Antioxidant Effects of Quercetin and Coenzyme Q10 in Mini Organ Cultures of Human Nasal Mucosa Cells

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Abstract. *Background: Oxidative DNA damage is a known risk factor of head and neck cancer. Antioxidants, such as coenzyme Q10 (CoQ_{10}) and quercetin, a member of flavonoids present in red wine and tea, are thought to play a significant role in protecting cells from oxidative stress induced by reactive oxygen species (ROS). The aim of this study was to investigate antioxidant effects of quercetin and CoQ_{10} on mini organ cultures (MOCs) of human nasal mucosa. Materials and Methods: Macroscopically healthy tissue of nasal mucosa was harvested from 20 patients undergoing surgery of the nasal turbinates. The tissue samples were cultured and incubated with quercetin (5 μM and 50 μM) and CoQ_{10} (1 μM and 10 μM). Aqua bidest. served as negative control. After incubation with H_2O_2 (1 mM) serving as ROS, DNA damage was evaluated by the Comet assay. The extent of damage was quantified using a digital analysis system. Results: After incubation for 1 hour both CoQ_{10} and quercetin reduced DNA damage after oxidative stress significantly at all concentrations used. Furthermore, no cytotoxicity was measured. Conclusion: This study provides considerable evidence that quercetin and CoQ_{10} have strong antioxidant effects on mucosal cells of the nasal turbinates. In consequence, further studies providing epidemiological and toxicological data are warranted to specify the role of quercetin and CoQ_{10} in complementary medicine.*

Complementary medicine has become an important issue adressing cancer patients. The inability of conventional medicine to treat all aspects of cancer has been regarded as a contributing factor as to why complementary medicine has become more popular over the last decades (1). Cancer prevention plays an important role in this subject. In this context, antioxidants and may help to prevent DNA damage caused by reactive oxygen species (ROS) (2) and flavonoids represent one of the most important group of substances. These low molecular weight compounds are found in seeds, citrus fruits, olive oil, tea and red wine, and to have possible antioxidant activities *in vivo* (3, 4). Antiproliferative effects on human cancer cell lines have been shown in various studies (5, 6). However, their antioxidant properties *in vitro* are known only in some cases (7). Controversy has arisen regarding the antioxidant properties of quercetin, which has been reported to act in a pro-oxidant or antioxidant manner depending on its concentration. The ability of quercetin to prevent DNA damage caused by ROS in human nasal mucosa cells is the subject of this study.

Another antioxidant that is possibly able to deactivate ROS is coenzyme Q10 (CoQ_{10}). This coenzyme is synthesized endogenously in human cells and is also found in virtually all aerobic organisms (8). Intracellularly, CoQ_{10} is found in the mitochondria. It is widely promoted for enhancing or modulating the immune system (9). Deficiencies of CoQ_{10} occur with age, the use of certain medications and with diseases, including cancer. In the diet, it is mainly derived from meat and poultry (10). It can be absorbed and then stored in low-density lipoproteins in the liver (11). Uncontrolled studies have suggested that CoQ_{10} may suppress tumor growth (12, 13). However, no studies have examined the antioxidant effects of CoQ_{10} on human mucosal cells *in vitro*.

This study was designed to investigate whether supplementation with quercetin or CoQ_{10} protects DNA of human nasal mucosa cells against oxidative stress caused by ROS.

Materials and Methods

Donors. Specimens of approximately 25 mm by 5 mm by 1 mm of human mucosa from the lower ridge of the inferior nasal turbinates (n=20; 6 female, 14 male patients; average age 37.9 years; no smokers) were harvested during surgery on the nasal air passage. The patients were otherwise healthy and only as much mucosa was resected as was necessary for the benefit of the patient, avoiding any

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additional risk to the patient. The patients had signed an informed consent statement. The study was approved by the Ethical Commission of the Medical School, Ludwig Maximilians University, Munich.

