originalarbeit

^{211}At-α-Dose Dependence of Poly-ADP-Ribosylation of Human Glioblastoma Cells in Vitro

Suitability in Cancer Therapy?

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Aim: It was intended to test the biological response (poly-ADP-ribosylation of cellular proteins) of α-particles from extracellular ^{211}At for enhanced damage to human glioblastoma cells in vitro and to discuss its suitability for potential application in therapy of high-grade gliomas.
Materials and Methods : Confluent cultures of human glioblastoma cells were exposed to different doses of α-radiations from homogeneously distributed extracellular ^{211}At. Cellular poly-ADP-ribosylation of all proteins including histones was monitored since it is an indirect but sensitive indicator of chromatin damage and putative repair in both normal and malignant mammalian cells.
Results: A significant diminution (average 85.6%) in poly-ADP-ribosylation of total cellular proteins relative to that for non-irradiated glioblastoma cells was observed following 0.025 to 1.0 Gy α-radiations. In the dose range of 0.0025 to 0.01 Gy there was an increase with a maximum value of approximately 119.0% at 0.0025 Gy. Below 0.0025 Gy no change in poly-ADP-ribosylation was observed.
Conclusions: Level of cellular poly-ADP-ribosylation of proteins at 0.025 to 1.0 Gy of α-radiation dose from ^{211}At appears to cause enhanced damage by creating molecular conditions which are not conducive to repair of DNA damages in human glioblastoma cells in vitro. Therefore, it is assumed that clinical application of ^{211}At at least in this dose range might enhance clinical efficacy in radiotherapy of cancer.

Key Words: ^{211}At-α-radiation · Glioblastoma cells in vitro · Poly-ADP-ribosylation · Radiotherapy

^{211}At-α-Dosis-Abhängigkeit der Poly-ADP-Ribosylierung von menschlichen Glioblastomzellen in vitro. Einsetzbar in der Krebstherapie?

Ziel: Es war die Absicht, die biologische Reaktion (Poly-ADP-Ribosylierung zellulärer Proteine) menschlicher Glioblastomzellen in vitro auf verstärkte Schadensbildung durch α-Teilchen von extrazellulärem ^{211}At zu testen und deren Berücksichtigung für eine potentielle Anwendung in der Therapie von malignen Glioblastomen zu diskutieren.
Material und Methode: Konfluente Kulturen menschlicher Glioblastomzellen wurden unterschiedlichen α-Dosen von homogen verteiltem extrazellulärem ^{211}At ausgesetzt. Die zelluläre Poly-ADP-Ribosylierung aller Proteine, einschließlich die der Histone, wurde bestimmt, da sie ein indirekter, aber empfindlicher Indikator für Chromatinschäden und mutmaßlich für die Reparatur in normalen und entarteten Zellen ist.
Ergebnisse: Eine signifikante Verringerung (durchschnittlich 85,6%) der Poly-ADP-Ribosylierung aller zellulären Proteine, relativ zu der der nichtbestrahlten Glioblastomzellen, wurde nach 0,025 bis 1,0 Gy α-Bestrahlung beobachtet. Im Dosisbereich von 0,0025 bis 0,01 Gy gab es einen Anstieg mit einem maximalen Wert von angenähert 119% bei 0,0025 Gy. Unterhalb von 0,0025 Gy wurde keine Änderung der Poly-ADP-Ribosylierung beobachtet.
Schlußfolgerungen: Das Niveau poly-ADP-ribosylierter Proteine im ^{211}At-α-Dosisbereich von 0,025 bis 1,0 Gy scheint eine erhöhte Schädigung dadurch zu bewirken, daß molekulare Bedingungen geschaffen werden, die der Reparatur von DNA-Schäden in menschlichen Glioblastomzellen in vitro nicht förderlich sind. Daher wird angenommen, daß die klinische Anwendung von ^{211}At – zumindest in diesem Dosisbereich – die Wirksamkeit der Radiotherapie von Krebs steigern könnte.

Schlüsselwörter: ^{211}At-α-Strahlung · Glioblastomzellen in vitro · Poly-ADP-Ribosylierung · Strahlentherapie

