TELOMERE DAMAGE INDUCED BY ACUTE OXIDATIVE STRESS AND CHROMOSOME INSTABILITY.

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Telomeres are nucleoprotein structures located at the end of linear chromosomes consisting of non-coding repetitive DNA sequences, such as TTAGGG in human, and telomeres binding proteins (Blackburn, 1991). Their primary role is to maintain chromosome and genome stability. A lot of study demonstrated that short or dysfunctional telomeres of two chromosomes/chromatids can fused together at their ends leading to a dicentric chromosome. Both the bases and the ribose components of DNA have been identified as being susceptible to oxidative damage, however guanine residues have been shown to be particularly sensitive; 7,8-dihydro-8-oxyguanine (8-oxoGua) or the deoxyribonucleoside form of this lesion (8-oxodG) being a common biomarker of oxidative stress. The high incidence of guanine residues in telomeric DNA sequences makes the telomere the preferential target for oxidative damage and the telomeric low efficiency in DNA damage repair, increase the probability of the accumulation of 8oxoGua. In fact oxidative stress was shown to accelerate telomere shortening in replicating fibroblasts "in vitro". This acceleration was attributed to the enhanced induction of telomeric single strand breaks by free radicals, leading to the loss of the distal fragments of telomeric DNA following replication.

With the aim to evaluate if the chromosome instability induced by oxidative stress is related to telomeric damage, we used human primary fibroblasts (MRC-5) treated with two doses of H_2O_2 (100 μM and 200 μM) for 1 hour. The evaluation of the cell cycle by citofluorimetric analysis showed no cell cycle effects after treatments. Our previous studies demonstrated a significant telomere shortening 48 hour after treatment and an increase of micronuclei (MN), nuclear buds (NBUDs) and nucleoplasmic bridges (NPBs) at the same time, leaving us to suppose a correlation between telomere shortening and chromosome instability. To better understand the correlation between chromosome instability and telomeric oxidative damage, we evaluate the persistence of oxidative stress induced damage up to 24 hours after treatment analyzing genomic DNA damage (SSBs) by alkaline comet assay and genomic FPG-sensitive sites by Formamidopyrimidine-DNA-glycosylase-modified comet assay. Furthermore, the measure up to 24 hours of the amount of oxidized residues specifically within telomeric DNA, that are recognized and excised by Formamidopyrimidine-DNA-glycosylase by qPCR, gave us the information about telomeric damage. The results obtained by comparing genomic and telomeric persistent oxidative damage could be interesting to evaluate if telomere dysfunction could be the principal target responsible of chromosome instability induced by oxidative stress.