Anti-Cancer Targeting Telomerase Inhibitors: β-Rubromycin and Oleic Acid

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Abstract: Telomerase is a ribonucleoprotein complex that elongates telomeric DNA and appears to play an important part in the cellular immortalization of cancers. In the screening of potent inhibitors of human telomerase, several inhibitors have been discovered from natural and chemical sources. Some compounds potently inhibit the activity of human telomerase. Rubromycins and fatty acids such as β-rubromycin and oleic acid, respectively, were found to be inhibitors of human telomerase. The IC_{50} values of β-rubromycin and oleic acid were 8.60 and 8.78 μM, respectively. A kinetic study revealed that these compounds competitively inhibited the activity of telomerase with respect to the substrate of the primer and dNTP. The energy-minimized three-dimensional structure of β-rubromycin and oleic acid was calculated and designed. The V-shaped curve and molecule length of 18.7–20.3 Å in these compound structures were suggested to be important for telomerase inhibition. The three-dimensional structure of the active site of telomerase (i.e., the binding site of the primer and dNTP substrate) might have a "pocket" that could "join" these compounds. These results appear to suggest a potential structure for the development of more potent inhibitors of human telomerase.

Keywords: Telomerase, β-rubromycin, oleic acid, enzyme inhibitor, three-dimensional accepted structure, anti-cancer drugs.

INTRODUCTION

Telomerase is an enzyme that adds DNA sequence repeats (TTAGGG in all vertebrates) to the 3' end of DNA strands in the telomere regions, which are found at the ends of eukaryotic chromosomes. This region of repeated nucleotides (called telomeres) contains non-coding DNA material and prevents constant loss of important DNA from chromosome ends. Hence, every time the chromosome is copied only 100-200 nucleotides are lost, which does not damage the DNA of the organism. Telomerase is a reverse transcriptase that carries its own RNA molecule. The RNA molecule is used as a template when it elongates telomeres, which are shortened after each replication cycle [1-3].

Telomerase consists of two molecules each of human telomerase reverse transcriptase (TERT), telomerase RNA (TR or TERC) and dyskerin (DKC1) [4]. The genes of telomerase subunits (TERT, TERC, DKC1, TEP1) are located on different chromosomes of the human genome. Human TERT gene (hTERT) is translated into a protein of 1132 amino acids. TERT proteins from many eukaryotes have been sequenced and TERT polypeptide folds with TERC, a non-coding RNA (451 nucleotides long in humans). TERT has a "mitten" structure that allows it to wrap around the chromosome to add single-stranded telomere repeats.

A reverse transcriptase is a class of enzyme that creates single-stranded DNA using single-stranded RNA as a template. Enzymes of this class (not TERT specifically, but those isolated from viruses) are utilized by scientists in the molecular biological process of reverse-transcription polymerase chain reaction (RT-PCR). RT-PCR allows the creation of several DNA copies of a target sequence using RNA as a template. As stated above, TERT carries its own template called TERC.

The high-resolution protein structure of the *Tribolium castaneum* catalytic subunit of telomerase TERT was decoded by Gillis *et al.* [5]. The structure revealed that the protein consists of four conserved domains [RNA-binding domain, fingers, palm and thumb], organized into a ring configuration that shares common features with retroviral reverse transcriptases, viral RNA polymerases and bacteriophage B-family DNA polymerases.

Using TERC, TERT can add a six-nucleotide repeating sequence, 5'-TTAGGG (in all vertebrates, the sequence differs in other organisms), to the 3' strand of chromosomes. These TTAGGG repeats (with their various protein binding partners) are called "telomeres". The template region of TERC is 3'-CAAUCCCAAUC-5' [6]. In this way, telomerase can bind the first few nucleotides of the template to the last telomere sequence on the chromosome, add a new telomere repeat (5'-GGTTAG-3') sequence, let go, realign the new 3'-end of telomere to the template and repeat the process.

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1: 97-5829/12 $58.00+.00 © 2012 Bentham Science Publishers

Cancer is a very difficult disease to fight because the immune system has problems recognizing it. Telomerase is necessary for the immortality of many cancer types, so it is thought to be a potential drug target. If a drug can be used to "turn off" telomerase in cancer cells, the telomere shortening described above will resume—telomere length will be lost as the cells continue to divide, mutations will occur and cell stability will decrease. Experimental drug and vaccine therapies targeting active telomerase have been tested in mouse models, and some have entered clinical trials. We established a telomerase assay method (telomeric repeat amplification protocol (TRAP)) and have been screening potent inhibitors of human telomerase from natural and chemical sources [7]. As a result, various low-molecular-weight compounds that inhibit the activity of human telomerase have been found. In this review, the inhibitory effects of novel classes of non-peptidic, non-nucleosidic small molecular inhibitors such as rubromycins and fatty acids on the catalytic activity of human telomerase are discussed.