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High-grade gliomas are the most commonly occurring primary neurological neoplasm in the adult population [20]. Regardless of therapy, their overall prognosis is dismal [17]. Fewer than 10% of patients with glioblastoma multiforme survive 2 years beyond diagnosis [4]. Although radiotherapy has long been a valuable adjunct to surgery in the clinical management of glioblastoma, such tumors have generally been found intrinsically radioresistent [4, 10, 14, 18, 25]. Tumor hypoxia, DNA repair, rapid cell proliferation and repopulation, all pose a formidable challenge to the therapeutic efficacy of usual low-LET radiations [3, 11]. It is recognized that high-LET quality radiations can potentially minimize or obviate the radiobiological limitations of conventional radiotherapy [3, 11], though clinical experience has been mixed [3, 8, 9, 13, 15]. Despite these facts, findings suggest that a cure may be achieved through the selective internalized targeting of high-LET radiations, such as α-particles, to glioblastoma, thereby effecting significant localized energy deposition in neoplastic cells with minimal irradiation of surrounding normal tissue. Although there is a paucity of suitable α-emitting isotopes, ^{212}Bi and ^{211}At have engendered much interest as potential endoradiotherapeutic agents [5–7, 12, 24]. In particular, the radiohalogen ^{211}At, with a half-life of 7.21 hours has proved an eminently suitable radionuclide for cancer therapy [5–7]. Its principle α-particle emissions have a mean energy of 6.8 MeV, with an average range in tissue of about 65 μm; the dose-average LET_∞ is approximately 100 keV/μm, near optimal for endoradiotherapeutic purposes [7]. The mean absorbed α-radiation dose from permanently sequestered ^{211}At is 40.7 μGy × g/Bq [5–7]. Metabolically-targeted ^{211}At therapy is currently being investigated as part of an integrated management strategy for high-grade gliomas. It is well recognized that cell death due to ionizing radiations is a consequence of nuclear damage [11]. In this respect, high-LET radiations are profoundly cytotoxic. Damage at a molecular level is predominantly due to direct α-particle ionizations at the critical target, DNA. Repair of DNA damage following lower doses of α-particles from internalized ^{211}At may be an important determinant of its potential clinical efficacy. Therefore, it may be of clinical relevance to find out a dose range where the damaging ability of α-radiation from ^{211}At is enhanced with reduced repair possibility.

Poly-ADP-ribosylation (PADPR), a post-translational modification of mainly chromosomal proteins, is an indirect but sensitive indicator of chromatin damage and putative repair in both normal and malignant mammalian cells [2, 19, 21]. PADPR of chromosomal proteins leads to profound changes in the structure of chromatin [15]. An enhancement of PADPR of histones may render DNA more accessible to repair enzymes [1, 16]. On the other hand, reduced level of PADPR represent a more condensed chromatin with reduced accessibility of DNA to repair enzymes. Increased levels of poly-ADP-ribosylated proteins observed after exposure of cells to low and high-LET quality radiation beams reflect initiation of DNA repair following chromosomal damage [19, 22]. In this study, poly-ADP-ribosylations of total cellular proteins including histones in human glioblastoma cells have been measured following in-vitro exposure to α-particle radiations from homogeneously distributed extracellular ^{211}At.

Materials and Methods

Astatine-211 and Exposure of Cells to α-Radiation

It was prepared by the ^{207}Bi(α,2n)^{211}At nuclear reaction using a scanning 27.5 MeV α-particle beam accelerated in the Nuffield 1.52 m diameter cyclotron in the School of Physics and Space Research, Birmingham University. ^{211}At was extracted from the bismuth target by dry distillation at 680 °C in an atmosphere of dry helium. Aqueous solutions of $Na^{211}At$ (> 150 GBq/μM) were maintained in an anionic state by the addition of 2 μmol Na_2SO_3 and buffered to physiological pH 7.4. Dose activities were determined by measurement of its 76.9 to 92.4 keV ^{211}Po K-L, M, N X-rays; γ-ray spectroscopy confirmed its radiochemical purity greater than 99.999%.

Treatment aliquots of ^{211}At were made up in Iscove's modified Dulbecco's medium (IMDM) containing 10% fetal calf serum (v/v) and 1% penicillin/streptomycin (w/v). Six days old human glioblastoma cells 86HG-39 (2.3×10^6) in Leighton culture tubes were exposed to 0.94 to 1880.0 kBq ^{211}At in order to deliver an absorbed α-dose of 0.0005 to 1.0 Gy over 1 hour, at 37 °C. 86HG-39 exhibited no intrinsic affinity for ^{211}At; its intracellular uptake is negligible (contributing $< 10^{-4}$% to the total absorbed radiation dose). Following irradiation, ^{211}At activity was completely removed by sequential washing of the cells with IMDM.

Assay of Poly-ADP-Ribosylation

Assay was performed immediately. The technique has been fully described elsewhere [19]. In brief, treated glioblastoma cells were washed with PBS and permeabilized at 0 °C by incubation in a buffer solution of 10 mM tris-HCl, 1 ml EDTA, 4 mM $MgCl_2$, and 30 mM 2-mercaptoethanol for 15 minutes. This was then replaced by 0.4 ml fresh buffer and 0.2 ml of solution containing 80 mM tris-HCl, 1 μM ^{32}P-NAD^+ corresponding to 222 kBq ^{32}P, and kept at 37 °C for 15 minutes. The PADPR reaction was stopped by the addition of 6 ml 15% ice cold trichloroacetic acid (TCA) and maintained for 30 minutes at 0 °C . The TCA-insoluble proteins were collected on GF/C filter discs and their radioactivity determined in a liquid scintillation counter (Tri-Carb 460). An identical procedure was carried out with the control samples in the absence of radioactivity (there are no stable astatine nuclides). In all cases, PADPR of proteins in the treated cells was expressed as a percentage of that of the controls.

