Protective effects of Ursolic acid and Luteolin against oxidative DNA damage include enhancement of DNA repair in Caco-2 cells.

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Abstract

Consumption of fruits and vegetables is associated with a reduced risk of developing a wide range of cancers including colon cancer. In this study, we evaluated the effects of two compounds present in fruits and vegetables, ursolic acid, a triterpenoid, and luteolin, a flavonoid, on DNA protection and DNA repair in Caco-2 cells using the comet assay.

Ursolic acid and luteolin showed a protective effect against H_2O_2-induced DNA damage. To evaluate effects on induction of base oxidation, we exposed cells to the photosensitiser Ro 19-8022 plus visible light to induce 8-oxoguanine. Luteolin protected against this damage in Caco-2 cells after a short period of incubation. Repair rate was increased by pre-treatment of cells for 24h with ursolic acid or luteolin (rejoining of strand breaks) in Caco-2 cells after treatment with H_2O_2. We also measured the incision activity of a cell extract from Caco-2 cells treated for 24h with test compounds on a DNA substrate containing specific damage (8-oxoGua), to evaluate effects on base excision repair activity. Preincubation for 24h with ursolic acid enhanced incision activity in Caco-2 cells. In conclusion, we demonstrated for the first time that ursolic acid and luteolin not only protect DNA from oxidative damage but also increase repair activity in Caco-2 cells. These effects of ursolic acid and luteolin may contribute to their anti-carcinogenic effects.

Keywords: ursolic acid; luteolin; DNA oxidation; antioxidants; DNA repair; comet assay.

1. Introduction

Colorectal cancer (CRC) is one of the main causes of cancer-related mortality in the western world and was the second most common cancer in Europe in 2006 [1]. Oxidative stress, defined as a disturbance in the equilibrium status of pro-oxidant and antioxidant systems in favour of pro-oxidant, can damage diverse cellular macromolecules such as DNA, lipids, and proteins. The various types of DNA damage that can be generated as a result of oxidative attack, if not properly removed, can lead to mutagenesis and/or cell death. 8-oxo-7,8-dihydroguanine (8-oxoGua) is one of the most abundant forms of DNA oxidation and can cause G to T transversions in several oncogenes and tumour suppressor genes [2]. The major mechanism repairing DNA oxidation damage is the base excision repair (BER) pathway. In BER, DNA glycosylases are responsible for cleavage of the *N*-glycosidic bond between the base and the pentose sugar, removing modified DNA bases and creating an apurinic or apyrimidinic site (AP site). Endonucleolytic activity of the glycosylases or an AP-endonuclease transforms AP sites to gaps in DNA that are filled by a DNA polymerase and sealed by a DNA ligase [3,4]. In the present study we have evaluated effects of two phytochemicals found in fruits, vegetables and spices on DNA oxidation and DNA repair.

Accumulating evidence from epidemiological studies as well as laboratory data suggest that consumption of fruits and vegetables is associated with a reduced risk of developing a wide range of cancers including colon cancer [5,6]. Dietary strategies for cancer prevention are considered attractive alternatives because the consumption of natural compounds with potential chemopreventive effects is associated with low toxicity, safety and good acceptance by the public [7,8].

Ursolic acid (UA), a natural pentacyclic triterpenoid acid, is widespread in nature and abundant in certain medicinal plants. UA has been reported to possess a wide range of biological activities, such as anti-inflammatory, anticarcinogenic, antihyperglycemic, hepatoprotective and neuroprotective activities [9-12].

Luteolin (Lut) is a flavons, a subclass of flavonoids, found in fairly large amounts in fruits, vegetables, olive oil, red wine and tea. Many studies have shown that Lut exhibits a variety of pharmacological activities, including anti-inflammatory, antibacterial,

antioxidant and anticancer activities [13-16]. Contrarily to Lut, UA is not an antioxidant at relevant cellular redox conditions.

Protection of DNA from damage and modulation of DNA repair enzyme capacities may be assumed to contribute to protection against mutations and to maintenance of genomic stability. In the current study we evaluated DNA-protective and repair-enhancing effects of Lut and UA in human colon cells (Caco-2) exposed to oxidative agents. DNA damage was evaluated by alkaline single-cell gel electrophoresis (comet assay). BER of oxidised DNA was measured using an *in vitro* assay for incision activity of a cell extract, incubated with a substrate containing oxidised DNA bases [17]. We also assessed the ability of cells to rejoin strand breaks induced in DNA by H_2O_2. UA and Lut were used in concentrations likely to be attained in gut when humans have a diet rich in fruits and vegetables.