Cultures. Specimens were microscopically dissected into cubes of 1 mm^3 using only mucosa, excluding any deeper submucosal layers and bony structures. Specimens were washed in bronchial epithelial cell growth medium (BEGM; Promocell, Heidelberg, Germany) three times and placed in 24-well plates, with one fragment in each well. Wells were coated with 0.75% Agar Noble dissolved in Dulbecco's modified Eagle's medium (Gibco, Eggenstein, Germany), 10% fetal calf serum (FCS) and nonessential amino acids, streptomycin and amphotericin B. These mini organ cultures (MOCs) floated in their medium and did not adhere to the plates. BEGM (250 ml) served as medium per mucosa fragment. Cultures were subjected to a temperature of 37˚C, in an atmosphere of 5% CO_2 with 100% relative humidity. BEGM was renewed every second day and the multiwell plates were replaced after 10-12 days. After 5 days, the initial mucosa fragments appeared completely coated with partly ciliated epithelium.

Before incubation, viability testing was performed for both cell types using trypan blue staining (14).

Incubation. Mini organs were incubated with either quercetin (5 μM and 50 μM, respectively) or CoQ_{10} (1 μM and 10 μM, respectively) solved in distilled water for 60 minutes. After incubation the cultures were washed three times in BEGM. Aqua bidest was used as the negative control. Concentrations of the substances were chosen according to pilot tests on mini organs, dose-response relations performed in our laboratory and prior studies. Viability was tested during culturing of the cells by recording the cilia function using inverse microscopy. After incubation, the cultures were exposed to oxidative stress using hydrogen peroxide (H_2O_2) at a concentration of 1 mM for an exposure time of one hour. Again, the cultures were washed in BEGM.

Comet assay. The samples underwent enzymatic digestion with collagenase P (1 mg/ml), hyaluronidase from bovine testes (1 mg/ml; Boehringer, Mannheim, Germany) and protease E type XIV from *Streptomyces griseus* (5 mg/ml; Sigma, Steinheim, Germany) for 45 min in a 37˚C shaking water bath (15). Enzymes were dissolved in BEGM and digestion was neutralized by FCS before cells were washed in phosphate-buffered saline twice.

The alkaline microgel electrophoresis assay (Comet assay) was used to measure DNA single strand breaks and alkaline labile sites as previously described in detail (15, 16). Therefore, special comet slides, designed with a frosting of 5 mm along the long edges (76 mm × 26 mm; Langenbrinck; Emmendingen, Germany), were prepared with 85 μl of 0.5% normal melting agarose (Biozym; Hameln, Germany). The cell aliquots were suspended with 75 μl of 0.7% low melting agarose (Biozym, Hameln, Germany) and applied to the prepared slides. Alkaline lysis with 10 ml dimethylsulfoxide (DMSO), 1 ml Triton®-X, and 89 ml alkaline lysis buffer (NaCl 0.9% , Na_2EDTA, Trizma base, *N*-lauroylsarcosin sodium salt) was carried out for 1 h.

The slides were placed into a horizontal gel electrophoresis chamber (Renner, Dannstadt, Germany), positioned close to the anode and covered with alkaline buffer solution containing 10 mM NaOH and 200 mM Na_2EDTA at pH 13.2. After a 20-min DNA "unwinding" period, electrophoresis was started at 0.8 V/cm and 300 mA for 20 min. Following neutralization (Trizma base, pH 7.5; Merck, Darmstadt, Germany), the slides were air dried and the DNA stained with ethidium bromide (Sigma, Steinheim, Germany). All slides were examined under a fluorescence microscope (magnification ×40; DMLB; Leica, Heerbrugg, Switzerland) with an adapted CCD camera (Cohu; San Diego, CA, USA). Eighty cells (40 cells/slide) were scored per sample.

Digital analysis. Depending on the degree of strand-break induction, the DNA fragments demonstrate different types of migration within the electric field, creating so-called comets (Figures 1 and 2), with single-strand breaks and alkali-labile sites in the tail of the comet. The comets were measured using an image analysis system (Comet ++; Kinetic Imaging, Liverpool, UK). To quantify the extent of DNA migration, Olive's tail moment (OTM), *i.e.* the relative amount of DNA in the tail of the comet multiplied by the median migration distance (17), was used.

Statistics. For statistical analysis, the SPSS™ 13.0 program was used (Chicago, IL, USA). To compare DNA protective impacts by different concentrations of the tested substances *vs.* negative controls, the Wilcoxon test was applied. The local level of significance was defined as $p \leq 0.05$. Standard box-plots (lower quartile, median, upper quartile) were used to illustrate the results. Dots mark mild statistical outliers (between 1.5 and 3 times interquartile range (IQR)), diamonds mark extreme statistical outliers (more than 3 times IQR).

