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BIOMEDICAL SCIENCES

Cellular bases of hypofractionated radiotherapy protocols for lung cancer

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Abstract: The extreme demand on health systems due to the COVID-19 pandemic has led to reconsider hypofractionation. Although the best clinical efficacy of these schemes is being demonstrated, the biological bases have not been established. Thus, after validating basic clinical parameters, through complementary *in vitro* models, we characterized the cellular and molecular mechanisms of hypofractionation protocols. Cell cultures of human lung cancer cell line A549 were irradiated with 0, 2, 4, 8, 12, 16 and 20 Gy. The clastogenic, cytotoxic, proliferative and clonogenic capacities and bystander effect were evaluated. In addition, we assessed survival and toxicity in a retrospective study of 49 patients with lung cancer. Our findings showed that the greater efficacy of ablative regimens should not only be attributed to events of direct cell death induced by genotoxic damage, but also to a lower cell repopulation and the indirect action of clastogenic factors secreted. These treatments were optimal in terms of 1- and 2-year overall survival (74 and 65%, respectively), and progression-free survival at 1 and 2 years (71 and 61%, respectively). The greater efficacy of high doses per fraction could be attributed to a multifactorial mechanism that goes beyond the 4Rs of conventional radiotherapy.

Key words: radiotherapy dose hypofractionation, 4Rs, Linear-Quadratic Model, cytotoxic effect, stereotactic body radiotherapy.

INTRODUCTION

The need to effectively tackle the COVID-19 pandemic is a major challenge for global health systems, in a vigorous change that forces the reallocation of the available resources and the prioritization of options in this health emergency. Radiotherapy (RT) as the most widely used cancer treatment is not an exception (Rathod et al. 2020, Maier et al. 2016). Under these circumstances, the value of shorter treatments, which are now reliable and effective, has reappeared given technological progress (Baskar et al. 2014, Swaminath et al. 2017). This constitutes a valuable strategy in terms of patient transit reduction, social distancing promotion and minimization of the risk of contagion, in addition to optimizing human resources and associated care costs (Brenner 2012, Hunter et al. 2018, Ko et al. 2011).

As applied, radiotherapy requires a tissue response that integrates the lethal effects directed at the tumor, with the possibility of recovery of healthy tissue that is reached by radiation (Baskar et al. 2014). This condition is based on dividing the therapeutic dose and refers to four phenomena ("4R"): DNA repair; cell repopulation (mainly in healthy tissue); recruitment of cells in phases of the cell cycle more sensitive to radiation; reoxygenation (mainly in the tumor) (Hall & Giaccia 2012). Fractionation takes advantage of the difference

in recovery rate between normal and tumor tissues. In this way, the different radiotherapy protocols have been empirically established and given the advances in the administration of radiation, with doses much more adjusted to the objective, interest is renewed in shortening conventional treatments (CRT) by means of protocols with fewer sessions but with higher doses (hypofractionation and ablative radiotherapy).

Lung cancer is a considerably lethal pathology. The therapeutic approach of RT is effective for all its variants, either as a single or adjuvant modality (Pesek & Muzik 2018, Brown et al. 2013). In this sense, attempts are currently being made to impose ablative (stereotactic body radiation therapy, SBRT) or moderate hypofractionated RT (HRT) protocols both in early stages without surgical resolution and in advanced stages (Iyengar et al. 2018). The efficacy of these regimens has been shown through phase II clinical trials, which demonstrated better progression-free survival and overall patient survival (Suh & Cho 2019, Folkert & Timmerman 2017, Cuccia et al. 2020). Although still under debate, few innovations in RT have had the impact of these modalities, which achieved 85-90% local control (Choi 2019, Murray et al. 2017, Weder et al. 2018).

Empirically-based advances in hypofractionation are supported by a growing number of papers describing its use, despite its cellular bases have not been fully determined (Ko et al. 2011, Bayo Lozano et al. 2012). The radiobiological model that would better interpret hypofractionation efficacy and the possibility of having additional biological effects to the classical 4Rs of RT are much discussed topics (Brown et al. 2014, Kirkpatrick et al. 2008, Otsuka et al. 2011, Shibamoto et al. 2012, Sia et al. 2020). In accordance with the new centralized radiobiological paradigm in the holistic approach (Mothersill & Seymour 2004, Prise & O'Sullivan 2009), the best results of these schemes would be based on a multifactorial mechanism that included a coordinated systemic and tissue response (Baskar et al. 2014, Prasanna et al. 2014). Thus, our objective was to explore the cytomolecular foundations underlying hypofractionated protocols, to better understand them and optimize their management.