ENZYMATIC ASSAY FOR HUMAN TELOMERASE

The catalytic activity of human telomerase extracted from K-562 cells was evaluated as described previously [7, 8] based on a modified version of the TRAP [9]. In brief, the reaction mixture was preincubated for 10 min at room temperature in the presence or absence of various amounts of test compounds that had been dissolved in dimethyl sulfoxide. The telomerase reaction was initiated by addition of a 200-μM concentration of each of the four 2′-deoxyribonucleoside 5′-triphosphates (dNTPs) and the modified telomerase substrate primer TS-A (5′-AAAAAAAAAAAAAATCCGTCGAGCAGAGTT-3′) in a total volume of 50 μl. The mixture was subsequently incubated for 30 min at room temperature. After the reaction had been quenched, TS-A oligonucleotides elongated by telomerase were extracted and then precipitated in ethanol. Precipitated materials were amplified by PCR using 6-FAM-lableled ACX primer (5′-GCGCGGCTTACCCTTACCCTTACCCTAACC-3′) as described by Ueno *et al.* [7]. The resultant PCR-amplified telomerase product was mixed with a fluorescent size standard. It was then separated and visualized by capillary electrophoresis. The amount of products was integrated and quantified using computer software. The catalytic activity of telomerase without the inhibitor was considered to be 100% and the remaining activity at each concentration of the inhibitor was determined relative to this value.

RUBROMYCINS AND THEIR ANALOGS

Production, Isolation, and Purification of Rubromycins and Their Analogs

A strain of *Streptomyces* species isolated from a field in Tatebayashi City (Gunma, Japan) was used to produce β- and γ-rubromycins [10, 11]. *Actinoplanes ianthinogenes* JCM3249 and *Streptomyces californicus* JCM6910 obtained from the Japan Collection of Microorganisms (Saitama, Japan) were used to produce purpuromycin [12] and griseorhodin A [13], respectively. α-Rubromycin was obtained by heating β-rubromycin in refluxing pyridine for 15 min as described previously [11] followed by purification by preparative thin-layer chromatography and recrystallization. Griseorhodin C was obtained by incubating griseorhodin A in trifluoroacetic acid for 16 h at room temperature in the dark as described previously [14] and was purified by silica gel column chromatography followed by HPLC. The chemical structures of these rubromycins and their analogs are shown in Fig. (**1**).

Effect of Rubromycins and their Analogs on the Activity of Human Telomerase

β-Rubromycin was the strongest inhibitor of human telomerase among the compounds tested, with 50% inhibitory concentrations (IC_{50}) of 8.60 μM (Fig. (**2**)). γ-Rubromycin and purpuromycin appeared to be moderately potent inhibitors, with IC_{50} values of 32.0 and 39.6 μM, respectively. In contrast, griseorhodins A and C, opening the spiroketal system of γ-rubromycin and having no carboxyl groups (Fig. (**1**)), resulted in marked reduction in the inhibitory potency toward telomerase (IC_{50} >200 μM), suggesting the essential role of this spiroketal system and a carboxyl group in telomerase inhibition. When the telomerase assay was conducted with $[^{32}P]$-dCTP and polyacrylamide gel electrophoresis as described by Kim *et al.* [9], virtually identical IC_{50} values (3.2 μM for β- and γ-rubromycins) were obtained, further confirming this modified version of the TRAP to be suitable for the present study.

FATTY ACIDS

Preparation of Fatty Acids

Linear-chain fatty acids were named using the nomenclature described by Weete [15] [(A:B ΔC1-C2), where A is the number of carbon atoms, B is the number of double bonds, and C1-C2 is the position of each double bond from the carboxyl end of the molecule. Saturated fatty acids, *cis*-unsaturated fatty acids, *trans*-unsaturated fatty acids and derivatives of C_{18} fatty acid were purchased commercially.

Effect of Fatty Acids on the Activity of Human Telomerase

A series of saturated fatty acids with hydrocarbon chain lengths from 10 to 24 were tested first. They showed only slight effects on inhibition against human telomerase-mediated addition of nucleotide repeats, with residual activities at 100 μM (Fig. (**3A**)) and IC_{50} values >500 μM.

We then investigated the inhibitory effect of unsaturated fatty acids of the *cis*-configuration with C12–24 lengths of hydrocarbon chains toward the telomerase. As shown in Fig. (**3B**), 100 μM C16 fatty acids such as palmitoleic acid (16:1 Δ9*cis*), C18-unsaturated fatty acids and C20 fatty acids such as eicosenoic acid (20:1 Δ11*cis*) significantly inhibited telomerase. These findings suggested that an unsaturated carbohydrate bond (i.e., double bond) in these fatty acids played a critical part in human telomerase inhibition. In particular, oleic acid (18:1 Δ 9*cis*) was the strongest inhibitor of telomerase in C18 unsaturated fatty acids. In contrast, fatty acids with hydrocarbon chain lengths of <14 and >22 showed no inhibition against telomerase at 100 μM concentration. No unsaturated fatty acids with a

