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Original article

Selenadiazole derivatives as potent thioredoxin reductase inhibitors that enhance the radiosensitivity of cancer cells

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ABSTRACT

Thioredoxin system is an attractive target to overcome radioresistance in cancer therapy. The redox enzyme thioredoxin reductase (TrxR) plays a vital role in restoring cellular thiol redox balance disrupted by radiation-induced reactive oxygens species (ROS) generation and oxidative damage. In this study, a series of 1,2,5-selenadiazoles have been synthesized and identified as highly effective inhibitors of TrxR to disrupt the intracellular redox balance, and thus significantly enhanced the sensitivity of cancer cells to X-ray. Upon irradiation, 1,2,5-selenadiazoles displayed a marked synergistic inhibitory effect on radioresistant A375 melanoma cell through enhancement of ROS overproduction, and subsequent induction of ROS-promoted apoptotic pathways, which triggered then mitochondrial dysfunction and caspase activation, finally resulted in augment of radiotherapeutic efficacy. Interestingly, we also found the interaction sites between 1,2,5-selenadiazole and the model peptide of TrxR, which can be confirmed by MALDI-ToF-MS. These results clearly demonstrate TrxR as a potential target for therapy of radioresistant cancers, and selenadiazole derivatives may be attractive radiosensitizing agent by targeting TrxR.

1. Introduction

Cancer is the second leading cause of death in the world after cardiovascular diseases. Today, millions of cancer people extend their lives attributing to early identification and treatment. Although radiation therapy is well-known to play an important role in cancer treatment, the common problem of resistance remains a major obstacle worthy of exploration [1,2]. Radiation-induced ionizations may act directly on the cellular component molecules or indirectly on water molecules, causing water-derived radicals, which react with nearby molecules in a very short time, resulting in oxidation of the affected molecules [3–5]. Reactive oxygen species (ROS) generated as a result of indirect damage is the principal mediator of radiation induced damage to biological systems. Generation of ROS creates oxidative stress and disturbs redox balance within the cells, triggering up-regulation of antioxidant systems [6] which conduce to the induction of resistance mechanisms. This makes the ubiquitous redox enzyme thioredoxin reductase an attractive drug target. In all thioredoxin system, thioredoxin (Trx) involves in redox reactions through its conserved active site with two thiol residues (–Cys–Gly–Pro–Cys–) which can be reduced by thioredoxin reductase (TrxR) using electrons from NADPH [7]. Previous works have showed that 1,2,5-selenadiazole derivatives exerted extensive biological activities against a wide variety of cancer cells [8,9], With TrxR-inhibition as the aim, drawing on these works, three novel benzimidazole group-containing 1,2,5-selenadiazole-oriented derivatives were designed and synthesized recently and were subjected to test for inhibition efficiency and specificity on A375 human melanoma cells, which have traditionally been considered radiation resistant so that radiation therapy has not commonly been employed to treat it [10]. In the present study, a series of 1,2,5-selenadiazole derivatives (Scheme 1) were synthesized and evaluated as *in vitro* radiosensitizer on the A375 cells as well as antiproliferation agents. The underlying molecular mechanisms accounting for the synergistic effects were also elucidated.

Chemoradiotherapy (CRT), the concurrent use of radiation therapy and chemotherapy, is a well-established field that in most cases works better than just using either of them alone and, actually contributes to the improvement in the overall health of the patient as well as extending the life expectancy in some patients

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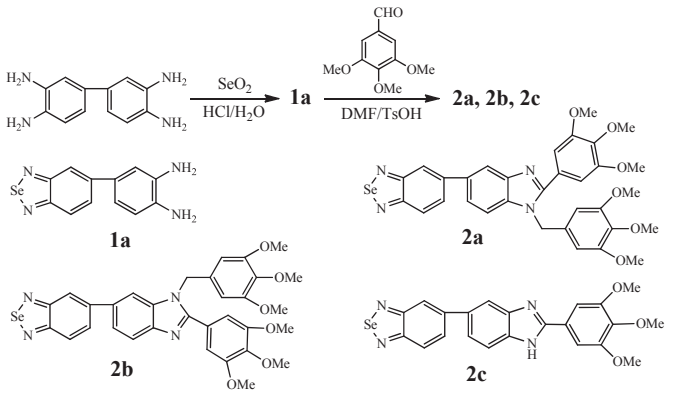

Scheme 1. General method for the synthesis of 1,2,5-selenadiazole derivatives (**1a**, **2a**, **2b** and **2c**).

[11,12]. Decades ago, researchers introduced a theoretical framework to describe the interaction of cytotoxic chemotherapy and radiotherapy [13], called "spatial cooperation", it proposed that the action of radiation and chemotherapeutic drugs is directed toward different target sites in the body and might interaction with each other. Radiation tends to target localized tumors, while chemotherapy drugs are likely to be more effective in eliminating micrometastases. However, relationship between radiotherapy and chemotherapeutic drugs in biological systems need to be further elucidated. In this study, a series of 1,2,5-selenadiazoles have been synthesized and identified as highly effective inhibitors of TrxR to disrupt the intracellular redox balance, and thus significantly enhanced the sensitivity of cancer cells to X-ray. Upon irradiation, 1,2,5-selenadiazoles displayed a marked synergistic inhibitory effect on radioresistant A375 melanoma cell through enhancement of ROS overproduction, and subsequent induction of ROS-promoted apoptotic pathways, which triggered then mitochondrial dysfunction and caspase activation, finally resulted in augment of radiotherapeutic efficacy. Taken together, these results clearly demonstrate TrxR as a potential target for therapy of radioresistant cancers, and selenadiazole derivatives may be attractive radiosensitizing agent by targeting TrxR.