MATERIALS AND METHODS

Patient study design

In terms of validating the clinical efficacy of different RT treatments for lung cancer, we conducted a retrospective study of 49 inoperable patients (21 women and 28 men; mean age, 67.8 years; range, 40-89 years) from the Deán Funes Medical Center, Córdoba Capital. Data were collected from January 2017 to September 2019 (Supplementary Material, Table SI). Most patients presented adenocarcinoma (69%) and squamous cell carcinoma (22%). They underwent different treatments: CRT (n = 5; 2 Gy), HRT (n = 22; dose range: 2.1 - 5 Gy) and SBRT (n = 22; dose range: 8.1 – 18 Gy). Only six patients received RT as a single treatment. The endpoints analyzed were overall survival, progression-free survival and acute toxicity (according to Common Terminology Criteria for Adverse Events; CTCAE). Considering the small number of patients undergoing CRT (5/49), these were not taken into account for the survival analysis. The study was performed in accordance with the core principles of the Good Clinical Practice Guidelines (Vijayananthan & Nawawi 2008) and following the principles of the Declaration of Helsinki.

Cell culture study design

The experimental design included *in vitro* models using logarithmic growth phase A549 cells. The

study was executed in two stages to assess the target (stage 1) and non-target (stage 2) effects of radiation treatments. In stage 1, cell cultures were irradiated with doses used in conventional radiotherapy, CRT, (2 Gy) and hypofractionation HRT (4 and 8 Gy) or ablative radiation therapy, SBRT, (12, 16 and 20 Gy) (Timmerman 2008). DNA damage, cell viability as well as proliferative and clonogenic capacities were evaluated (Luo 2019). In stage 2, cell cultures were irradiated with 2, 8, 12, 16 and 20 Gy and incubated for 3 h to generate irradiated cell conditioned medium (ICCM), with which non-irradiated receptor cultures of the same cell line were treated for 24 h. DNA damage was assessed in just irradiated cells, to evaluate genotoxicity; 3 h after irradiation, to estimate DNA repair; and 24 h after treatment with ICCM, to study bystander effects. In each case, at least three independent experiments were performed with their corresponding duplicates.

In addition, in order to evaluate the effects of the total doses of 60 Gy of a radiotherapy treatment, a 1-week practicable experiment was designed. Thus, cell cultures were irradiated with a fractional dose of 20 Gy, separated by 48 h from each other, in order to reproduce a SBRT treatment as most similar to the clinical one. From these samples, the genotoxic damage was analyzed, which was calculated as damage index and damage class, and cell viability was also evaluated.

Cell culture

The human A549 lung cancer cell line from the American Type Culture Collection was obtained from the cell collection of the *Instituto Multidisciplinario de Biología Celular*, La Plata, Argentina. It was maintained in Dulbecco's Modified Eagle Medium/Nutrient Mixture (DMEM/F-12; Microvet SRL, Argentina) containing 10% fetal bovine serum (Natocor-Microvet SRL, Argentina), penicillin-G sodium 1,000,000 IU (Klonal, Argentina) and streptomycin sulfate (RICHET®, Argentina).

Irradiation procedure

Cell irradiation was performed with a VarianClinac® 6MV linear accelerator (Varian Medical Systems, USA) at a dose rate of 300 cGy/min. The plates containing cells were placed inside a water phantom (density equivalent to soft tissue and depth greater than the buildup zone). The system was irradiated from the bottom with isocentric photon beams. The deviation of the absorbed dose was compatible with the therapeutic objective (< 5%).

DNA damage

Genotoxic damage in A549 cells was assessed immediately after irradiation by the alkaline comet assay according to Singh et al. (1988) with slight modifications (Ocolotobiche et al. 2019). The slides were coded and then scored blind. Each experimental point was evaluated in duplicate (200 cells/condition). The cytomolecular analysis of comets was performed by visual classification, given its good correlation with image analysis programs (Azqueta et al. 2011), and high reproducibility between laboratories (Kumaravel et al. 2009). Each cell was classified into 5 classes, from class 0 (no DNA migration) to class 4 (maximum DNA migration). Genetic damage was measured with the damage index (DI), calculated with the formula $DI = [(1x\mathit{n1})+(2x\mathit{n2})+(3x\mathit{n3})+(4x\mathit{n4})]/(\mathit{n0}+\mathit{n1}+\mathit{n2}+\mathit{n3}+\mathit{n4})x100$, where *n* indicates the number of cells in each class. Damage class (DC) was calculated as the sum of cells with comet grade 0 (null damage), 1 and 2 (moderate damage), and 3 and 4 (severe damage).