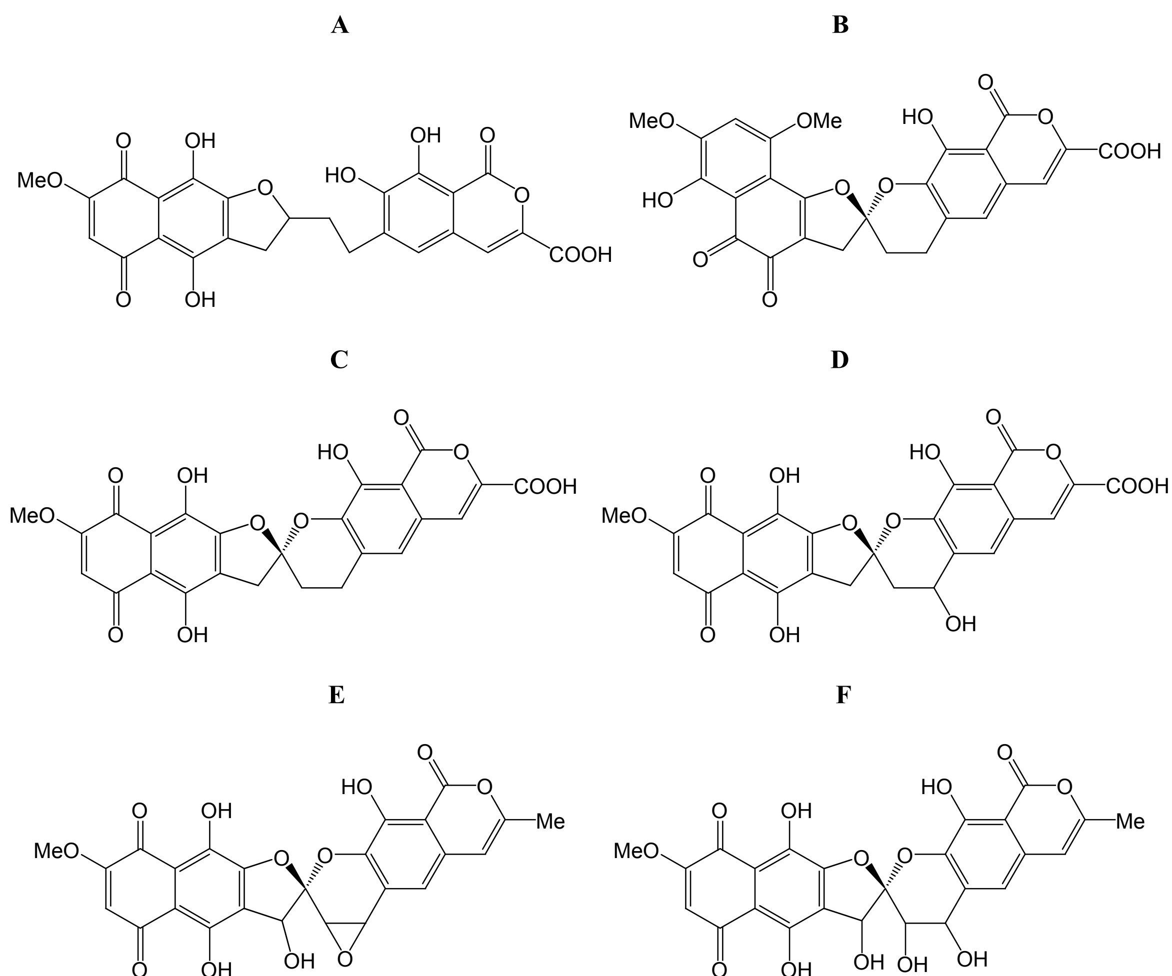

Fig. (1). Structures of rubromycins and their analogs. (**A**) α-Rubromycin, (**B**) β-rubromycin, (**C**) γ-rubromycin, (**D**) purpuromycin, (**E**) griseorhodin A and (**F**) griseorhodin C.

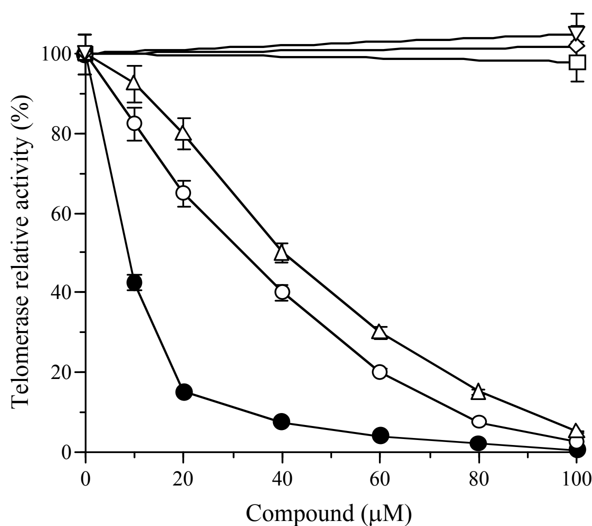

Fig. (2). Dose–response curves of rubromycins and their analogs on human telomerase. α-Rubromycin (open square), β-rubromycin (closed circle), γ-rubromycin (open circle), purpuromycin (open triangle), griseorhodin A (open diamond) and griseorhodin C (open reverse-triangle). A modified version of the TRAP was employed to measure telomerase activity. Data are the means ± SE of three independent experiments.