2. Results and discussion

2.1. Chemistry

In terms of the synthesis of 1,2,5-selenadiazole derivatives, **1a** was synthesized by 3, 3'-Diaminobenzidine (214 mg, 1 mmol) and selenium dioxide (111 mg, 1 mol) in 0.2 N HCl. Importantly, the following condensation reaction of **a** and 3,4,5-Trimethoxybenzaldehyde (molar ratio: 1:1.2) which catalyzed by p-TSA using DMF as solution with aim of introduction of benzimidazoles group ultimately gained three products (Scheme 1). This is different from previous literatures [14] in terms of classical condensation reaction of o-phenylenediamine with aldehydes, which mostly acquired only one or two products. **2a** and **2b** are isomerides which were further separated by HPLC (Agilent Edipse XDB-C18, 21.2 × 250 mm, 7 μm) using water and acetonitrile (from 72:28 to 36:64, 8.0 ml/min) as mobile phase and ultraviolet as detector (320 nm).

2.2. In vitro anticancer activities of selenadiazoles in combination with X-ray

The antiproliferative activities of the 1,2,5-selenadiazole (**2a**, **2b** and **2c**) alone or in combination with X-ray (8 Gy) were firstly screened against A375 cells by means of MTT assay (Fig. 1A, B and C). The results showed that CRT had significantly greater inhibitory effects than using corresponding radiotherapy or chemotherapy alone on A375 cells. IC_{50} value of **2a**, **2b** and **2c** (Fig. 1D) reduced from 28.3 μg/ml, 26.4 μg/ml, 12.4 μg/ml to 9.2 μg/ml, 8.0 μg/ml and 2.2 μg/ml (18 h) respectively after combining with 8 Gy. Apparently, the dosage of agents decreased greatly, so that systemic toxicity was considerably lowered down while the antiproliferative activities of the agents were highly enhanced. Agents have brought great alteration into the IC_{50} value, especially for **2c**, which in combination with 8 Gy was only approximately one sixth (1/6) of free **2c**. In addition, **2a** and **2b**, which are structural isomerides and have extreme similarity in the chemical structure bore a remarkably close resemblance to each other in the inhibitory effect on cell viability. Morphological examination of the cells (Fig. 1E) after corresponding treatment showed the typical feature of cell death such as cell shrinkage and rounding up of the cells. Noteworthily, The structure of **2a**, **2b** or **2c** remained the same after irradiation.

Anticancer drugs exert at least part of their cytotoxic effect by triggering apoptosis in susceptible cells [15]. Therefore, flow cytometric analysis were performed to determine whether apoptosis was involved in cell death induced by 1,2,5-selenadiazoles and corresponding CRT. As shown in Fig. 2A and Sub-G1 cell populations (see Supporting information), there was hardly any apoptosis observed in A375 cells which exposed to 8 Gy alone without treatment with **2b** or **2c**, just 2.3%. Nevertheless, treatment with **2b** (10 μg/ml) alone only triggered 11.7% of apoptotic cells, amazingly, in the presence of 8 Gy, this section substantially increased to 57.6%. Likewise, treatment with **2c** (4 μg/ml), the proportion of apoptotic cells rose from 36.2% in the absence of 8 Gy to 57.1% in the presence of 8 Gy. This apparently indicated that in the presence of 8 Gy, they significantly increased the apoptosis-inducing effect in comparison with that in the absence of 8 Gy and definitely the apoptosis is the major mode of cell death induced by **2b** or **2c** in the absence or presence of 8 Gy.

2.3. Compounds (*2b*/*2c*) in combination with 8 Gy enhance the apoptosis-inducing effect

There are two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway [16]. Each requires specific triggering signals to begin an energy-dependent cascade of molecular events. Since that CRT could significantly amplify the *in vitro* anticancer and apoptosis-inducing effects of **2b** or **2c**, we subsequently conducted further experiments to understand the molecular mechanism.

Caspases, closely associated with apoptosis, are aspartate-specific cysteine proteases and members of the interleukin-1beta-converting enzyme family [17]. The caspase-cascade system plays central roles in the induction, transduction and amplification of intracellular apoptotic signals. In this study, activation of two initiator caspases: caspase-8 (Fas/TNF-mediated) caspase-9 (mitochondrial-mediated), and an executor caspase caspase-3 were therefore measured by fluorometric assay for the apoptotic program. Fig. 2B indicated that Exposure of A375 cells to **2c** (2 μg/ml) alone can increase the activation of caspase-3/8/9, resulted in the involvement of both intrinsic and extrinsic apoptotic pathways. Furthermore, treatment of **2c** in the presence of 8 Gy apparently enhanced the activation caspase-3/8/9 to a higher degree. These results were further confirmed by caspases and cleavage of PARP as examined by Western blotting. As shown in Fig. 2C, exposure of A375 cells to **2c** in the absence or presence of 8 Gy both caused increase in the activation of caspase-3, caspase-8 and caspase-9, and apparently the latter way was more noticeable,