Cell viability

Trypan blue exclusion assay

After irradiation, the cell suspensions were treated with 0.04% trypan blue and the number of total and viable cells (unstained) were determined using a hemocytometer to calculate percentage of vital cells.

MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

After treatment, cell viability of A549 cells was determined with the MTT assay, as described by Mosmann (1983) with minor modifications (Banegas et al. 2018). Plates with growing cells were irradiated and incubated for 6 days. Each group comprised 12 duplicate wells.

Cell proliferation

A549 cells were seeded into 12 well plates (40,000 cells/well). After 24 h, the plates were irradiated and incubated for 1 to 5 days. For each experimental point, cells from three wells were removed every 24 h and the number of viable cells was determined by the trypan blue exclusion assay.

Clonogenic capacity

The technique described by Puck & Marcus (1956) was implemented. Briefly, 600 log-phase cells were plated in 6-cm plates for 4 h. The plates were then irradiated and incubated for 7 days. Colonies were counted and survival fraction was calculated.

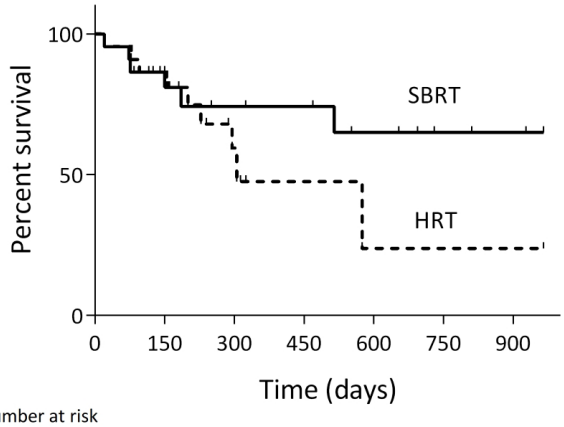
Statistical analysis

Data from at least three independent experiments performed in duplicate are expressed as mean ± SD. The one-way analysis of variance (ANOVA) or two-way ANOVA and multiple comparisons test of Tukey were used to determine statistical significance. For survival analysis, Kaplan-Meier plots were generated and Mantel-Cox test was implemented.

RESULTS

Clinical efficacy of HRT and SBRT irradiation protocols

Mean time of patient follow-up was 295 days for HRT (range, 20-960) and 469 days for SBRT (range, 20-928). Figure 1 shows HRT and SBRT overall survival curves (1-year survival, 47.6 and 74.2%; 2-year survival, 23.8 and 64.9%, respectively), differences between protocols were not significant (Chi-square = 1.241). Median HRT survival time was 305 days, while that of SBRT could not be defined because it is calculated with 50% overall survival. Regarding HRT and SBRT progression-free survival, it was 50.3 and 71.3% at 1 year, and 25.1 and 61.1% at 2 years, respectively (NS; Chi-square = 0.8195). Median



Number at risk

Percent survival (%)

HRT	22 (100.0)	17 (86.4)	6 (59.5)	3 (47.6)	2 (23.8)	2 (23.8)	2 (23.8)
SBRT	22 (100.0)	16 (81.0)	11 (74.2)	10 (74.2)	7 (64.9)	4 (64.9)	3 (64.9)

Figure 1. Survival curves. Survival analysis stratified by the type of radiotherapy treatment, plotted as the proportion of patients surviving from the date of treatment versus time in days using the Kaplan-Meier method. Below the graph, the table indicates the number of patients at risk and the percentage of survival for each treatment versus time elapsed since treatment.

HRT progression-free survival time was 295 days, while that of SBRT could not be defined. Only low-grade toxicities were observed: CRT 2/5; HRT and SBRT 4/22. No differences in side effects between treatments were detected (Chi-square = 0.72).