Results

Controls. Cell viabilty and cilia function remained uncompromised for all tested substances. Cell viability ranged from 92% to 99% in the specimens. No significant DNA damage could be observed with the negative control (DMSO; OTM 1.41). No DNA damage was caused after incubation with quercetin (5 μM OTM 1.76 and 50 μM OTM 1.68, respectively) or coenzyme Q10 (1 μM OTM 1.69 and 10 μM OTM 1.36, respectively) without addition of H_2O_2. Taking into account that the comet assay indicates relevant DNA damage and therefore genotoxic effect only at OTM >2.0 (17), these components did not reveal relevant genotoxic effects by the tested substances. Incubation with H_2O_2 (1 mM) showed significant DNA damage (OTM 16.64), with persistant good cell viability counts (91-99%) (Figures 3-5).

Antioxidant effects of quercetin. In order to investigate the antioxidant effects of quercetin, the specimens were incubated with this compound at two different concentrations (5 μM and 50 μM) for one hour. The initial DNA damage caused by H_2O_2 alone (OTM 16.6) was significantly reduced to an OTM of 11.5 at 5 μM ($p<0.001$) and of 8.1 at 50 μM ($p<0.001$) (Figure 6).

Antioxidant effects of coenzyme Q10. Antioxidant effects of CoQ_{10} were evaluated using concentrations of 1 μM and 10 μM. Again, a significant reduction of DNA damage was found compared to H_2O_2 alone (OTM 17.85; 1 mM) with an OTM of 13.57 at 1 μM ($p<0.001$) and of 12.90 at 10 μM ($p<0.001$) (Figure 7).

Figure 1. *Practically undamaged DNA of a nasal mucosa cell after incubation with Aqua bidest. (negative control) (magnification ×400).*

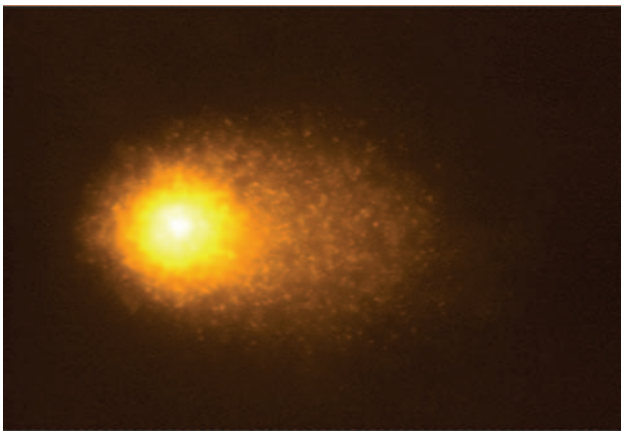

Figure 2. *Damaged DNA of a nasal mucosa cell after incubation with H_2O_2 (1 mM, OTM 14.5) (magnification ×400).*

