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Decomposition of the Telomere-Targeting agent BRACO19 in physiological media results in products with decreased inhibitory potential

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Abstract

The stability of the acridine-based telomere-targeting agent BRACO19, a G-quadruplex stabilizing substance, was tested at different pH, temperature and in different dissolution media. Analysis was performed by HPLC. Decomposition products were examined by LC/MS and NMR. The TRAP assay was used to determine the inhibitory potential of the decomposition products on telomerase activity. The results show that the stability of BRACO19 strongly depends on pH and temperature. Decomposition was fastest at physiological pH and temperature while the type of dissolution medium had no major influence on stability. The most probable mechanism for this decomposition seems to be a hydrolysis of the amide bonds in position 3 and 6 of the acridine ring and/or a deamination of the phenyl ring. The decomposition products showed a reduced inhibitory potential compared to the parent compound BRACO19. The results demonstrate that the preparation of dosage forms and their storage conditions will have an important influence on the stability – and hence biological efficacy – of BRACO19 and related substances.

Keywords: Acridine; Stability; G-quadruplex; Hydrolysis; Deamination; TRAP assay

1. Introduction

During the last years the concept of telomerase inhibition for the treatment of cancer became an area of intensive research. Telomerase is a reverse transcriptase composed of the catalytic subunit human *te*lomerase *r*everse *t*ranscriptase (hTERT) and the template RNA *h*uman *t*elomerase *R*NA (hTR). Its substrates, the telomeres, are located at the ends of each chromosome. Telomeres are repetitive TTAGGG/AATCCC sequences that end in a 3′-$(TTAGGG)_n$ single strand overhang. They form a so-called *t*-loop, a structure stabilized by associated proteins, where the single strand invades the double stranded region. The *t*-loops prevent the chromosome ends from end-to-end fusions and being recognized as damaged DNA. Due to the end replication problem during DNA duplication telomeres erode at each cell cycle until they approach a certain limit. Reaching this limit is an important signal for a cell to enter the state of cellular senescence. Cells that do not stop dividing will experience severe chromosomal damages leading to cell death (apoptosis). Since most malignant cells express telomerase they are able to maintain their telomeres above this limit and therefore escape these pathways (Shay and Wright, 2004; Blackburn, 2005; Pendino et al., 2006). Various telomerase inhibitors have been developed and successfully tested. They either target hTERT, hTR or the telomeres (Shay and Wright, 2006).

Our studies concentrate on the topical treatment of non-small cell lung cancer (NSCLC) via the inhalative route. A search for suitable drug candidates led to the telomere targeting 3,6,9-aminoacridine derivative BRACO19 ((9-[4-(*N*,*N*-dimethylamino)phenylamino]-3,6-bis(3-pyrrolodino-propionamido) acridine; Fig. 1) which acts by G-quadruplex stabilization (Gowan et al., 2002; Piotrowska

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et al., 2005). G-quadruplexes are planar G-quartet motifs that can form in guanine-rich DNA sequence like the telomeres. These structures interact with the inhibitor via π–π stacking. Positively charged side chains of the inhibitor interact with the negatively charged phosphate DNA backbone and thereby stabilize the G-quadruplex–inhibitor complex. Due to this mechanism telomeres are not able to form their native structure and hence fail to protect the chromosomes (Cuesta et al., 2003; Riou, 2004).

BRACO19 has shown some promising results in studies in tumor cell cultures and mouse xenografts. It was reported that a treatment with BRACO19 not only resulted in telomerase inhibition but also in general telomere dysfunction that led to atypical mitosis and consequently to apoptosis (Burger et al., 2005).

In a previous study we characterized BRACO19 with respect to its biopharmaceutical properties and found it to be a typical class III drug substance with a good aqueous solubility but a very poor permeability across epithelial cell monolayers (Taetz et al., 2006). During these studies we also found that BRACO19 has stability problems when dissolved in aqueous media at physiological pH.

In the present study we examined the influence of pH, temperature and dissolution media on the stability of BRACO19. We have identified potential decomposition products and evaluated whether they can contribute to the inhibitory action of this drug candidate.

2. Materials and methods

2.1. BRACO19

BRACO19 ((9-[4-(*N*,*N*-dimethylamino)phenylamino]-3,6-bis(3-pyrrolidino-propionamido) acridine) × 3HCl; Fig. 1) was synthesized by ENDOTHERM GmbH (Saarbruecken, Germany) according to Harrison et al. (2003). Identity and puritiy were proven by NMR (Table 3) and HPLC, respectively, in comparison with the original compound.