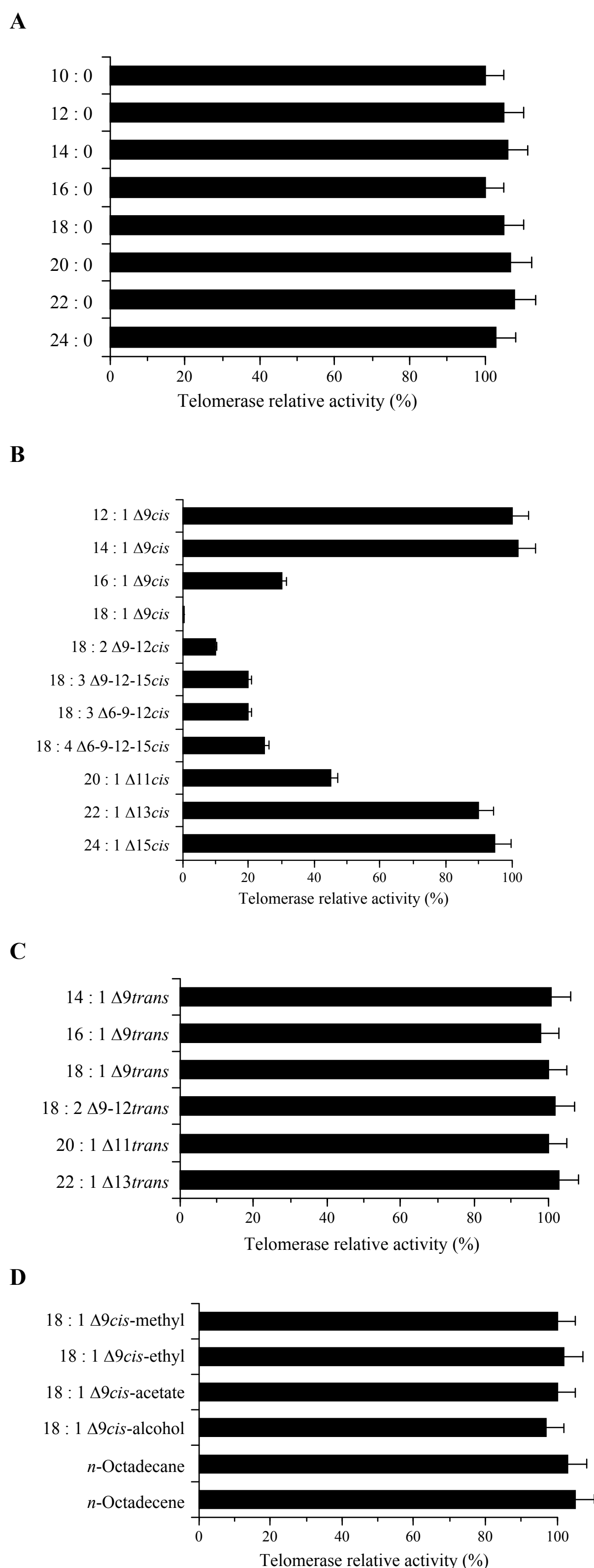

Fig. (3). Inhibitory effect of C10 to C24 fatty acids (100-μM each) on human telomerase. (**A**) Saturated fatty acids. (**B**) *Cis*-type unsaturated fatty acid. (**C**) *Trans*-type unsaturated fatty acids. (**D**) C18 fatty acid derivatives. A modified version of the TRAP was employed to measure telomerase activity. Data are the means ± SE of three independent experiments.

trans-configuration (100 μM) contributed to the inhibition of telomerase activity (Fig. (**3C**)). These results suggested that isomeric configurations (*cis* versus *trans*) and length of the carbohydrate chain of the fatty-acid structure were quite important for providing a potent inhibitory effect for telomerase.

Conversely, inhibition of telomerase activity by C18 fatty acids was more potent when the carboxyl group was free (Fig. (**3D**)). When the carboxyl group was modified chemically to add a methyl ester, ethyl ester, acetate or alcohol, or when it was removed completely, these hydrocarbons could not inhibit telomerase activity. These results showed the importance of the free carboxyl group in inhibition.

