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Exogenous 8-oxo-dG is not utilized for nucleotide synthesis but enhances the accumulation of 8-oxo-Gua in DNA through error-prone DNA synthesis

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Abstract

7,8-Dihydro-8-oxoguanine (8-oxo-Gua) and its nucleoside in cytosol are derived from the repair of oxidative DNA and the cleanup of oxidatively damaged DNA precursors, respectively. While the harmful effects of 8-oxo-Gua present in DNA have been studied extensively, few have reported its effects on cytosolic function. Our previous study showed that the addition of 8-oxo-dG to culture media caused an accumulation of 8-oxo-Gua in nuclear DNA in several leukemic cells including KG-1, which lack 8-oxoguanine glycosylase 1 (OGG1) activity due to mutational loss. However, the mechanism underlying 8-oxo-Gua level increases in DNA has not been addressed. In this study, we elucidated the metabolic fate of 8-oxo-Gua-containing nucleotide and the effect of exogenous 8-oxo-dG on DNA synthesis in KG-1 cells. We found that 8-oxo-dGMP was rapidly dephosphorylated to 8-oxo-dG rather than phosphorylated to 8-oxo-dGDP, thus indicating that 8-oxo-Gua-cotaining molecule is not used as a substrate for DNA synthesis in KG-1 cells. In fact, radiolabled 8-oxo-dG was incubated but radioactivity was not detected in nuclear DNA of KG-1 cells, showing that 8-oxo-dG is not directly incorporated into DNA. Interestingly, the activity of DNA polymerase β, which synthesize DNA with low fidelity increased in KG-1 cells treated with 8-oxo-dG, whereas the expression of DNA polymerase α decreased. In addition, the accumulation of 8-oxo-Gua in KG-1 DNA was completely inhibited by a specific inhibitor of DNA polymerase β. Thus, our findings address that the insertion of 8-oxo-dG into KG-1 DNA is not due to the direct incorporation of exogenous 8-oxo-dG, but rather to the inaccurate incorporation of endogenous 8-oxo-dGTP by DNA polymerase β. It further suggests that 8-oxo-dG in the cytosol may function as an active molecule itself and perturb the well-defined DNA synthesis.

Keywords: DNA polymerase β; OGG1; Oxidative stress; 8-oxo-deoxyguanosine

1. Introduction

7,8-Dihydro-8-oxoguanine (8-Oxoguanine; 8-oxo-Gua) is one of most abundant oxidative DNA adducts and thus is used as a marker of oxidative DNA damage [1]. 8-oxo-Gua in DNA is promutagenic since it

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promotes the incorporation of dATP instead of dCTP opposite this lesion during replication, inducing a GC to TA transversion [2,3]. 8-oxo-Gua in DNA also has an oxidative damage-inducing effect on the neighborhood bases due to its electron-rich nature [4,5]. In addition, 8-oxo-Gua in DNA can induce the formation of reversible topoisomerase1-linked DNA single-stranded breaks [6,7]. Therefore, 8-oxo-Gua in DNA is a harmful residue that can lead to carcinogenesis and potentially perturb cell functions to cause even cell death. However, cells are equipped with repair systems to removes 8-oxo-Gua in DNA by OGG1 (which repairs 8-oxo-Gua:C) [8], OGG2 (8-oxo-Gua:A) [9], and NTH1 (8-oxo-Gua:G) [10]. In addition, nucleotide excision repair [11] and endonuclease [12] are found to finally generate a mononucleoside form, 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG).

In contrast to 8-oxo-Gua in DNA, we do not have information on the actions of cytosolic 8-oxo-Gua-containing nucleoside, for example, 8-oxo-dG. In fact, nucleosides of purine and pyrimidine analogues have been introduced as chemotherapeutic agents, which are 2-chloro-2′-deoxyadenosine (CdA), 2-fluoro-9-β-D-arabionofuranosyladenine (F-AraA), 1-β-D-arabiofuranosylcytosine (AraC), 2′,2′-difluorodeoxycytidine (dFdC), and 9-β-D-arabionofuranosylguanine (AraG) [13,14]. These analogues are taken up into cells and phosphorylated to nucleoside triphosphates. The phosphorylated analogues can exert cytotoxic activity by being incorporated into DNA and RNA and altering their structures, by interfering with various enzymes involved in the synthesis of nucleic acids such as DNA polymerases and ribonucleotide reductase, or by modifying the metabolisms of physiological nucleosides [15]. These actions result in the inhibition of synthesis of nucleic acids and eventually cell death. Therefore, we hypothesized that 8-oxo-dG can be also cytotoxic possibly due to its incorporation into DNA.