2.2. Buffers and cell culture medium for stability studies

For stability studies different HBSS (Hank's balanced salt solution) buffers and diluted McIlvain buffer were used.

The basic compositions of HBSS buffers were: 137.0 mM NaCl, 5.36 mM KCl, 4.26 mM $NaHCO_3$, 0.18 mM $Na_2HPO_4 \times 7H_2O$, 0.44 mM KH_2PO_4, 5.55 mM Glucose, 0.13 mM $CaCl_2 \times 2H_2O$, 0.05 mM $MgCl_2 \times 6H_2O$, 0.04 mM $MgSO_4 \times 7H_2O$. For the adjustment of the appropriate pH values in HBSS buffers the buffering substances were exchanged as follows: pH 7.4 and 7.0: 10.0 mM HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]), pH 6.0: 10.0 mM MES (2-morpholinoethanesulfonic acid), pH 4.0 and 5.0: 10.0 mM sodium acetate. pH values were adjusted with 1 M NaOH or 1 M HCl, respectively.

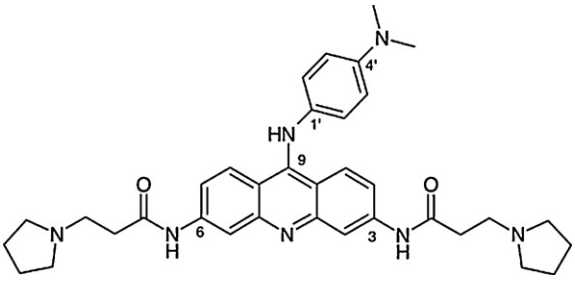

Fig. 1. Structure of BRACO19 ((9-[4-(*N*,*N*-dimethylamino)phenylamino]-3,6-bis(3-pyrrolodino-propionamido) acridine).

Diluted McIlvain buffer contained 50 mM citric acid and 1.4 mM Na_2HPO_4. The pH was 2.8. Phosphate buffer was composed of 5 mM KH_2PO_4 and the pH was adjusted with NaOH to pH 7.4.

RPMI cell culture medium was purchased from PAA Laboratories GmbH (Pasching, Austria) and supplemented with 10% FCS (Sigma–Aldrich Chemie GmbH, Taufkirchen, Germany). The pH of the cell culture medium was 7.5.

2.3. HPLC-DAD analysis of BRACO19 and decomposition products

BRACO19 was analyzed by reversed phase HPLC using an isocratic Dionex HPLC system consisting of an ASI 100 automated sample injector with adjustable temperature, UVD 340U diode array detector (DAD) and P680 pump with Chromeleon® software (version 6.60 SP1 build 1449) (Dionex, Idstein, Germany).

We used a Gemini® RP-18 column/150 × 4.6 mm/5 μm/110 Å (Phenomenex, Aschaffenburg, Germany). The mobile phase was composed of 80:20 (v/v) methanol:borate buffer pH 10.0 (100 mM). At a flow rate of 0.6 ml/min the retention time of BRACO19 was 8.9 ± 0.2 min. The wavelength of the detector was set at 268 nm. The detection and quantification limit of BRACO19 were 0.010 and 0.025 μg/ml, respectively. Quantification was linear in the range from 0.025 to 12 μg/ml.

2.4. Decomposition experiments

All decomposition experiments were performed with the HPLC-DAD system described above in HPLC brown glass vials (hydrolysis grade 1; CS-Chromatography, Langerwehe, Germany). Decomposition experiments over 7 h were performed at 37° with HBSS buffer of different pH values and diluted McIlvain buffer of pH 2.8. The influence of temperature on BRACO19 decomposition was assessed at 4 °C in comparison to the decomposition at 37 °C in HBSS buffer pH 7.4. BRACO19 was dissolved at a concentration of 12 μg/ml in each respective buffer. 20 μl samples were drawn every 45 min for 7 h and analyzed by HPLC as described above. Each experiment was done in triplicate.

Long-term decomposition experiments were performed for 4 days in HBSS buffer pH 7.4, phosphate buffer pH 7.4 and RPMI cell culture medium containing 10% FCS (pH 7.5), respectively. The concentration of BRACO19 was 12 μg/ml and the temperature was set to 37 °C. Samples were drawn every 2.54 h. All experiments were performed in triplicate.