Target effects related to CRT, HRT and SBRT dose fractions

Genotoxic effect was evaluated in cell cultures treated with the different radiation doses. Fig. 2A shows an increase in DNA damage ($p<0.0001$) with HRT or SBRT doses, compared with CRT. DNA damage generated by 8 and 20 Gy was 6 (192 AU) and 8 (247 AU) times greater, respectively, than that produced by 2 Gy (32 AU). Furthermore, the effect of the 2 Gy dose did not differ from the control without irradiation (8 AU), and there were no differences between the 12, 16 and 20 Gy doses either (215, 224 and 247 AU, respectively). On the other hand, results of genotoxic damage evaluated 3 h after irradiation (Fig. 2B) showed that the effect of 2 and 8 Gy doses (17 and 36 AU, respectively) did not differ from the control without irradiation (7 AU). However, the 12, 16 and 20 Gy doses (137, 153 and 169 AU, respectively)

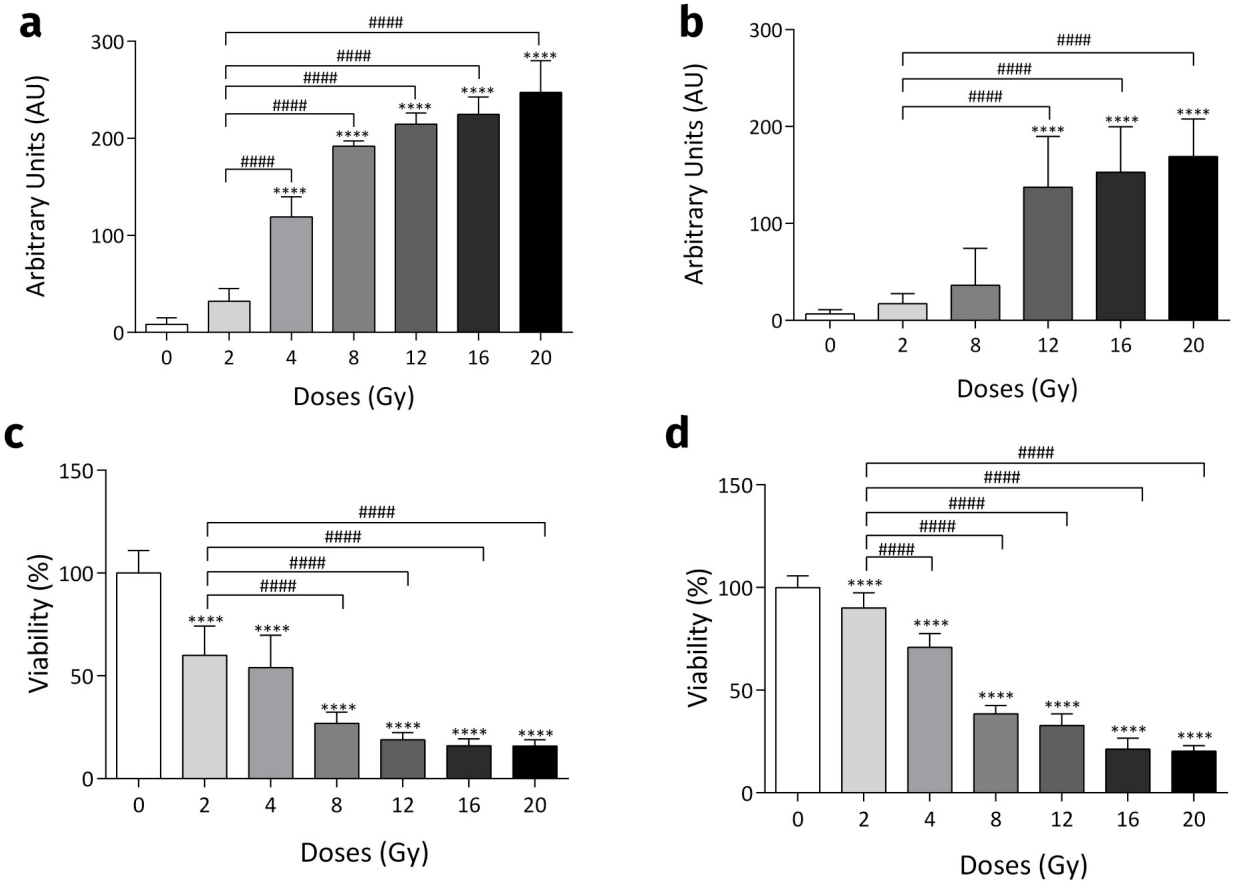


Figure 2. Genomic damage, repair and cell viability. Upper panel: Radiation-induced genotoxic effect evaluated in the A549 cell line just after irradiation (Panel a) and 3 h post irradiation (Panel b). Results were plotted as damage index vs Gy dose irradiation and expressed as mean ± SD. Lower panel: Percentage of viable A549 cells after irradiation with different Gy doses. Cell viability was measured by integrity of the plasma membrane (Panel c) and mitochondrial activity (Panel d). Results are expressed as mean ± SD. One-way ANOVA and post-hoc Tukey's test, **** $p < 0.0001$ versus untreated control cells and #### $p < 0.0001$ versus cells treated with 2 Gy.

increased DNA damage compared with the control ($p<0.0001$) and in relation to the 2-Gy dose. The detected damage was lower than that observed in Fig. 2A, probably mediated by the cellular DNA repair machinery.