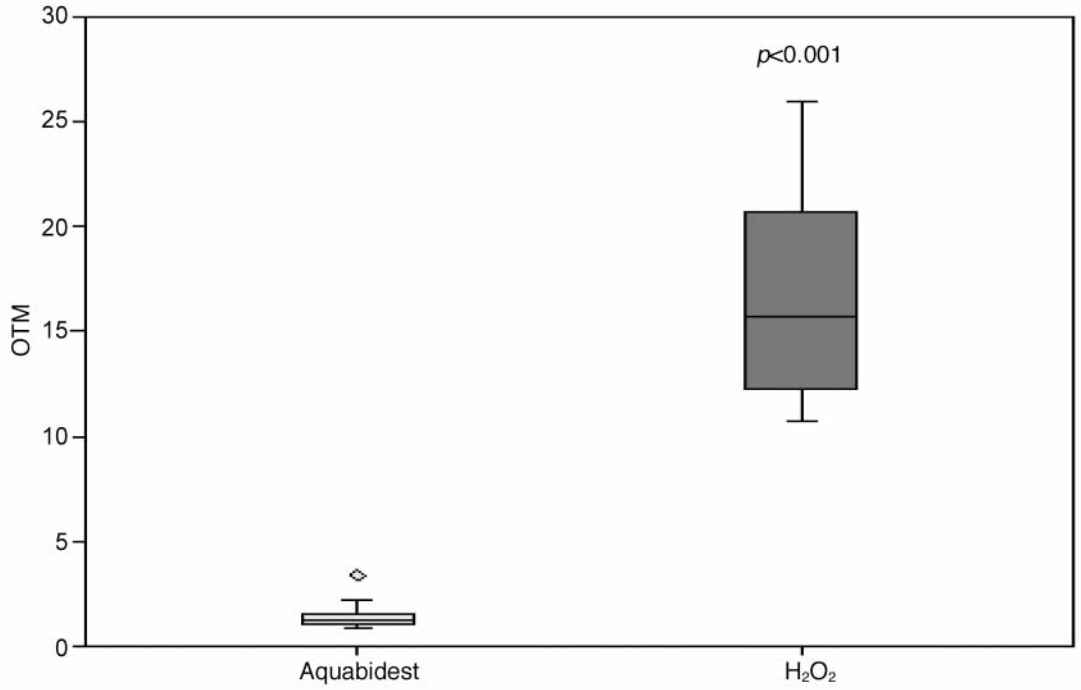

Figure 3. *DNA damage in human nasal mucosal cells after incubation with H_2O_2 (1 mM) (n=20).*

Discussion

Nasal epithelium is the first target for inhaled carcinogens and therefore is constantly exposed to potentially mutagenic compounds. Oxidative stress caused by ROS can damage lipids, proteins, enzymes, carbohydrates and DNA in cells and tissues, resulting in diverse damage of human cells. Human antioxidant defense is equipped with enzymatic scavengers such as superoxide dismutase, catalase and gluthatione peroxidase, but also non-enzymatic ones. They not only include several minerals, vitamins and coenzymes, such as CoQ_{10}, but also polyphenols, which mainly consist of flavonoids and phenolic acids (18). These agents can significantly reduce damage due to oxidants. They deactivate oxidants before they react with biological targets, preventing the induction of chain reactions and the activation of oxygen to highly reactive products (19).