2. Material and methods

2.1. *Chemicals*

UA (purity ≥ 90%), hydrogen peroxide, Dulbecco's Modified Eagle Medium (DMEM), penicillin/streptomycin, trypsin solution and 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Lut (purity > 90%) was from Extrasynthese (Genay, France). Fetal bovine serum (FBS) was purchased from Biochrom KG (Berlin, Germany). Ro (photosensitizer Ro19-8022) was from F.Hoffmann-La Roche (Basel, Switzerland). SYBR Gold (nucleic acid gel stain) was from Invitrogen Molecular probes (Oregon, USA). All other reagents and chemicals used were of analytical grade.

2.2. *Cell culture*

Caco-2 cells (derived from human colon carcinoma) were maintained as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and antibiotics (100U/ml penicillin and 100µg/ml streptomycin), under an atmosphere of 5% CO_2 at 37ºC. Cells were trypsinised when nearly confluent.

Cells were seeded onto 12-well plates, with 1 ml/well at a density of 0.2×10^6 cells/ml, and incubated with different concentrations of test compounds in complete DMEM medium to test for possible direct cytotoxicity, genotoxicity, and for effects on induced DNA oxidation, and for modulation of DNA repair. Stock solutions of UA and Lut were prepared in dimethyl sulphoxide (DMSO) and aliquots kept at -20ºC. The final concentration of DMSO in medium was <0.5%).

2.3. *Cell toxicity assay*

The test compound's cytotoxicity was assayed in 12-multiwell culture plates seeded with $0.\ 2 \times 10^6$ cell /well. Twenty-four hours after plating, the medium was discarded and fresh medium containing test compounds at different concentrations was added. After 48h of incubation with test compounds, cytotoxicity was evaluated by MTT test. The number of viable cells in each well was estimated by the cell capacity for reduction of MTT as described by [18]. The results were expressed as a percentage of cell viability relative to control (cells without any test compound).

2.4. *Comet assay*

The alkaline version of the single cell gel electrophoresis assay was used to evaluate DNA damage as previously described [19] with some modifications. Briefly, Caco-2 cells were trypsinized, washed, centrifuged, and the pellet suspended in low melting point agarose; about $2x10^4$cells were placed on a slide (pre-coated with 1% normal melting point agarose and dried), and covered with a coverslip. After 10 min at 4 ºC, the coverslips were removed and slides were placed in lysis solution (2.5M NaCl, 100mM Na_2EDTA, 10mM Tris Base, pH 10 plus 1% Triton X-100) for 1h at 4ºC. When oxidised bases were to be measured, after lysis slides were washed three times with buffer (40mM HEPES, 0.1M KCl, 0.5mM EDTA, 0.2mg/ml BSA, pH 8.0) and incubated with 30µl of formamidopyrimidine DNA glycosylase (FPG) in this buffer or with buffer only for 20 min at 37ºC. Slides were then placed in horizontal electrophoresis chamber with electrophoresis solution (300mM NaOH, 1mM Na_2EDTA, pH >13) for 30 min at 4ºC for the DNA to unwind before electrophoresis was run for 30 min at 25V and ~300mA. After electrophoresis, slides were washed two times with PBS and dried at room temperature. For analysis of the comet images, slides

were stained with SYBR Gold solution for 30 min at 4ºC; after drying, slides were analysed in a fluorescence microscope and Comet 4 analysis system (Perceptive software) was used to calculate the parameter % tail intensity. Generally, 100 randomly selected cells are analyzed per sample.

2.5. *Genotoxic effects of UA and Lut*

Caco-2 cells were incubated for 24h at 37ºC with UA and Lut at different concentrations. Cells were collected by trypsinization and DNA damage (strand breaks, SBs) was evaluated by the alkaline version of the comet assay. Digestion with FPG allowed detection of oxidized purines [20].

2.6. *Effects of UA and Lut on DNA oxidation.*

To evaluate protection against oxidative damage, Caco-2 cells were preincubated with 5 and 10 µM UA or 10 and 20 µM Lut for 24h (long period of incubation) or 2h (short period of incubation) at 37ºC. Cells were washed with PBS and treated with H_2O_2 (75 µM in PBS) for 5min on ice to induce SBs, or with 1µM Ro (photosensitizer Ro19-8022, prepared in PBS from a stock solution at 1 mM in ethanol) plus visible light from a 500 W tungsten-halogen source (1.5min on ice) at 33cm to induce 8-oxoGua. DNA damage (SBs and 8-oxoGua) was evaluated by the comet assay without or with FPG, respectively.