Results of cell viability by the integrity of the plasma membrane and mitochondrial activity are presented in Fig. 2. A similar behavior can be seen, *i.e.*, all treatments decreased cell viability ($p <0.0001$). It is important to note that the 8 Gy dose produced an abrupt fall in cell viability (27%) just after irradiation (Fig. 2C). The 8 and 20 Gy doses display a decline of 55 and 75% respectively, compared to the 2 Gy dose. Similarly, a significant decrease in cell viability was observed 6 days after irradiation ($p <0.0001$), produced by doses from 4 Gy onwards with respect to the control (100%) and the 2 Gy dose (90%) (Fig. 2D). Again, a sharp drop in cell viability was noted with the 8 Gy dose (38%), and a 58 and 77% reduction elicited by 8 and 20 Gy, respectively, with respect to the 2-Gy dose.

Survival fraction data, evaluated by the clonogenic assay, are shown in Fig. 3A. An abrupt reduction at 8 Gy can be seen, but loss of clonogenic capacity was observed only after 12 Gy. To evaluate the adjustment of the survival values obtained to the linear quadratic (LQ) model, parameters α and β were calculated (0.1505 and 0.0445, respectively). The adjustment showed a very good approximation, despite deviations observed with 12 Gy (Fig. 3A). Moreover, survival was zero with 16 Gy and higher doses and did not correspond to the LQ model, which is unable to predict this value because it is an asymptotic curve to the x axis. Finally, evaluation of the proliferative capacity of cells showed that proliferation curves began to separate from the third day, observing a lower growth rate of irradiated cells with respect to the control, with differences between control and 16 Gy ($p<0.01$), and no differences between control and 2 Gy (Fig. 3B). On the fourth day, the separation was even greater, the growth rate of irradiated cells decreased, and differences between control and 20 Gy were observed ($p<0.0001$). Additionally, the number of counted cells decreased when comparing the conventional 2 Gy and the 20 Gy doses ($p<0.01$). Again, there was a significant drop in the number of cells from 8 Gy onwards.

In general terms, a significant change in cellular behavior was observed from 8 Gy onwards in relation to CRT 2 Gy doses, which was reflected in genomic damage, cell viability and proliferative and clonogenic capacities. Likewise, differences between the SBRT doses

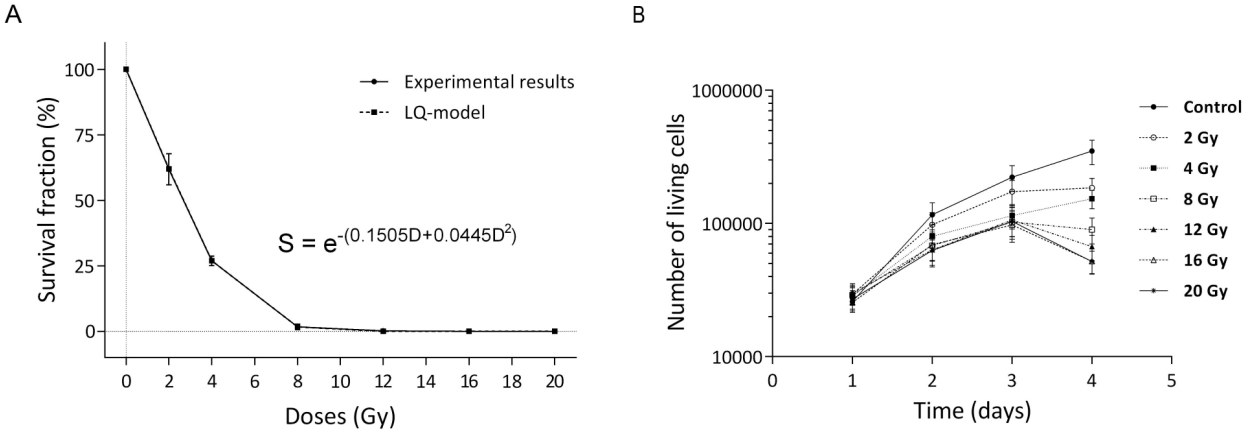


Figure 3. Clonogenic and proliferative capacities. a. Survival fraction based on the dose given (full line). Adjustment of the values to the LQ model (dotted line). b. Number of living cells as a function of time (days), for each dose delivered. Results are expressed as mean ± SEM.

