INHIBITORS OF TOPOISOMERASE 1 INDUCE ARREST OF COLON CANCER HCT116 CELLS AT THE END OF MITOSIS

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Topoisomerase I (Top1) inhibitors, used in cancer therapy for the treatment of a variety of solid tumours, are semi-synthetic drugs derived from the alkaloid camptothecin obtained from the Chinese tree Campotheca acuminate. Their main molecular mechanism of action relies on stabilization of the cleavable complex formed by Top1 and DNA with generation of single strand breaks. Irreversible DNA damage occurs when the stabilized drug-DNA-Top1 complex collides with replication machineries, leading to replication fork stalling and eventually double strand breaks. Tumour cell treatment with Top1 poison may cause not only cell cycle arrest or delay and cell death, but also chromosome damage. Even though DNA damage and consequently chromosome aberrations mainly occur during the S phase of cell cycle at sites where the replication fork is arrested, DNA can be damaged also during the G2 phase. However, the mechanism underlying this effect has not been clarified yet. Two hypotheses have been proposed: a) latest part of DNA duplication might take place also in the G2 phase, b) since the inhibition of Top1 interferes also with the stability of genes during their transcription, camptothecins may induce breaks at the level of genes expressed in G2. Moreover, these drugs frequently induce karyotype alterations, for example polyploidy, through the total inhibition of sister chromatid separation, or aneuploidy, when the phenomenon concerns only some chromosomes.

Different clones of the colon cancer HCT116 cell line, proficient or deficient for the mismatch repair component MLH1, poly(ADP-ribose)polymerase 1 (PARP-1) and the tumour suppressor p53 were studied . Tumour cells were exposed to graded concentrations of SN38, the active metabolite of irinotecan, which is a camptothecin derivative commonly used for colon cancer chemotherapy. Initially, we determined the SN38 concentrations that caused significant chromosomes damage (treating cells in G2 for 3 hours), but that allowed cells to reach partly mitosis without causing a complete G2 arrest. Besides chromosome damage (as control of the efficacy of the drug) we analysed mitosis progression in control cultures and in those treated with SN38. The progression through mitosis was evaluated counting the percentage of cells in the different phases of mitosis (prophase, metaphase, anaphase, telophase and cytodieresis). Cells were stained with DAPI and using antibodies recognizing tubulin, to better characterize the different phases of mitosis, or centromere protein F (CENPF) that labels cells in G2 and mitosis, and phosphorylated H2AX as a marker of DNA damage.

SN38 treatment induced an increase in the percentage of cells accumulated at the second part of mitosis after metaphase/anaphase transition checkpoint. This effect was not observed in cells exposed to bleomycin, which is known to cause a strong G2 arrest, or aphidicolin that blocks cells mainly in early S phase. The arrest in the last phase of mitosis (cytodieresis) has been associated also to damage of telomeres, that can be targeted by camptothecins. Thus, telomere sensitivity to SN38 was analysed in HCT116 derived cell clones by FISH.

MMR deficient HCT116 cells (devoid of hMLH1 expression) were more sensitive to SN38 as compared to MMR proficient counterpart. Stable silencing of PARP1 expression resulted in higher sensitivity to SN38 clastogenicity and a higher percentage of cells arrested at the end of mitosis, compared either to control cultures and also with those expressing MLH1. PNA-FISH analysis showed that SN38 may induce a decrease in the number of telomeres positive for the PNA probes, suggesting a damage at that level.