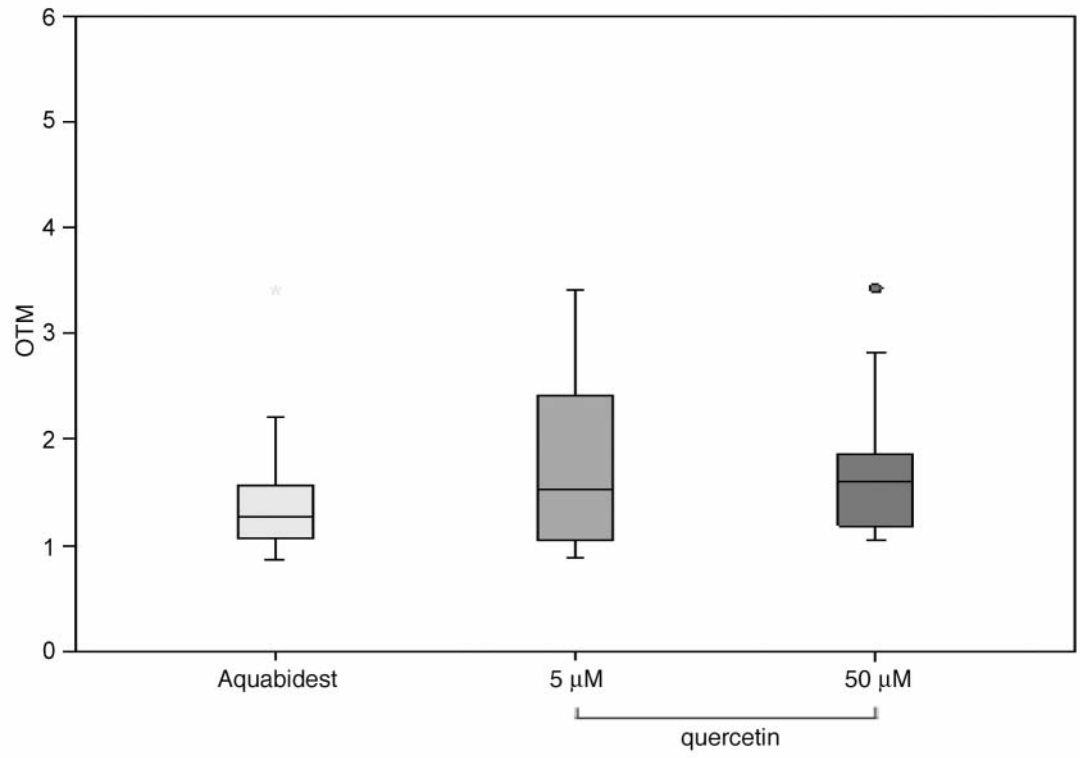

Figure 4. *DNA damage in human nasal mucosal cells after incubation with quercetin (5 µM and 50 µM without H_2O_2) (n=20).*

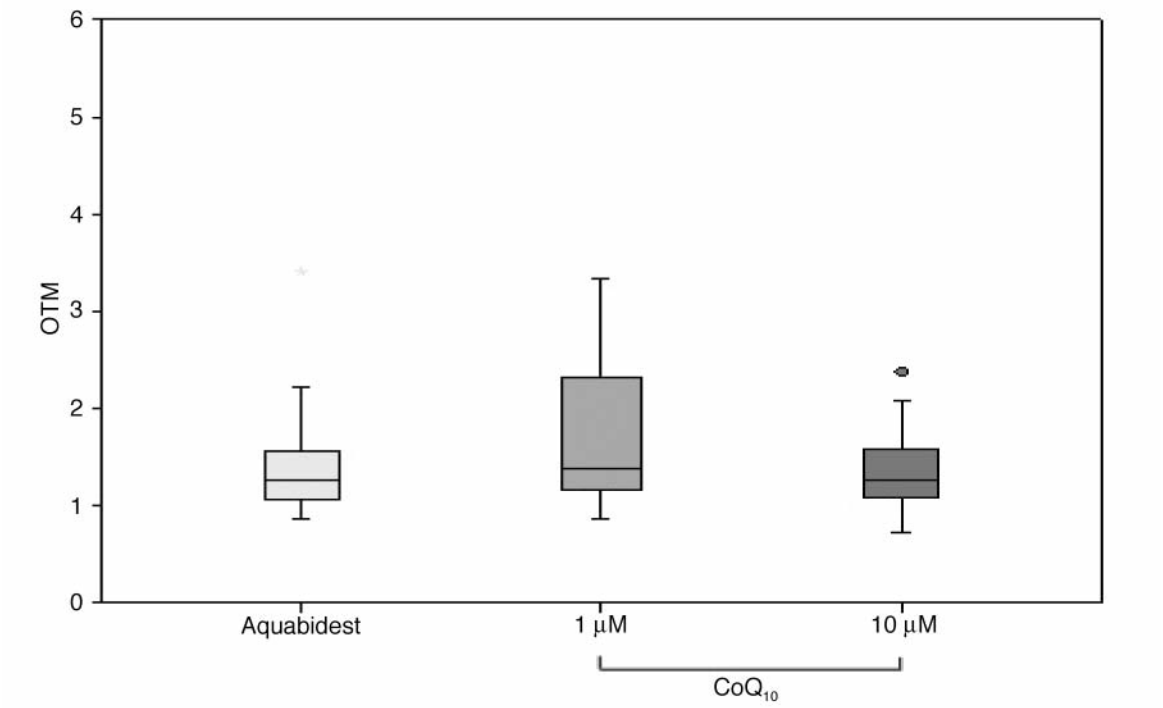

Figure 5. *DNA damage in human nasal mucosal cells after incubation with CoQ_{10} (1 µM and 10 µM without H_2O_2) (n=20).*