(12, 16 and 20 Gy) were not significant in relation to the same evaluated parameters.

Non-target effects related to CRT, HRT and SBRT dose fractions

The ICCM collected from cell cultures 3 h after irradiation was used to treat fresh cell cultures. After 24 h of treatment, genomic damage was measured (Fig. 4). No differences were found between the 2 Gy dose (8 AU) and the non-irradiated control (2 AU). Genotoxicity of ICCM with 8, 12, 16 and 20 Gy doses (50, 101, 114 and 111 AU, respectively) was greater than with the control and the 2 Gy dose. In addition, significant differences were observed in the genotoxic effect of ICCM between HRT and SBRT doses, but no differences were observed between 12, 16 and 20 Gy.

Effects related to SBRT total dose

Cell cultures were irradiated with a fractional dose of 20 Gy, separated by 48 h from each other, in order to reproduce a normal SBRT treatment. Fig. 5A shows an increase in DNA damage with

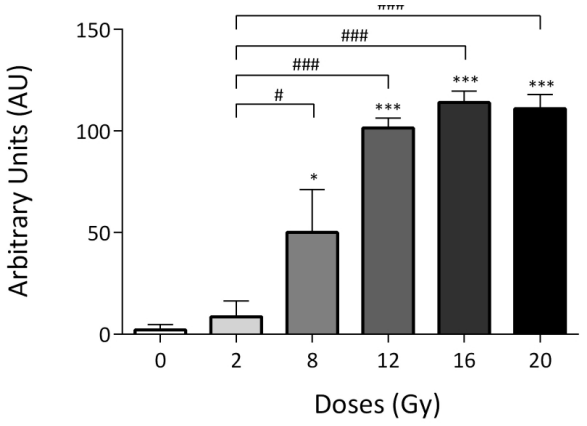


Figure 4. Non Target Effects. ICCM-induced genotoxic damage evaluated in the A549 cell line, plotted like damage index (AU) vs irradiation dose (Gy) and expressed as mean ± SD. One-way ANOVA and post-hoc Tukey's test, * p < 0,05 and *** p < 0.001 versus untreated control cells; # p < 0,05 and ### p < 0,001 versus cells treated with 2 Gy.

doses of 20, 40 and 60 Gy, compared to 0 Gy (p <0.0001). The DNA damage generated by a cumulative dose of 60 Gy was greater than that produced by 20 Gy (p <0.001). In Fig. 5B the genotoxic damage classified by Damage class is shown, it can be seen that with 20 and 40 Gy the damage generated is mainly of moderate class (p <0.001), and with 60 Gy of severe type (p <0.001). The results of cell viability evaluated by the integrity of the plasma membrane are presented in Fig. 6. A similar behavior can be observed, that is, a cumulative dose of 60 Gy significantly decreased cell viability with respect to the dose of 0 and 20 Gy (p <0.0001).

DISCUSSION

The poignant and unexpected COVID-19 pandemic has forced health systems to optimize their resources and prioritize strategies adapted to this crisis. In this context, treatment rationalization and evaluation of therapeutic options for RT have been suggested. In this sense, reconsideration of hypofractionation is now recommended (Faivre-Finn et al. 2020) because they allow less movement of patients, medical personnel and transport, thus reducing health risk. Although the efficacy of these treatments for non-small cell lung cancer is supported by several studies (Iyengar et al. 2018, Brown et al. 2014), we believed it was appropriate to empirically document their results and then delve into their foundations. We verified that SBRT presents optimal therapeutic results. Coinciding with other studies (Wang et al. 2016, Suh & Cho 2019, Palma et al. 2019, Kalinauskaite et al. 2020, Uzel et al. 2019), we obtained good overall survival rates and progression-free survival, with no significant increases in toxicity. Only four out of 22 individuals developed mild side effects (esophagitis, pyrosis, dysphagia or cough with mucus), with no fatal or rare effects,