2.7. *Effects of UA and Lut on cellular repair.*

In the cellular repair assay two different treatment regimes were used: First, pre-treatment with UA or Lut followed by exposure to H_2O_2 and recovery in fresh medium. Caco-2 cells were preincubated with UA or Lut for 24h at 37ºC. Cells were washed with PBS and treated with H_2O_2 (75 µM) for 5 min on ice to induce SBs. The H_2O_2 was removed and cells were washed with PBS and then incubated in fresh culture medium for 0, 10, 30 or 60 min at 37ºC. Thus we evaluated the effect of pre-incubation in UA or Lut on the ability of cells to rejoin SBs [19]. In the second approach, to look for a possible direct effect of UA or Lut on enzyme activity, H_2O_2 treatment was performed before cells were incubated with the test compounds. Briefly, Caco-2 cells were treated

with H_2O_2 (75 µM) for 5 min on ice to induce SBs. Cells were washed with PBS to remove H_2O_2 and then incubated with UA or Lut for 0, 10, 30 or 60 min at 37ºC. Results were expressed as % of repair DNA damage that was calculated using the follow formula:

% of repair DNA damage = $(T_0 - T_{30})/ (T_0 - C_{30}) \times 100$; where T_0 represents DNA damage before recovery period, T_{30} represents DNA damage after 30 min of recovery and C_{30} represents DNA damage of the control after 30 min of recovery.

2.8. *Effects of UA and Lut on BER (in vitro assay)*

This assay measures the excision repair activity of an extract prepared from cells treated with test compounds by providing the extract with a DNA substrate (agarose-embedded nucleoids) containing specific damage [17]. In this case, the substrate DNA was from cells previously exposed to Ro plus visible light to induce 8-oxoGua that is repaired by BER, and was prepared as described by Gaivão et al. [21]. Incision at damage sites, detected using the alkaline comet assay, indicates the capacity of glycosylase in the extract to initiate BER.

2.8.1. *Cell extract preparation*

Extracts were prepared as described previously [17] with some modifications. Briefly, for extract preparation, Caco-2 cells were incubated with 10µM UA, 10µM Lut or 0.5% DMSO (control) for 24h at 37ºC. Cells were washed with PBS, trypsinized and resuspended in PBS. Cells were divided into aliquots of $1x10^6$cells in 1ml and after centrifugation (14000g; 5min at 4ºC) the dry pellets were frozen in liquid nitrogen and stored at -80 ºC.

2.8.2. *Substrate preparation*

Substrates for BER assay were preparated as described previously [17]. Briefly, HeLa cells cultivated in flasks, when near to confluence were treated with Ro plus visible light (5 min at 33cm on ice) to induce 8-oxoGua. Cells were washed with PBS, trypsinised and resuspended in medium. Cells were centrifuged, the pellet resuspended in freezing medium (DMEM medium supplemented with 20% FBS and 10% DMSO)

and aliquots of $1x10^6$cells in 1ml frozen slowly and stored at -80ºC. HeLa cells without Ro treatment were also frozen in freezing medium and stored at -80ºC.

2.8.3. *Substract incubation with cell extract*

On the day of the experiment, extracts were resuspended in 65 µl of extraction buffer (45mM Hepes, 0.4M KCl, 1mM EDTA, 0.1mM dithiothreitol and 10% glycerol, pH 7.8) plus Triton X-100 (0.25%), mixed 5sec on vortex at top speed and incubated 5 min on ice. After centrifugation (~14,000xg, 4ºC, 5min) 55 µl of supernatant was removed and mixed with 220µl of cold reaction buffer (40mM HEPES, 0.1M KCl, 0.5 mM EDTA and 0.2mg/ml bovine serum albumin, pH 8). Two gels per slide containing $2x10^4$ substrate cells /gel (with or without treatment with Ro) were placed on slides pre-coated with normal melting point agarose and lysed for 1h. Slides were washed three times with reaction buffer and 30µl of extract was added to each gel and incubated 20 min at 37ºC in a moist box. FPG and reaction buffer were included as positive and negative controls, respectively. After incubation, slides were transferred immediately to alkaline electrophoresis solution and the normal comet assay was run [17, 22.]

2.9. *Statistical analysis*

Results were expressed as mean ± SEM at least 3 independent experiments. Significant differences ($P<0.05$) were evaluated by Student's t-test.

3. Results

3.1. *Cytotoxic effects of UA and Lut*

In order to choose the concentrations of UA and Lut that can be used in protective studies, evaluations of test compounds' toxicity were done using MTT test. When Caco-2 cells were incubated for 48h, UA and Lut significantly decreased cell viability only at concentrations higher than 50 and 100µM, respectively (Fig. 1). For the follow experiments non-cytotoxic concentrations of UA and Lut were used.

3.2. *Genotoxicity of UA and Lut*

The effects of UA and Lut on induction of SBs and oxidized bases were evaluated. For this, Caco-2 cells were incubated with UA (5 and 10µM) or Lut (10 and 20µM) for 24 h at 37ºC and DNA damage assessed by the comet assay with and without FPG treatment. At tested concentrations UA and Lut did not induce either SBs or oxidized purines (FPG-sensitive sites) (Fig.2).