Fig. (**4A**) shows the dose–response curves for each of the *cis*-configured mono-unsaturated fatty acids. Among the C14 to C24 mono-unsaturated fatty acids tested, oleic acid (C18 fatty acid) was the most potent inhibitor of telomerase, with 50% inhibition occurring at 8.78 μM. C16 and C20 fatty acids moderately inhibited, and fatty acids with >22 or <14 carbons did not influence the inhibition of telomerase, suggesting that the length of the carbohydrate chain was critical for telomerase inhibition. The effect of the numbers of *cis*-unsaturated bonds in a fatty acid on the inhibition potential against human telomerase using mono-, di-, tri- and tetra-unsaturated C18 fatty acids was also examined. A mono-unsaturated fatty acid with a *cis*-configuration (i.e., oleic acid) was the strongest inhibitor of telomerase among the C18 fatty acids tested (Fig. (**4B**)). *Cis*-octadecenoic acids have an ethylenic double bond at position 6 (petroselinic acid), 9 (oleic acid) or 11 (vaccenic acid); the IC_{50} values for telomerase inhibition were 15, 8.8 and 16 μM, respectively (data not shown). Oleic acid showed the strongest inhibition among these *cis*-octadecenoic acids, suggesting that the position of the double bond was also quite important for inhibition. *cis*-Di-, tri- and tetra-C18 fatty acids moderately inhibited telomerase activity, whereas a saturated C18 fatty acid (i.e., stearic acid) did not affect telomerase inhibition. One double bond of *cis*-configuration in unsaturated fatty acids appears to play an important part in the molecular mechanism of telomerase inhibition.

MECHANISM OF ACTION OF HUMAN TELOMERASE INHIBITION BY β-RUBROMYCIN AND OLEIC ACID

β-Rubromycin and oleic acid showed the most potent inhibition of human telomerase activity among the rubromycin analogs tested and fatty acids investigated, respectively. Hence, the inhibitory mode of these compounds was investigated. Steady-state kinetic analyses to inhibit the telomerase-mediated addition of hexameric nucleotide repeats (TTAGGG)*n* to the ends of TS-A were examined. Also, the extent of inhibition as a function of the TS-A primer or dNTP substrate concentration was studied (Table **1**).

Double reciprocal plots of the data obtained showed that the inhibition of human telomerase activity by β-rubromycin was competitive with respect to the TS-A primer and dNTP substrate. For the TS-A primer, the apparent maximum velocity (V_{max}) was unchanged at 3.85×10^{-7} area, whereas an