Our previous study showed that exogenous 8-oxo-dG added to KG-1 cell (a human acute leukemia cell) in culture, caused G1 cell cycle arrest, apoptotic cell death, and accumulation of 8-oxo-Gua in nuclear DNA [16,17]. This cell is deficient in OGG1 (8-oxoguanine glycosylase 1, a repair enzyme of 8-oxo-Gua) activity and thus is incapable of removing 8-oxo-Gua from DNA. Other leukemic cell lines with low OGG1 activity, i.e. H9, CEM-CM3, and Molt-4, were also found to have similar cytotoxic responses to exogenous 8-oxo-dG, whereas U937 or Jurkat cells with normal OGG1 activity were not [17]. The cytotoxicity induced by 8-oxo-dG could be explained due to a low capacity of OGG1 to cope with an accumulation of 8-oxo-Gua in DNA.

However, little information is available regarding the mechanism by which exogenous 8-oxo-dG increases the level of 8-oxo-Gua in DNA. In this study, we examined whether 8-oxo-Gua-containing molecules such as 8-oxo-dGMP is utilized as a substrate for the enzymes involving in DNA synthesis and then directly incorporated into DNA, or whether 8-oxo-dG indirectly affects DNA synthesis. We found that 8-oxo-dGMP was not phosphorylated into 8-oxo-dGDP but instead, dephosphorylated to 8-oxo-dG and confirmed that exogenous 8-oxo-dG was not directly incorporated into DNA. However, the KG-1 treated with exogenous 8-oxo-dG showed the increase of DNA polymerase β activity and the increase of 8-oxo-Gua in DNA by added 8-oxo-dG was blocked by dideoxythymidine, a DNA polymerase β inhibitor. These results suggest that 8-oxo-dG is not metabolized and itself can cause the accumulation of 8-oxo-Gua in DNA through the activation of non-replicative DNA synthesis, which may enhance the inaccurate incorporation of endogenous 8-oxo-dGTP into DNA.

2. Materials and methods

2.1. Materials and cell lines

2′-Deoxyguanosine (dG), 7,8-dihydroxy-8-oxo-2′-deoxyguanosine (8-oxo-dG), deoxyguanosine 5′-triphosphate (dGTP), 2′,3′-dideoxythymidine, guanylate kinase, nucleotidase, purine nucleoside phosphorylase and nuclease P1 were obtained from Sigma. 5′-[α-^{32}P]-dGTP (800 Ci/mmol), [1′,2′-^{3}H]-dGTP (40 Ci/mmol), and deoxy[methyl-^{3}H] thymidine 5′-triphosphate (dTTP) were purchased from Amersham Pharmacia Biotech. Alkaline phosphatase was purchased from Roche Molecular Biochemicals. U937 and KG-1 cells were obtained from the American Type Culture Collection and maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum at 37 °C in 5% CO_2.

2.2. Preparation of radiolabeled 8-oxo-dG and 8-oxo-dGMP

^{3}H-labeled 8-oxo-dG was prepared by the oxidation of ^{3}H-labeled-dG according to the modified procedure [18]. α-^{32}P-labeled 8-oxo-dGMP was prepared by the oxidation of α-^{32}P-labeled dGTP and its subsequent dephosphorylation of oxidized dGTP according to the previously described procedure [19,20]. All radiolabeled nucleosides and nucleotides were purified by high performance liquid chromatography (HPLC).

2.3. Guanylate kinase assay

8-oxo-dGMP was tested whether this oxidized nucleotide can be a substrate for guanylnate kinase using crude cell extract of KG-1 cells as an enzyme source. To prepare crude cell extract, KG-1 cells (2×10^{8}) was suspended in a lysis buffer