3.3. *Effects of UA and Lut on oxidatively induced-DNA damage.*

To evaluate possible effects of UA or Lut on oxidatively induced-DNA damage, Caco-2 cells were incubated for 24h (a long) or 2h (a short) periods with the compounds before treatment with H_2O_2 or Ro.

Both 5µM UA and 20µM Lut, with a long period of incubation, significantly decreased DNA SBs induction by H_2O_2 (Fig.3A). With a short period of incubation (2h), the effects of UA and Lut were even more pronounced (Fig.3B). The protective effects were not dose-dependent.

In the assay with Ro plus light, Caco-2 cells were also pre-treated for a long or short period with UA or Lut. With a long period of incubation, compounds at tested concentrations did not protect DNA from damage induced by Ro (Fig.4A). With a short incubation Lut significantly decreased oxidized DNA bases induced by Ro, while UA (10 µM) showed a tendency to protect Caco-2 cells (Fig.4B).

3.4. *Effects of UA and Lut on repair ability.*

3.4.1. *Cellular repair assay*

The ability of Caco-2 cells to rejoin strand breaks induced by H_2O_2 was assessed by measuring damage remaining at different times of recovery (0, 10, 30 and 60 min). SBs decreased with the time of recovery and at 60 min the levels of SBs were similar to the control (without H_2O_2 treatment) (data not shown). To assess effects of the test compounds on the ability of Caco-2 cells to rejoin DNA strand breaks, two different treatments were used. First, cells were treated with compounds for 24 h before H_2O_2 exposure and recovery in fresh medium for 30 min at 37ºC. For recovery time we chose 30 min because it is within the linear phase of SB repair (Fig. 5A). Caco-2 cells treated only with H_2O_2 (control cells), after 30 min of recovery had rejoined ~ 50% of SBs.

Cells pre-incubated with 5µM UA or 10µM Lut had rejoined 86% and 88% respectively, representing a relative increase in the extent of DNA rejoining of 65% and 68% compared with the control cells, respectively. The highest concentrations of UA and Lut show a tendency ($p \leq 0.1$) to increase the ability to rejoin SBs (Fig.5B).

In the second treatment, cells were incubated with test compounds for different times after H_2O_2 exposure. No difference was found when cells were incubated with compounds during the recovery period when compared with cells incubated with fresh medium after H_2O_2 exposure (data not shown) indicating the absence of any direct influence on repair enzymes.

3.4.2. *BER activity measured in vitro*

The ability of Caco-2 cells to repair oxidised bases by BER was measured by a modified comet assay, the *in vitro* BER assay. In this assay a DNA substrate containing specific damage, 8-oxoGua, induced by Ro plus visible light was incubated with an extract of Caco-2 cells (treated with UA or Lut for 24h at 37 ºC). Figure 6 shows, first, that substrate when treated with FPG (positive control) increased SBs detected by comet assay compared with substrate incubated only with reaction buffer (negative control). Second, extract from Caco-2 cells treated only with DMSO led to an increase in SBs in substrate DNA when compared with the negative control. This means that the extract from Caco-2 cells has BER activity. And third, extracts obtained from cells pre-treated with 10 µM UA showed significantly increased excision repair activity, by 24% when compared with an extract of Caco-2 cells treated with DMSO, while repair activity was not significantly affected by pre-treatment with Lut. There was no increase in SBs when extracts were incubated with substrate without 8-oxoGua (data not shown), indicating that the increase of breaks observed for UA corresponds to 8-oxoguanine DNA glycosylase 1 (OGG1) enzyme activity and confirming the absence of nonspecific nucleases in cell extracts.

4. Discussion

The integrity of DNA is critically important for DNA replication and cell division. Oxidative DNA damage in addition to a defective DNA repair mechanisms are known to be associated with carcinogenesis [23, 24]. Dietary antioxidants have the possibility

to prevent oxidation, but this requires that they are in proximity to the DNA in an active form. Several authors have reported that a compound's lipophilicity is a determinant characteristic for biological activity of the compounds. UA and Lut represent two classes of phytochemicals with different chemical and biological properties. Lut has free radical scavenging activity, whereas UA is virtually inactive as a free radical scavenger. Both are, however, highly lipophilic [25-27]. We evaluated the effects of both compounds, UA and Lut, against oxidative damage in Caco-2 cells at two levels: DNA protection and DNA repair. In this work, we show that ursolic acid and luteolin not only protect DNA from oxidative damage after a short period of pre-incubation but also increase repair activity in Caco-2 cells.

