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Research paper

Inhibition of mammalian thioredoxin reductase by black tea and its constituents: Implications for anticancer actions

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Abstract

Black tea is recently reported to have anti-carcinogenic effects through pro-oxidant property, but the underlying mechanisms remain unclear. Mammalian cytosolic thioredoxin reductase (TrxR1) is well -known for its anti-oxidation activity. In this study, we found that black tea extract (BTE) and theaflavins (TFs), the major black tea polyphenols, inhibited the purified TrxR1 with IC_{50} 44 μg/ml and 21 ± 1 μg/ml, respectively. Kinetics of TFs exhibited a mixed type of competitive and non-competitive inhibition, with K_{is} 4 ± 1 μg/ml and K_{ii} 26 ± 5 μg/ml against coenzyme NADPH, and with K_{is} 12 ± 3 μg/ml and K_{ii} 27 ± 5 μg/ml against substrate DTNB. In addition, TFs inhibited TrxR1 in a time-dependent manner. In an equilibrium step, a reversible TrxR1-TFs complex ($E * I$) forms, which is followed by a slow irreversible first-order inactivation step. Rate constant of the inactivation was 0.7 min^{-1}, and dissociation constant of $E * I$ was 51.9 μg/ml. Treatment of NADPH-reduced TrxR1 with TFs decreased 5-(Iodoacetamido) fluorescein incorporation, a fluorescent thiol-reactive reagent, suggesting that Sec/Cys residue(s) in the active site may be involved in the binding of TFs. The inhibitory capacity of TFs depends on their structure. Among the TFs tested, gallated forms had strong inhibitory effects. The interactions between TFs and TrxR1 were investigated by molecular docking, which revealed important features of the binding mechanism of theaflavins. An inhibitory effect of BTE on viability of HeLa cells was observed with IC_{50} 29 μg/ml. At 33 μg/ml of BTE, TrxR1 activity in HeLa cells was decreased by 73% at 22 h after BTE treatment. TFs inhibited cell viability with IC_{50} 10 ± 4 μg/ml for HeLa cells and with IC_{50} 20 ± 5 μg/ml for EAhy926 cells. The cell susceptibility to TFs was inversely correlated to cellular levels of TrxR1. The inhibitory actions of TFs on TrxR1 may be an important mechanism of their anti-cancer properties.

Keywords: Thioredoxin reductase; Black tea; Theaflavins; Anticancer; Pro-oxidant

1. Introduction

Mammalian cytosolic thioredoxin reductase (TrxR1) is a well-known antioxidant protein that regulates many cellular functions, including cell growth and apoptosis [1]. Up-regulation of TrxR1 is found to be associated with increased cell proliferation, transformation and anti-apoptosis [2], which occurs in certain types of tumor cells [3]. Recent research findings suggest the possible involvement of TrxR1 in tumor progression [4] and in drug-resistance of cancer cells [5]. TrxR1 is a homo-dimeric selenoprotein. Its active site contains

Abbreviations: AP-1, activator protein-1; ASK1, apoptosis signal-regulating kinase 1; BTE, black tea extracts; CL-TrxR1, calf liver thioredoxin reductase; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); IAF, 5-(Iodoacetamido) fluorescein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenol tetrazolium bromide; Ref-1, Redox Factor-1; rr-TrxR1, recombinant rat cytosolic thioredoxin reductase; Sec, selenocysteine; TFs, theaflavins; Trx, thioredoxin; TrxR, thioredoxin reductase.

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one disulfide in N-terminal −Cys−x−x−x−Cys− motif and one selenenylsulfide in C-terminal −Gly−Cys−Sec−Gly motif [6]. These two bridges undergo reversible reduction, which is involved in the catalytic mechanism of TrxR1 [7]. Interruption of the selenenylsulfide formation by site-directed mutagenesis or by thiol-modifying reagents abolished TrxR1 activity [8,9]. Some chemotherapeutic agents clinically used today, such as arsenic trioxide [10] and cis- diamminedichloroplatinum (II) [11], inhibit TrxR1 through the binding to the C-terminal Sec residue. Unfortunately, nearly all of the anticancer drugs in use today have the potential to produce significant toxic effects on normal healthy cells apart from the desired killing effects on cancer cells. Thus, it is necessary to reduce or eliminate noxious substances in the treatment of cancer in order to lessen the recovery period and potentially promote the efficiency of the treatment.