Data

The results shown are mean ± SEM of 5 independent experiments each with 10 replicates. All experiments were done with 6-day-old cultures and it is assumed that states of confluence of cells were identical in each experiment. The data were subjected to Student's t-test to calculate the levels of significance of differences.

Results and Discussion

Human glioblastoma cells (86HG-39) as confluent monolayers were used in the investigation. The state of confluence is shown by nearly constant number of cells in each culture tube between 6th and 8th day after seeding. During this peri-

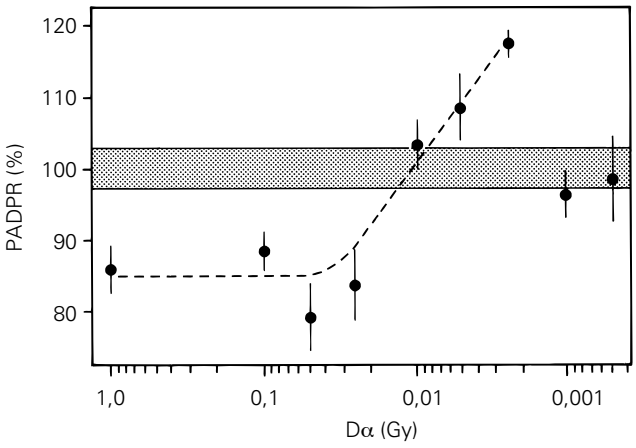

Figure 1. Poly-ADP-ribosylation (PADPR) of total proteins in human glioblastoma cells as a negative logarithmic function of absorbed α-radiation dose (Dα) from extracellular ^{211}At. The PADPR in non-irradiated controls was 100 ± 2.8 (SEM) %.

Abbildung 1. Poly-ADP-ribosylierung (PADPR) der gesamten Proteine in menschlichen Glioblastomzellen als negative logarithmische Funktion der absorbierten α-Strahlendosis (D_α) von extrazellulärem ^{211}At. Die PADPR in den nichtbestrahlten Kontrollen betrug 100 ± 2,8 (SEM) %.

od the majority of cells were in G_0/G_1 phase of cell cycle with the following pattern of distribution: G_0/G_1: 79.3 ± 3.3%; S: 11.2 ± 1.7%; G_2/M 9.8 ± 2.4%. Cell cycle parameters were not significantly different after ^{211}At treatment. Cell viability (87 ± 1%), as determined by the trypan blue exclusion test, remained invariant throughout the experiment. It was calculated that in the ^{211}At treatment protocol cells were exposed to α-particle fluxes of 1,500 to 3 × 10^6 α/cm^2 corresponding to absorbed doses of 0.0005 to 1.0 Gy.

The total cellular PADPR of proteins in 86HG-39 cells, relative to non-irradiated controls (100 ± 2.8%), as a function of absorbed α-radiation dose (D_α) is shown in Figure 1. Exposure of glioblastoma cell monolayers to an absorbed dose range of 0.025 to 1.0 Gy led to a significant reduction in PADPR of proteins to 85.6 ± 4.6% compared with the basal metabolic level in non-irradiated control cells ($p < 0.005$). Reduced relative PADPR did not appear to be dose-dependent. By considering the nuclear geometry of 86HG-39 cells in monolayer (nuclear cross-section 103.9 ± 7.5 μm^2) such α-particle fluxes averaged approximately 0.1 to 3.2 α-particle traversals per cell nucleus. Since high-LET radiation induced inactivation of enzymes are known, it is possible that poly(ADP-ribose) polymerase and poly(ADP-ribose) glycohydrolase, essential enzymes of PADPR metabolism, were partially inactivated leading to reduced PADPR level (Figure 1). Radiation induced enzyme inactivation is a known phenomenon and has been reported earlier [23]. Alternately, intense DNA damages caused by α-particles may have induced automodification of poly(ADP-ribose) polymerase leading to its partial inactivation and consequent reduction of PADPR of proteins [1, 2].

Following lower α-particle doses (0.0025 to 0.025 Gy) there existed a negative exponential relationship with relative levels of PADPR ($r = -0.992$; $p < 0.005$) reaching a maximum level of 119 ± 2.2% at 0.0025 Gy (see Figure 1). Enhanced level of PADPR of proteins have been shown to favor repair of DNA damages [1, 2, 16, 21]. Therefore, it is assumed that in this particular α-dose range repair of DNA damages is likely to be favored [19, 22). Below these doses down to 0.0005 Gy, the statistical probability of nuclear interactions and consequent DNA damage is extremely low (< 0.01 α-traversals per cell nucleus) and beyond the biochemical sensitivity of the PAPDR assay.

In conclusion, the in vitro results presented here show that α-radiation dose below 0.025 Gy has no clinical relevance as indicated by the level of PADPR of proteins which indicates possible repair of DNA damages. For doses above 0.025 Gy, lower level of PADPR of proteins is likely to create molecular conditions leading to a relatively more condensed chromatin. This may prevent repair of α-induced DNA damages enhancing thereby extent of DNA damage and consequent cell killing. Thus, α-radiation from ^{211}At, at least in the dose range of 0.025 to 1.0 Gy, might be suitable in cancer radiotherapy.

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