Concerning DNA protection, after a short incubation period (2 h) UA and Lut had a strong protective effect against H_2O_2-induced DNA damage. After a long period of incubation (24 h) both compounds showed a protective effect, but the percentage protection was smaller than with a short incubation period. In a previous paper, we showed that UA had chemoprotective activity against *t*BHP-induced DNA damage in HepG2 cells [24]. Our results are in agreement with other reports that also show that UA protects against H_2O_2-induced DNA damage [28,29] and decreased the level of AZT (3'-azido-3'-dideoxythymidine)-induced SBs in Caco-2 and HepG2 cells [30]. The protective effect of Lut against H_2O_2-mediated DNA SBs in Caco-2 cells is also in agreement with results obtained with other cell lines [26,31-35].

Besides DNA SBs, 8-oxoGua is one of the most abundant forms of oxidative damage and has been shown to cause G to T transversions. To evaluate effects on DNA protection against 8-oxoGua formation, we exposed Caco-2 cells to Ro plus visible light (to induce 8-oxoGua). Lut protected against Ro-induced DNA damage in Caco-2 after a short period of pre-incubation while UA showed a similar tendency. However, this protective effect was not observed with a long period of pre-incubation for either compound. Moon et al. [36] reported that dietary antioxidants such as quercetin, rutin and resveratrol as well as UA inhibit single strand breaks and 8-oxoGua in U937 cells exposed to 3-morpholinosydnomine N-ethylcarbamide (SIN-1). The protective effects of Lut against 8-oxoGua found in Caco-2 are in agreement with others authors. Cai et al. [37] showed that Lut, quercetin and genistein decrease oxidative damage to DNA, and among the test compounds, Lut had the most potent quenching effect on Fenton reaction-induced 8-oxoGua formation. Also Min and Ebeler [38] showed that several

flavonoids including Lut slightly inhibited 8-oxoGua formation in calf thymus DNA at low, physiologically relevant concentrations.

Phytochemicals such as flavonoids and triterpenoids can act as antioxidants in cells by modulating the activity of enzymatic and non-enzymatic cellular antioxidants and activating (phase I) enzymes and detoxifying (phaseII) enzymes involved in xenobiotic metabolism [24,39,40].

The protective effect of UA has been attributed to the ability of UA to increase levels of non-enzymatic antioxidants such as glutathione (GSH) and to increase the activity of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) [29,41,42]. Martin-Aragon et al. [43] reported that UA restores hepatocyte antioxidant levels preventing carbon tetrachloride-induced liver damage. Also, Saravan et al. [44] showed that UA has a hepatoprotective effect against chronic ethanol-mediated toxicity in rats. UA increased levels of circulatory antioxidants such as reduced glutathione, ascorbic acid and alpha-tocopherol improving the antioxidant status of alcoholic rats.

Németh et al. [45] reported that Lut and quercetin were incorporated in small intestinal epithelial cells and located in the nuclei, decreasing 8-oxoGua formation. Lut has been reported as able to modulate antioxidant status, increasing the activities of antioxidant enzymes GPX, glutathione-S-transferase (GST), glutathione reductase (GR), SOD and CAT or attenuating the decrease of antioxidant levels (e.g. GSH) induced by toxic agents [14,26,46].

In our study Lut and UA seem to exert effects through cellularly mediated mechanisms that can be lost with time. Despite the differences of antiradical capacity between the two compounds, both showed a strong protector effect against oxidation of DNA, reinforcing the notions that cellularly mediated effects and the degree of hydrophobicity and consequently uptake into the cell are important factors to be taken into account when assessing the effectiveness of antioxidant protection.

DNA damage combined with defects in repairing oxidative damage to DNA has been associated with a development of several diseases including cancer [47,48]. Cells have multiple DNA repair pathways for specific classes of lesions that mitigate the deleterious consequences of damage accumulation. Effects of natural compounds on DNA repair are still poorly understood; some reports show that polyphenols such as

curcumin and quercetin increase DNA repair activity [27,49]. To our knowledge, there are no studies reporting the effects of UA and Lut on DNA repair activity in colon cells.

In our present study, 24 h of pre-treatment with UA or Lut increased the rate of rejoining of strand breaks in Caco-2 cells after treatment with H_2O_2. However, when cells were incubated with test compounds after H_2O_2-induced damage, no such effects were observed. This suggests an effect of the compounds on induction of repair activity not due to direct interactions between UA or Lut and the repair enzymes.

The major mechanism that cells use to repair oxidative damage lesions is the BER pathway. Here, we have measured the incision activity of a cell extract from Caco-2 cells treated for 24 h with test compounds on a DNA substrate containing specific damage (8-oxoGua), to evaluate induction of BER activity. For the first time we report that UA, but not Lut, has a BER-inductive effect, increasing incision activity in Caco-2 cells. In accordance with our results, Silva et al. [34] did not find effects of Lut on BER activity in neuronal cells. However, Leung et al. [50] found that Lut increased the mRNA expression of DNA base excision repair enzymes, such as hOGG1 and apurinic endonuclease in human lung carcinoma cells.

