Telomerase represents a selective target for novel anticancer approaches. Consistent experimental data have demonstrated that in the vast majority of tumor tissues and metastasis this RNA dependent DNA-polymerase is active. Normal tissues do not show detectable activity of this enzyme, while germinal and staminal cells constitutively express low levels of it. Telomerase adds repetitive hexanucleotides (TTAGGG in humans), copying a template sequence carried within its integral RNA, to the chromosome 3' ends. This single-strand synthesis by telomerase and accompanying complementary -strand synthesis can balance the telomere erosion inherent in incomplete end-replication, allowing telomere length homeostasis. Telomeres consist of hexanucleotide TTAGGG repeats (with an average length of 5-15 kb in humans) and associated protein components. They serve to protect the ends of the chromosomes from recombination, fusion, or being recognized as damaged DNA. In normal diploid cells, gradual telomere loss occurs at each cell division (about 100 bases) so limiting their lifespan, resulting in a permanent growth arrest known as replicative senescence. Therefore the telomere protection is a potent anti-senescent signal that allows the cells to proliferate indefinitely (Sprung 1999, Kim 1994).

Three mayor components of human telomerase have been identified: human telomerase RNA, hTERC (Feng et al, 1995); telomerase-associated protein, TEP1 (Harrigton et al, 1997; Nakayama et al, 1997); and human telomerase reverse transcriptase, hTERT (Meyerson et al, 1997; Nakamura et al, 1997). Among these components, hTERT has been considered a catalytic subunit of telomerase and a rate-limiting determinant of the enzymatic activity (Bodnar et al, 1998;Vaziri and Benchimol, 1998). Telomerase plays a protective role against DNA damage and subsequent apoptosis (Holt et al, 1999).

Telomerase quantification may be of prognostic value in some cancers, but not in all. Cisplatin has played a pivotal role in chemotherapy and there has been widespread interest in metal-based drugs in recent years. There could be some possible advantages in using transition metals other than platinum involving additional coordination sites, alterations in ligand affinity and substitution kinetics. In this study we analyzed whether some recently synthesized water-soluble osmium clusters are able to inhibit the activity of telomerase. We tested the following complexes: HOs$(CO)_{6}$(3-aminoquinoline) [P(C_{6}H_{5}SO_{3}Na$^{+}$)], HOs$(CO)_{6}$(3-aminoquinoline) [P(OCH_{3}CH_{2}NMe_{3}J)], HOs$(CO)_{6}$(3-aminoquinoline) [P(OCH_{3}CH_{2}NMe_{3}J)], HOs$(CO)_{6}$(3-aminoquinoline) [P(OCH_{3}CH_{2}NMe_{3}J)], HOs$(CO)_{6}$(3-aminoquinoline) [P(OCH_{3}CH_{2}NMe_{3}J)]. All these molecules consist of a nucleobase-like ligand (3-aminoquinoline or quinoxaline), a phosphine or phosphite able to impart water solubility and a huge, chemically inert triosmium nonacarbonyl hydrido core (Rosenberg 2000).

These compounds differ from other metal-based drugs because they lack DNA alkylating properties (intra- or inter-cross linking). These molecules were designed with no leaving groups at all.

The intention was to obtain molecules characterized by steric bulk able to interfere with the catalytic subunit of the enzyme (hTERT).

The cytotoxic effects of the above said complexes as well as the corresponding free phosphorous ligands were assayed in vitro on human mammary adenocarcinoma MCF-7 using the MTT test to measure growth capability. Experiments were conducted in parallel with telomerase activity assays performed with the TRAP method (Telomerase Repeat Amplification Protocol Assay) (Kim 1994).

This protocol measures qualitatively and quantitatively the telomerase presence and activity; synthetic telomere is elongated from the enzyme present in cell lysates and the ladder products are amplified with PCR and resolved on gel. Relative inhibition has been determined with a densitometer by comparing the intensity of hexameric repeat ladders in the treated samples and the untreated control. We used two different experimental approaches, two biochemical assays with the isolated telomerase and a different enzyme, Taq DNA-polymerase, and an in vitro cell line model. The molecules that have a non-specific inhibition of Taq polymerase, enzyme present in the final step of the amplification of telomerase products, were excluded. Cells were treated with continuous exposure of 6, 24 and 48 hours and results showed that among the different complexes only HOs$(CO)_{6}$(3-aminoquinoline) [P(C_{6}H_{5}SO_{3}Na$^{+}$)] possessed significative antitelomerasic activity both on the isolated enzyme and on the residual enzyme extracted after the treatments. The optimal inhibition was achieved with 24 hour of continuous incubation. None of the
complexes tested under these conditions, in the range of concentrations $10^{-5}$-$10^{-9}$M, possessed significative cytotoxic effects, thus suggesting that these molecules have no unspecific toxic effects and underlining a possible specific interaction with the enzyme. HOs$_3$(CO)$_9$(3-aminoquinoline) [P(C$_6$H$_5$SO$_3$Na$^+$)$_3$] was able to inhibit telomerase activity from 64% to 34% in the concentration range $10^{-5}$M - $10^{-7}$M. Furthermore we have evidence of a strict dose-dependency of the effect. Our data show that osmium clusters could offer interesting hints for new and more selective approaches in the treatment of cancer. Further studies will focus on the biological properties of these compounds, their mechanisms of action and the development of more active and selective molecules.

References


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