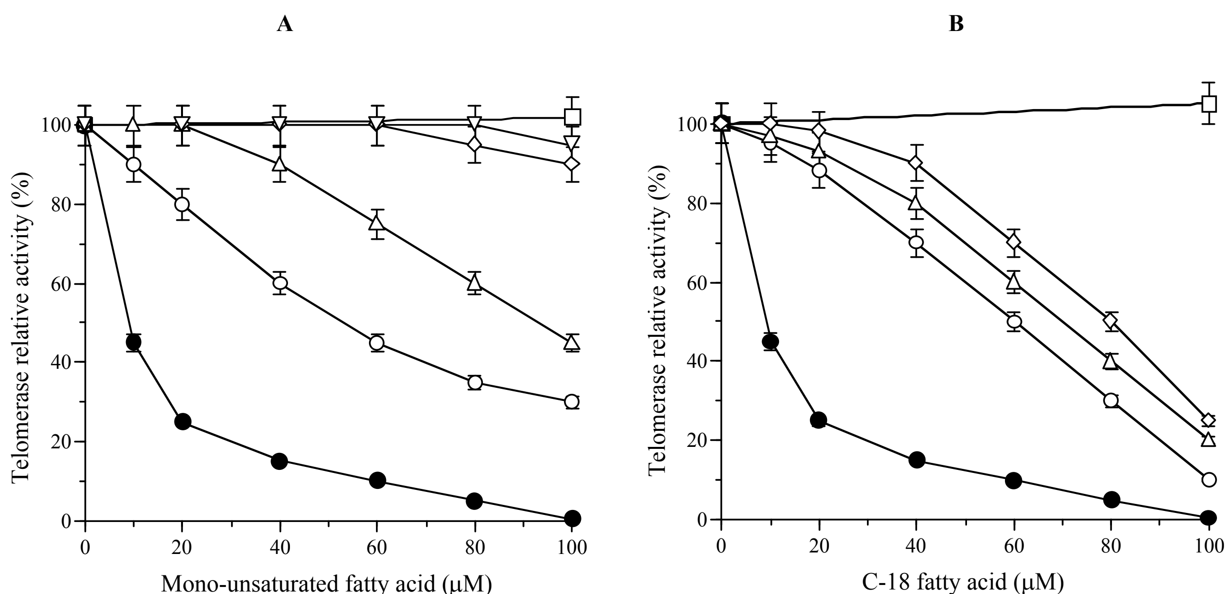

Fig. (4). Dose–response curves of fatty acids on human telomerase. (**A**) C14 to C24 mono-unsaturated fatty acid: myristoleic acid (14:1 Δ9*cis*, open square), palmitoleic acid (16:1 Δ9*cis*, open circle), oleic acid (18:1 Δ9*cis*, closed circle), *cis*-11-eicosenoic acid (20:1 Δ11*cis*, open triangle), erucic acid (22:1 Δ13*cis*, open diamond) and nervonic acid (24:1 Δ15*cis*, open reverse-triangle). (**B**) C18 fatty acids: stearic acid (18:0, open square), oleic acid (18:1 Δ9*cis*, closed circle), linoleic acid (18:2 Δ9-12*cis*, open circle), α-linolenic acid (18:3 Δ9-12-15*cis*, open triangle) and stearidonic acid (18:4 Δ6-9-12-15*cis*, open diamond). A modified version of the TRAP was employed to measure telomerase activity. Data are the means ± SE of three independent experiments.

Table 1. Kinetic Analyses of the Inhibitory Effects of β-Rubromycin and Oleic Acid on Telomerase as a Function of TS-A-Primer Dose and dNTP Concentration

Compound	Substrate	Compound (μM)	K_m [a] (μM)	V_{max} [a] (Area, $\times 10^{-7}$)	K_i [b] (μM)	Inhibitory Mode
β-Rubromycin	TS-A primer	0	0.217	3.85	0.740	Competitive
		2	0.476			
		4	1.11			
		6	2.86			
	dNTP	0	2.84	4.55	3.68	Competitive
		2	4.17			
		4	8.33			
		6	16.7			
Oleic acid	TS-A primer	0	0.217	3.57	0.862	Competitive
		2	0.323			
		4	0.667			
		6	1.96			
	dNTP	0	2.84	4.17	3.58	Competitive
		2	3.85			
		4	6.25			
		6	12.5			

Telomerase reactions were conducted with K-562 lysate equivalent to 5000 cells and various concentrations of the TS-A primer or dNTP substrate.

[a] These data were obtained from the Lineweaver–Burk plot.

[b] These data were obtained from the Dixon plot.

increase of 2.19-, 5.12- and 13.2-fold in the Michaelis constant (K_m) was observed in the presence of β-rubromycin at 2, 4 and 6 μM, respectively. The V_{max} for the dNTP substrate was unchanged at 4.55×10^{-7} area, whereas the K_m for the dNTP substrate increased from 2.84 μM to 16.7 μM in the presence of 0–6 μM β-rubromycin. The inhibition constant (K_i) obtained from Dixon plots was found to be 0.740 μM and 3.68 μM for the TS-A primer and dNTP substrate, respectively. Because the *Ki* value for the TS-A primer was approximately 5-fold smaller than that for the dNTP substrate, the affinity of β-rubromycin was greater for the enzyme–TS-A primer binary complex than for the enzyme–dNTP substrate complex.

Similarly, inhibition of the activity of human telomerase by oleic acid was competitive with the TS-A primer because there was no change in the apparent V_{max} (3.57×10^{-7} area), whereas the K_m increased from 0.217 μM to 1.96 μM for the TS-A primer in the presence of 0–6 μM oleic acid (Table **1**). Inhibition of telomerase activity by oleic acid was competitive with respect to the dNTP substrate (V_{max} was unchanged at 4.17×10^{-7} area). Dixon plots revealed the K_i value to be 0.862 μM and 3.58 μM for the TS-A primer and dNTP substrate, respectively. Therefore, the affinity of oleic acid was more than 4-fold greater for the enzyme–TS-A primer binary complex than for the enzyme–dNTP substrate complex. Similar results were obtained with other *cis*-unsaturated fatty acids that could inhibit telomerase activity (data not shown).

The telomerase-inhibiting activity of β-rubromycin and oleic acid was not influenced when excess protein (100 μg/mL bovine serum albumin) or MS2 RNA was added to the reaction mixture (data not shown). This finding suggested that by competing with the primer without non-specific binding of low-molecular-weight inhibitors with proteins or nucleotides, β-rubromycin and oleic acid could selectively bind or interact with the oligonucleotide primer binding site rather than the dNTP substrate binding site in TERT of telomerase.

THREE-DIMENSIONAL STRUCTURES OF β-RUBROMYCIN AND OLEIC ACID

Computational analyses were undertaken using molecular simulation and surface analysis software to obtain information about the molecular basis of the inhibitory properties of β-rubromycin and oleic acid. Fig. (**5**) shows the energy-minimized three-dimensional (3D) structures by comparing the electrostatic potential surfaces of these compounds. The electrostatic potential at each point on a constant electronic density surface (approximating the van der Waals surface for each arrangement) is represented graphically in red and corresponds to regions where the electrostatic potential is most negative; blue corresponds to the most positive regions.