In summary, we demonstrated for the first time that UA and Lut not only protect DNA from oxidative damage but also increase repair activity in Caco-2 cells. These protective effects of UA and Lut may contribute to their anti-carcinogenic effects. Modulation of DNA repair by these compounds and other phytochemicals needs to be further explored. *In vivo* studies in animals or humans, making use of functional biomarker assays such as the comet assay can provide a better understanding of the potentially important impact of phytochemicals on DNA repair pathways and cancer prevention.

Conflict of interest

There are no conflicts of interest to report.

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Figure legends

Figure 1 – Effects of UA and Lut on cellular viability (as % of control) of caco-2 cells as measured by MTT test. Results are expressed as mean ± SEM, of at least three independent experiments.

Figure 2 – DNA damage (SBs and FPG-sensitive sites) in Caco-2 cells treated for 24h with UA and Lut. Results are expressed as mean ± SEM, of at least three independent experiments.

Figure 3 – Effects of 24h (A) or 2h (B) of treatment with UA or Lut on DNA damage induced by 75µM H_2O_2 (5 min, on ice) in Caco-2 cells. Results are expressed as mean ± SEM, of at least three independent experiments.

Figure 4 – Effects of 24h (A) or 2h (B) of treatment with UA or Lut on DNA damage induced by 1µM Ro19-8022 plus light (1.5min, on ice) in Caco-2 cells. Results are expressed as mean ± SEM, of at least three independent experiments.

Figure 5 – Kinetic of SBs rejoining (A); and extent of repair of H_2O_2–induced damage in Caco-2 cells after preincubation with UA or Lut (B). Results are expressed as mean ± SEM, of at least three independent experiments.

Figure 6 – *In vitro* DNA repair: incision by extracts from Caco-2 cells pre-incubated with 10µM of UA and Lut. Extracts were incubated for 20min with gel-embedded nucleoid DNA containing 8-oxoGua lesions. Results are expressed as mean ± SEM, of four independent experiments.

Figure 1

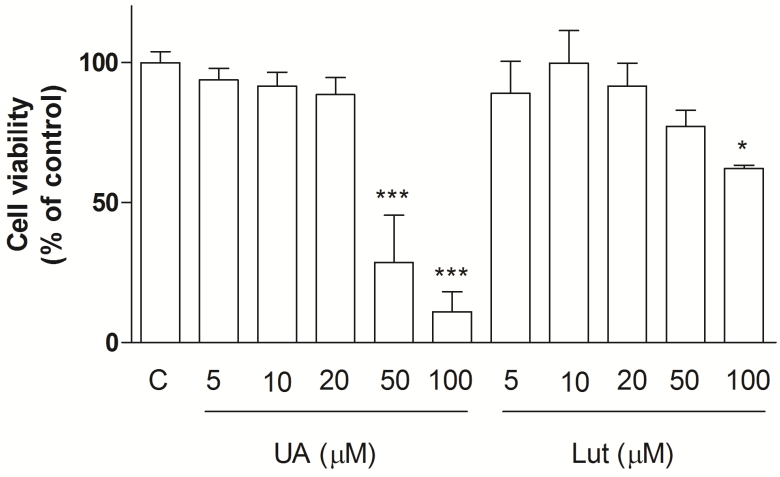
Cell viability
(% of control)
100
50
0
***
***
*
C
5
10
20
50
100
5
10
20
50
100
UA (μM)
Lut (μM)

Figure 2

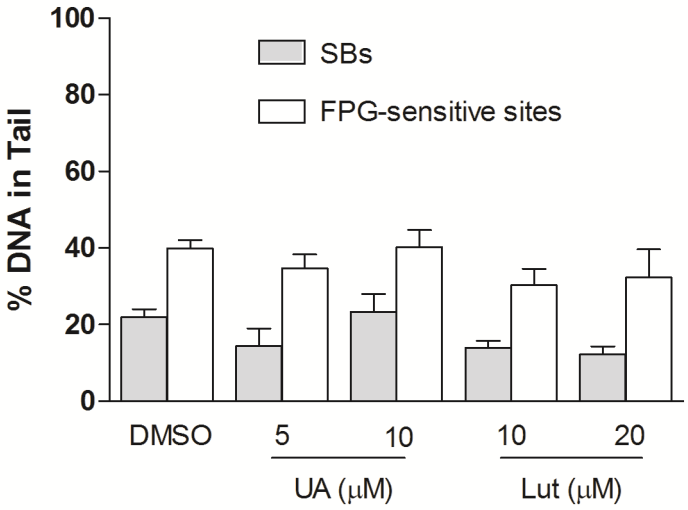
100
80
60
40
20
0
% DNA in Tail
SBs
FPG-sensitive sites
DMSO
5
10
10
20
UA (μM)
Lut (μM)