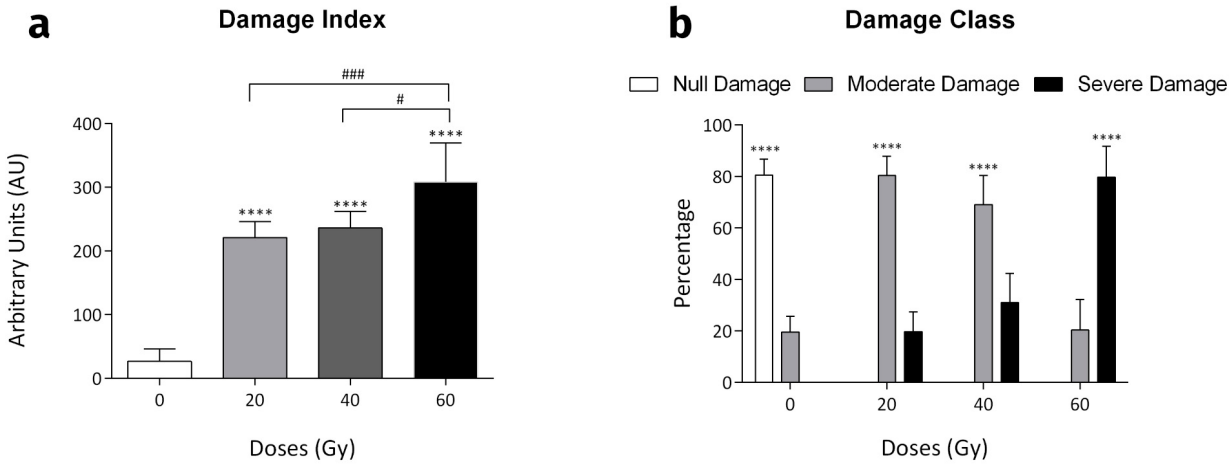


Figure 5. Genomic damage after total dose of SBRT. Radiation-induced genotoxic effect evaluated in the A549 cell line just after irradiation. a. Results were plotted as damage index vs Gy dose irradiation and expressed as mean ± SD. One-way ANOVA and post-hoc Tukey's test, **** p < 0.0001 versus untreated control cells and ### p < 0.001 and # p < 0.1 versus cells treated with 20 and 40-Gy, respectively. b. Results were plotted as damage class vs Gy dose irradiation and expressed as mean ± SD. Two-way ANOVA and post-hoc Tukey's test, **** p < 0.0001 versus the other class of damage at the same irradiation dose.

as mentioned by Loi et al. (2020). Although the basic parameters of this modality and HRT do not differ, the trend towards ablation could probably be statistically demonstrated with longer follow-up times. In this way, and mindful of the circumstances we are going through, not only effective but also safe and profitable therapeutic alternatives should be supported (Uzel et al. 2019, Swaminath et al. 2017, Kalinauskaite et al. 2020).

On the other hand, since hypofractionation and CRT patterns of radiation differ, it is necessary to know whether their radiobiological foundations also differ. The distribution of the total dose according to the time of treatment and the number of fractions depends partly on DNA damage repair and cell repopulation that occur between consecutive sessions (Hall & Giaccia 2012, Luo 2019). In this way, we implemented complementary *in vitro* experiments to clarify the cytotoxic and genotoxic bases of these treatments (Tubiana 2008).

The objective of RT is to induce lethal DNA damage to curb the clonogenic capacity of tumor cells. High doses not only alter this condition, but also the cellular function of all irradiated cells. Ablative treatment alludes to this (Baskar et al. 2014, Timmerman 2008). As expected, the results obtained clearly demonstrate not only that genomic damage increases in correlation with the dose, but also that cell behavior differs from CRT (2 Gy) from 8 Gy onwards. We could empirically ensure that ablation is substantiated through the induction of complex and potentially lethal lesions, a significant decrease in mitochondrial activity, and a functional loss of the plasma cell membrane. It has been suggested that these high doses would be reached with prolonged exposure times, enabling DNA repair and reducing the cytotoxic effect (Brown et al. 2014, Cosset 2017, Shibamoto et al. 2012, 2016). However, our results do not coincide with this. The analysis of residual genotoxic damage 3 h post-irradiation showed less efficiency of repair processes above the 8 Gy dose. Simultaneously, we observed a synchronous result in viability tests, without differences between 12, 16 and 20 Gy doses.