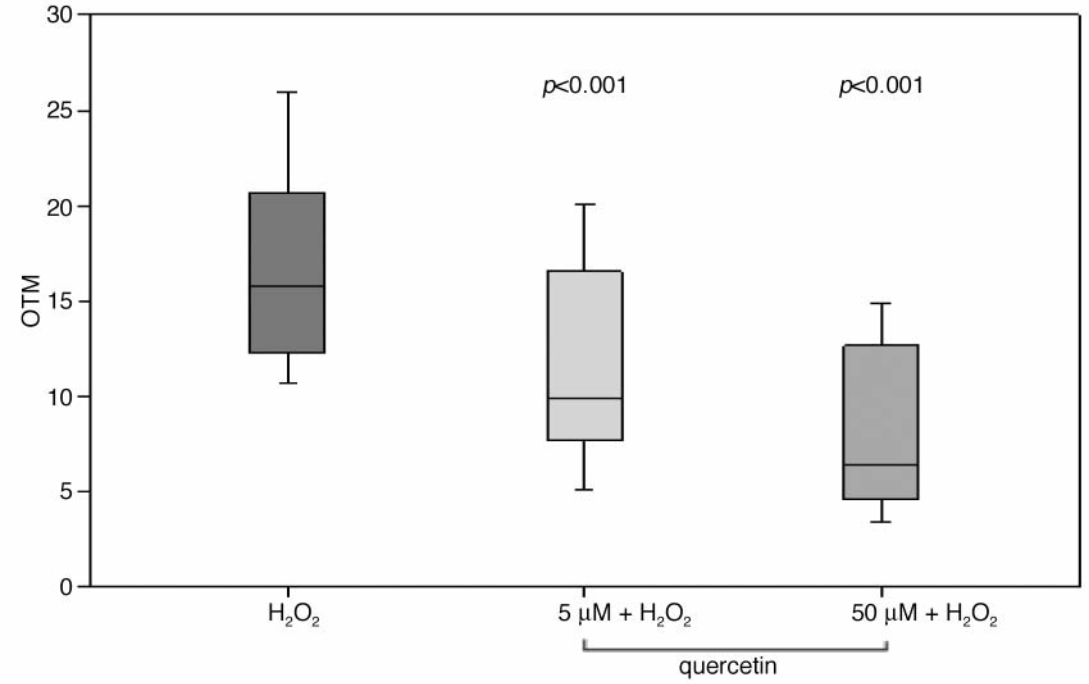

Figure 6. *Antioxidant effect of quercetin (5 µM and 50 µM) with incubation of H_2O_2 (1 mM) on human nasal mucosal cells (n=20).*