Figure 3

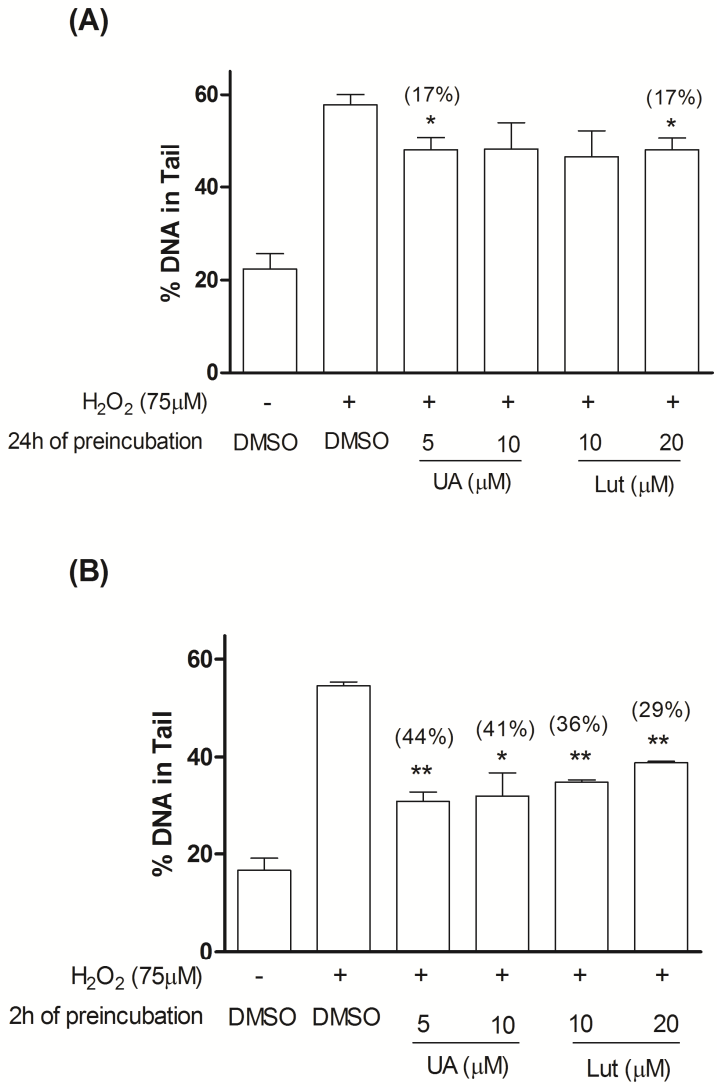
(A)
% DNA in Tail
60
40
20
0
(17%)
*
(17%)
*
H2O2 (75μM)
-
+
+
+
+
+
24h of preincubation
DMSO
DMSO
5
10
10
20
UA (μM)
Lut (μM)
(B)
% DNA in Tail
60
40
20
0
(44%)
**
(41%)
*
(36%)
**
(29%)
**
H2O2 (75μM)
-
+
+
+
+
+
2h of preincubation
DMSO
DMSO
5
10
10
20
UA (μM)
Lut (μM)