We focused on the calculated log P (Clog P) value (partition coefficients for octanol/water) and pKa (acid dissociation constant) of β-rubromycin and oleic acid as chemical properties (Table **2**). The value of Clog P (which indicates hydrophobicity) in β-rubromycin and oleic acid was different (differential value of 1.17). Therefore, the Clog P value did not affect telomerase inhibitory activity. The pKa value of these compounds was in a similar range (4.78–5.61) so the acidity of the compounds might be essential for inhibition. β-Rubromycin and oleic acid each have a carboxyl group (arrows in Fig. (**5**)), and this group configures the negatively charged surface area in both molecules (Fig. (**5**)). In the telomerase inhibitor study, we found several inhibitor compounds and propose that an acidic group (e.g., carboxyl group) in a telomerase inhibitor molecule is highly important for telomerase inhibition.

The 3D structures from which the energy-minimized compounds were calculated as shown in Table **2**. β-Rubromycin and oleic acid are almost the same length (18.7–20.3 Å), but the width and depth of the β-rubromycin molecule is approximately 2-fold larger than that of oleic acid. The inhibitory effect (i.e., IC_{50} and K_i values) of these compounds on human telomerase was similar, so the 3D length of the molecules must be important for the inhibition of telomerase. The 3D inhibition mechanism of TERT of telomerase by β-rubromycin and oleic acid remains unclear and will require further study.

DISCUSSION

Telomerase plays a key part in the cellular immortalization of cancers [16–19]. One of the major components of telomerase uses the reverse transcriptase protein subunit (hTERT) to add repeats to the single-stranded telomere end. The second major component of telomerase is an endogenous 455-nucleotide RNA subunit (hTR), which is closely associated with hTERT and with other proteins [20, 21]. As tumor cells divide, telomerase is required for the continued proliferation of tumor cells to compensate for the loss of telomeres resulting from the end replication problem, the exonuclease processing of 5Vends of DNA, as well as by shortening owing to oxidative stress [22, 23]. In the absence of telomerase, chromosome ends shorten with each cell division, eventually resulting in growth arrest. This telomere length-initiated growth arrest is termed “replicative senescence” and is postulated to be a tumor-protective mechanism *in vivo* [24-26]. Telomerase is active in 85% to 90% of all human tumors but not in normal somatic cells, with the exception of germline and selective progenitor cell populations [9, 27]. It has been suggested that tumor cells have unlimited proliferative potential as a consequence of the reactivation or up-regulation of telomerase. Activation of telomerase does not promote carcinogenesis, it only allows a cell to continue dividing and attain proliferative immortality, a necessary step for the conversion of normal human cells into tumor cells [28, 29]. Although tumor cells express telomerase, they typically have a short but stable telomere length, whereas normal cells do not express telomerase and have long, slowly shortening telomeres. These differences between cancer cells and normal cells make the former more sensitive to telomerase inhibitors and may allow a substantial therapeutic window for telomerase inhibition-based treatments. These features make telomerase a potentially universal and relatively safe anti-cancer target [30].

Recently, several classes of telomerase inhibitors have been designed and evaluated. These include direct small-

molecule inhibitors [31-34], compounds targeting putative telomeric DNA G-quadruplex structures [35–38], dominant-negative hTERT genes [39], antisense oligonucleotides targeting hTR or hTERT mRNA [40, 41], ribozymes targeting hTR [42, 43] or hTERT mRNA [44, 45] and hTR-directed peptide nucleic acid oligonucleotides [46]. In this review, we focused on direct small-molecule inhibitors and established a screening method for inhibitors of telomerase catalytic activity using a modified version of the TRAP: we found rubromycins and fatty acids.

β-Rubromycin can inhibit the proliferation of cancer cells *in vitro* as assessed with the [^{3}H]-thymidine incorporation assay. When K-562 cells and HeLa cells were exposed to this compound for 3 days and 4 days, respectively, β-rubromycin significantly inhibited the proliferation of K-562 cells and HeLa cells with 50% lethal dose (LD_{50}) values of 19.5 and 22.7 μM, respectively. These findings suggested that the cytotoxic activity toward these human cancer cells might be caused by telomerase inhibition. In addition, when the cytotoxicity of β-rubromycin was examined by counting the cells under a microscope and by measuring the metabolic activity of cellular enzymes using tetrazolium dye, LD_{50} values obtained for K-562 cells and HeLa cells were comparable to those determined by the [^{3}H]-thymidine incorporation assay (data not shown). These findings suggested that the effect of this compound on the proliferation of these cancer cells was cytotoxic rather than cytostatic. In comparison, β-rubromycin did not influence the growth of normal human umbilical vein endothelial cells and human dermal fibroblasts with 48 h incubation (data not shown). These results suggested that direct small-molecule inhibitors of telomerase such as β-rubromycin would be potent anti-cancer chemotherapy drugs. Recently, the chemical synthesis of (±)-γ-rubromycin was achieved [47]. Therefore, the derivatives based on (±)-γ-rubromycin (including β-rubromycin) may provide valuable information for the development of drug design strategies. This compound could be effective anti-cancer chemotherapy in combination with oleic acid, which is a major food component and nutrient. Therefore, oleic acid might be a functional food for anti-cancer regimens.