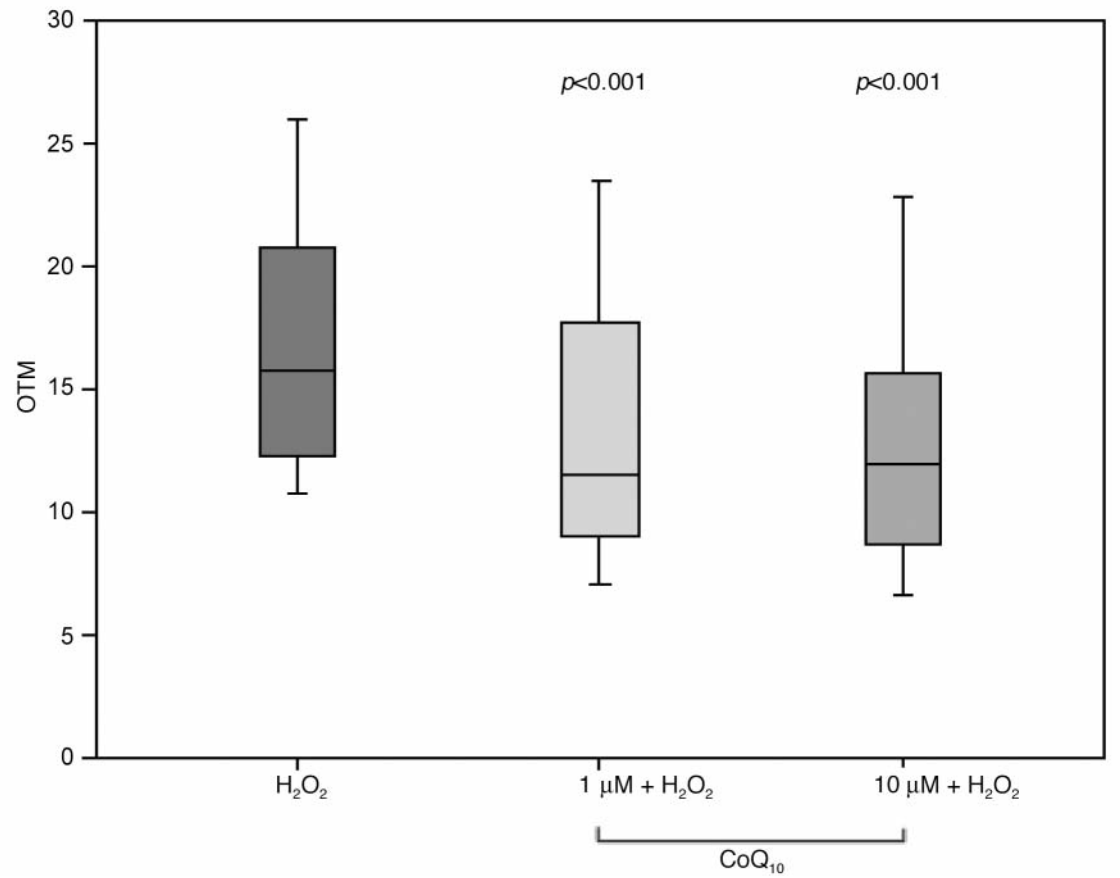

Figure 7. *Antioxidant effect of CoQ_{10} (1 µM and 10 µM) with incubation of H_2O_2 (1 mM) on human nasal mucosal cells (n=20).*

Quercetin, as a member of the flavonoid family is widely discussed as an antioxidant. The average plasma concentration of quercetin varies from 0.1 μM to 0.2 μM in healthy adults but can be enhanced with an oral uptake of 1 g per day to 1.5 μM. Maximum plasma levels occur 7 h after ingestion (20). Higher plasma levels can only be reached by intravenous application. Gugler *et al*. showed that a single intravenous dose of 100 g led to a serum concentration of 12 μM (21). The elimination half-life of quercetin has been suggested to be approximately 25 h (22). In our study, we found antioxidant potency of quercetin in human nasal mucosa at both concentrations used (5 μM and 50 μM) after 24 h incubation. The chosen concentrations are significantly higher than average plasma concentrations and can hardly be reached by oral uptake of quercetin. On the other hand, most *in vitro* studies with quercetin used even higher concentrations to prove antiproliferative effects. Significant inhibition of growth in cancer lines of the head and neck were found for concentrations higher than 100 μM (23, 24). Antioxidant effects of quercetin *in vitro* were shown by Alia *et al*. in a human hepatoma cell line (25). Wilms *et al*. found protective effects of quercetin against induction of oxidative DNA damage in human lymphocytes caused by ROS (26). Whereas genotoxic effects of quercetin are described in various studies (27, 28), no such effects of quercetin could be seen in our investigation, as OTM was not significantly enhanced by incubation with quercetin alone.

Just like quercetin, CoQ_{10} showed antioxidant effects in our study on human nasal mucosal cells. OTMs were reduced significantly by both concentrations used after cells were stressed by oxidants. No significant difference was found between the two concentrations (1 μM and 10 μM). *In vivo* supplementation by diet covers most of the daily CoQ_{10} demand. Intrinsic production taking place in the mitochondria is only responsible for 20-30% of the daily requirements. Normal CoQ_{10} plasma concentration in healthy adults lies at around 1-2 μM, but plasma levels can be significantly enhanced by increased oral intake (29). Deficiencies of CoQ_{10} in humans occur with age, the use of certain medications and diseases including cancer (30). Folkers *et al*. showed a significantly higher incidence of CoQ_{10} deficiency in cancer patients compared to healthy control patients (9). Other uncontrolled studies have suggested that CoQ_{10} may suppress tumor growth (31-33) and antioxidant effects have been reported in various studies (34, 35). No genotoxic effects of CoQ_{10} have been reported so far.

Conclusion

In conclusion, both flavonoids, quercetin and CoQ_{10}, showed strong antioxidant effects on human mucosal cells of the nasal turbinates. Neither toxicity nor genotoxic capacity of the compounds could be investigated. Favouring quercetin and CoQ_{10} as potential dietary antioxidants, further clinical studies are warranted evaluating epidemiological and toxicological data to specify the role of quercetin and CoQ_{10} in complementary medicine.

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