Figure 4

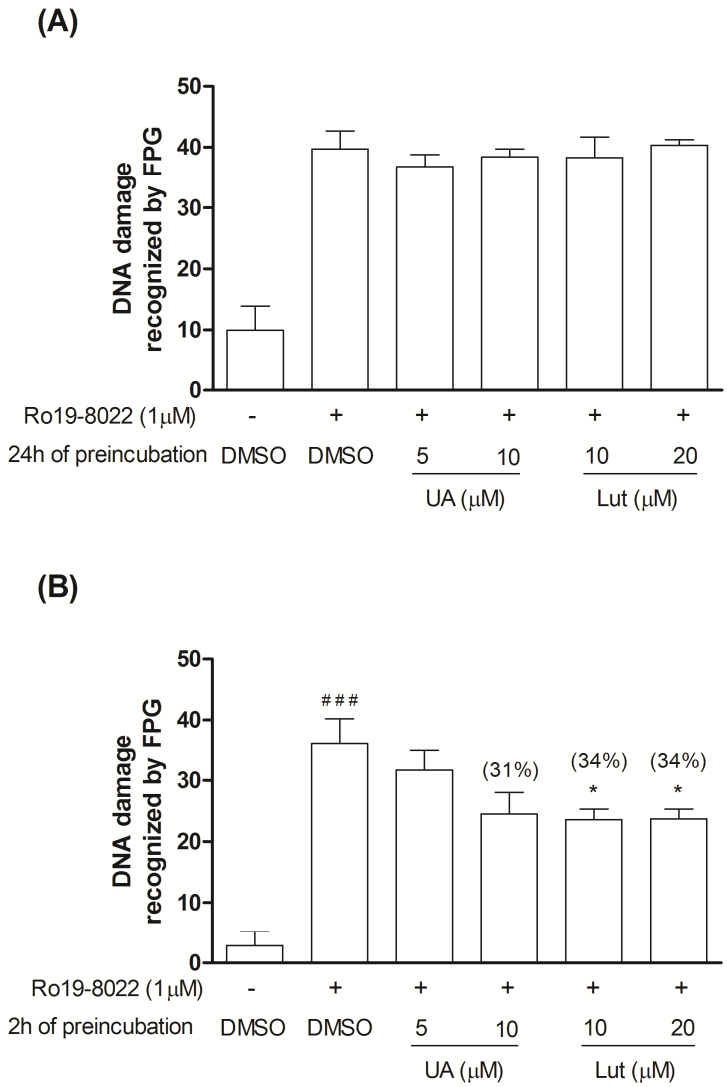
(A)
DNA damage recognized by FPG
50
40
30
20
10
0
Ro19-8022 (1μM) - + + + + +
24h of preincubation DMSO DMSO 5 10 10 20
UA (μM)
Lut (μM)
(B)
DNA damage recognized by FPG
50
40
30
20
10
0
###
(31%)
(34%)
*
(34%)
*
Ro19-8022 (1μM) - + + + + +
2h of preincubation DMSO DMSO 5 10 10 20
UA (μM)
Lut (μM)

Figure 5

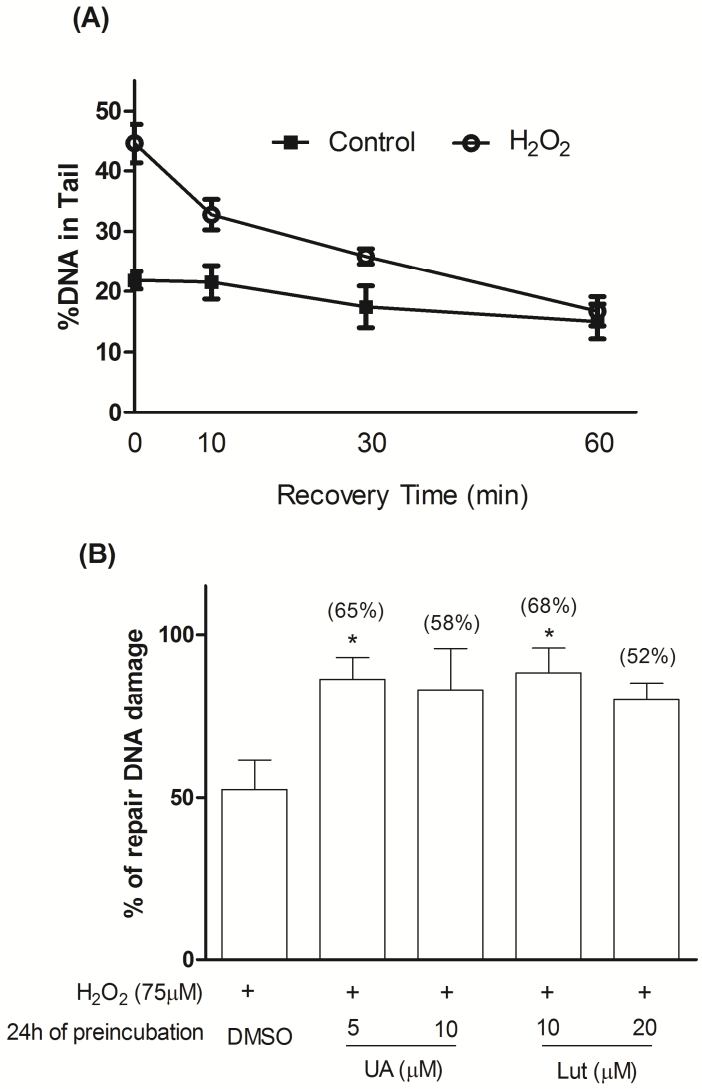
(A)
%DNA in Tail
50
40
30
20
10
0
Control
H_2O_2
0
10
30
60
Recovery Time (min)
(B)
% of repair DNA damage
100
50
0
(65%)
(58%)
(68%)
(52%)
*
*
H_2O_2 (75μM)
24h of preincubation
DMSO
5
10
10
20
UA (μM)
Lut (μM)

Figure 6