CONCLUSION

β-Rubromycin and oleic acid were the strongest inhibitors of human telomerase among the rubromycin analogs tested and fatty acids investigated, respectively. They competed with the primer and dNTP substrate. Moreover, these compounds could be considered to bind directly to the primer and dNTP binding site in telomerase. In this review, 3D structure modeling analyses of these compounds were used to speculate a model of telomerase and its potent inhibitors. The energy-minimized 3D structures of β-rubromycin and oleic acid obtained by computer modeling are shown in Fig. (**5**). These compounds formed a "V-shaped curve" and the molecular length was 18.7–20.3 Å (Table **2**). The primer and dNTP binding sites of TERT in telomerase must have a "pocket" that can bind to these compounds. The size of the pocket must have sufficient length, width and depth to join to these inhibitor molecules. Also, the pocket must comprise hydrophilic amino acids to bind to the oligonucleotide primer/dNTP substrate as well as a hydrophobic polypeptide sheet to bind to the hydrophobic region of these compounds. Moreover, the carboxyl group of these compounds must bind to the hydrophilic amino acid in the pocket in competition with the substrate primer because the free carboxyl group was important for telomerase inhibition (Figs. (**2**) and (**3**)). These are predictable for future X-ray crystal and NMR analyses of telomerase.

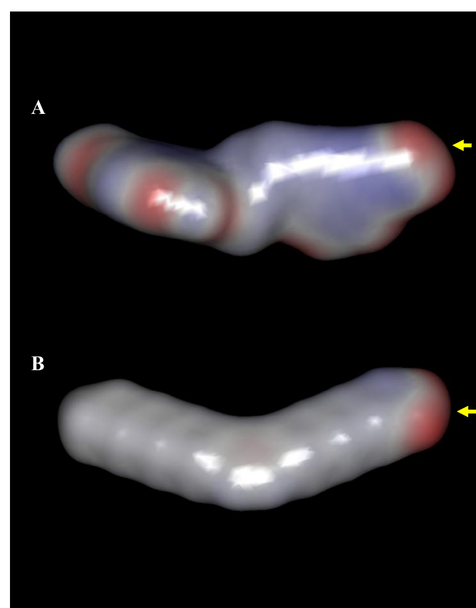

Fig. (5). Computer graphics of the energy-minimized three-dimensional structures of inhibitors of human telomerase. Electrostatic potential over molecular surfaces of β-rubromycin (**A**) and oleic acid (**B**). Blue areas are positively charged, red areas are negatively charged and white areas are neutral. Arrow indicates a carboxyl group. These panels were analyzed and built using Discovery Studio 2.5 modeling software (Accelrys, San Diego, CA, USA).

Table 2. Clog P Values, Calculated pKa Values and the Molecular Length, Width and Depth of the Three-Dimensional Structures of β-Rubromycin and Oleic Acid

Compound	Clog P	pKa	Length (Å)	Width (Å)	Depth (Å)
β-Rubromycin	3.654 ± 1.463	5.61 ± 0.40	18.7	6.5	6.5
Oleic acid	7.421 ± 0.199	4.78 ± 0.10	20.3	3.1	3.1

Unless otherwise noted, the Clog P values and pKa values of β-rubromycin and oleic acid were obtained from the calculated properties in SciFinder Scholar, which were originally calculated using Advanced Chemistry Development (ACD/Lab) Software V8.14 for Solaris (ACD/Labs). Energy-minimized three-dimensional structures of these compounds were prepared using Discovery Studio 2.5 modeling software (Accelrys, San Diego, CA, USA).

Telomerase is an attractive target for cancer chemotherapy. Therefore, clarifying the molecular mechanism of inhibition could provide clues to create an "ideal" artificial cancer chemotherapy agent. Therefore, 3D structural information on low-molecular-weight inhibitors of telomerase such as β-rubromycin and oleic acid may facilitate computer designs of new molecular probes capable of functioning as anti-neoplastic agents.

CONFLICT OF INTEREST

The authors declare that they do not have financial interests in any of the compounds reviewed in this article.

ACKNOWLEDGEMENTS

Y.M. acknowledges Grant-in-Aids for Scientific Research (C) (grant number 24580205) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) in Japan, Takeda Science Foundation (Japan) and the Nakashima Foundation (Japan). This research was supported in part by the MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2012-2016.

ABBREVIATIONS

3D	=	Three-dimensional
dNTP	=	2'-deoxyribonucleoside 5'-triphosphate
IC_{50}	=	50% inhibitory concentration
LD_{50}	=	50% lethal dose
RT-PCR	=	Reverse-transcription polymerase chain reaction
TERT	=	Telomerase reverse transcriptase
TRAP	=	Telomeric repeat amplification protocol
TR or TERC	=	Telomerase RNA

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Received: February 28, 2012 Revised: July 05, 2012 Accepted: July 05, 2012