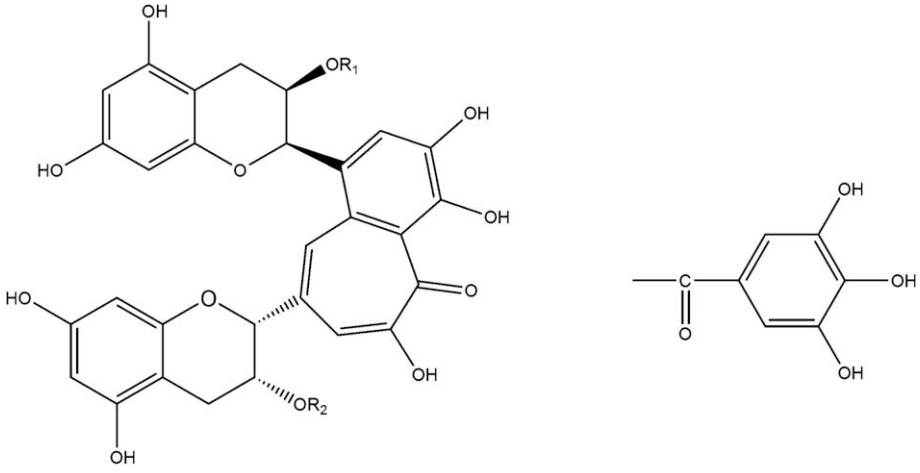
Tea (*Camellia sinensis*) is not toxic and tea consumption is suggested to prevent carcinogenesis. Of all tea products in the world, black tea accounts for 78% and is consumed in the largest quantity, especially in the Western countries [12]. Black tea polyphenols were found to strongly inhibit DNA synthesis in HTC rat hepatoma cells and DS19 mouse erythroleukemia cells [13]. Oral administration of black tea not only inhibited DNA synthesis, but also enhanced apoptosis in both non-malignant and malignant tumors in tumor-bearing mice [14]. The major black tea polyphenols are theaflavins (TFs, shown in Fig. 1), including theaflavin, theaflavin-3-monogallate, theaflavin-3′-monogallate and theaflavin-3,3′-digallate [15]. The key difference among the four compounds is the quantity and position of the galloyl moiety in the molecule. The galloyl moiety was essential for potent antioxidant activities of TFs [16]. The reduction potential of TFs was reported to be comparable to the well -known reducing agent alpha-tocopherol [17]. As oxidative stress is important in the pathogenesis of cancer, it is logical to consider that cancer chemo-preventive activities of TFs are due to their antioxidative properties [18]. However, recent research showed that certain TF induced generation of cellular reactive oxygen species (ROS), this oxidative stress is responsible for its inhibitory effect on tumor cell growth [19]. It would be considerable interest to reveal a physiological target of black tea and TFs.

In this study, we have evaluated the potential of black tea and TFs to inhibit TrxR1. The relationship between cellular TrxR1 activity levels and cell susceptibility to TFs is also evaluated by using the human endothelial EAhy926 cell line and the human cervical cancer HeLa cell line as models, because EAhy926 cell line has been proven to contain TrxR1 as a major selenoenzyme [20], and HeLa cell line has been found to be susceptible to TrxR1 inhibitors, such as 1-methyl-1-propyl-2-imidazolyl disulfide [5], curcumin [21] and mercury [22].

2. Materials and methods

2.1. Chemicals and reagents

5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB), NADPH, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenol tetrazolium bromide (MTT), 5-(Iodoacetamido) fluorescein (IAF) and TFs (mixture of theaflavin, theaflavin-3-monogallate, theaflavin-3′-monogallate and theaflavin-3,3′-digallate, purity ≥80%) were purchased from Sigma−Aldrich Co. (St Louis, MO, USA). IAF was dissolved in 50 mmol/l



Theaflavins Galloyl

Theaflavins	R1	R2
Theaflavin	H	H
Theaflavin-3-gallate	Galloyl-1	H
Theaflavin-3'-gallate	H	Galloyl-2
Theaflavin-3,3'-digallate	Galloyl-1	Galloyl-2

Fig. 1. Structure of theaflavins.