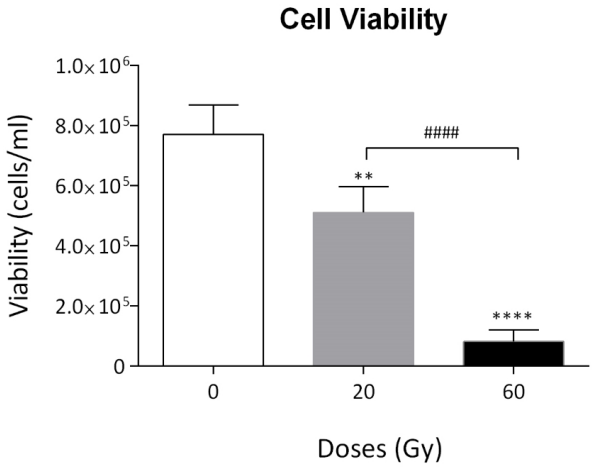


Figure 6. Cell viability after total dose of SBRT. Cell viability was measured by integrity of the plasma membrane and expressed as viable A549 cells by ml of culture. Results are expressed as mean ± SD. One-way ANOVA and post-hoc Tukey's test, **** p < 0.0001 versus untreated control cells and #### p < 0.0001 versus cells treated with 20-Gy.

Likewise, we empirically tested differential repopulation between different exposures by means of proliferation experiments. As time passed, cell cultures grew slower and ablative treatments differed. In line with this, a marked decrease in residual viability at 96 h ($p<0.0001$ for 20 Gy) was also observed for these doses, which in turn coincided with their decreasing clonogenic capacity. Regarding the LQ model, commonly used for CRT, its application for hypofractionation in lung cancer is highly debated (Guckenberger et al. 2013, Lu et al. 2019, Otsuka et al. 2011, Shibamoto et al. 2012, 2016). Our experience confirms studies that contemplate its validity up to 8-10 Gy doses per fraction (Cosset 2017, Prasanna et al. 2014, Sheu et al. 2013, Suh & Cho 2019). We believe that it is essential to understand the effects of each dose at molecular, cellular and integral level to face the complete radio-induced response. Although the classical radiobiology is based on the DNA damage of target cell, current research considers the holistic effect of RT (Baskar et al. 2014, Grass et al. 2016, Mothersill & Seymour 2004, Prasanna et al. 2014).

While some authors (Brown et al. 2014) understand that the effects of hypofractionation can be interpreted by the well-known 4Rs (repair, repopulation, recruitment, reoxygenation), we agree with those who think that it would be appropriate up to fractions from 3 to 6 Gy (Kim et al. 2015, Prasanna et al. 2014). We understand that ablation integrates these processes into a broader framework that considers other phenomena. Irradiated cells are known to release clastogenic factors that can alter neighboring cells and amplify the toxic effect of the treatment (Baskar et al. 2014, Grass et al. 2016, Prasanna et al. 2014). We demonstrated that conditioned medium from cell cultures exposed to 8, 12, 16, and 20 Gy had a greater genotoxic effect than medium exposed to conventional doses, and that indirect mechanisms were more robust in ablative doses. Our findings could be conceived as part of the pharmacodynamic effects reported by several authors (Baskar et al. 2014, Prasanna et al. 2014), proposing a major role of the tumor microenvironment (Brown et al. 2014, Kim et al. 2015, Kirkpatrick et al. 2008, Prasanna et al. 2014, Song et al. 2014). While the precise mechanism of intercellular communication after radiation exposure is still unclear, recent work also demonstrates the involvement of exosomes in increased levels of reactive oxygen species (ROS) and further DNA damage (Nakaoka et al. 2021). Conversely, the CRT would not be able to orchestrate this response (Grass et al. 2016, Song et al. 2014).

Finally, to complete and optimize the study, we decided to recreate in vitro exposure to a total dose for the lung (60 Gy), respecting the SBRT protocol. Thus, considering that one of the principles on which the fractionation of the tumoricidal dose rests is to prevent the recovery of the tumor between successive doses, we

decided to evaluate the genomic damage induced by 60 Gy, delivered in three sessions, spaced 48 hours apart. It was shown that as treatment progresses, the cumulative dose correlates with increased clastogenic damage and does not lead to DNA repair. This damage, which qualifies as "severe", may correspond to lethal injuries that support the decrease in cell viability (Iliakis 1988, Hall & Giaccia 2012). In this way it can be said that the fractionation of the dose imparted during one week, induced a cellular regression of the analyzed population, analogous to the reduction of the tumor volume, sought in the treatments with this therapy.

CONCLUSIONS

The results obtained suggest a greater probability of SBRT for tumor control in relation to conventional protocols, sustained through a multifactorial mechanism of action. Although significant questions remain, these findings contribute to a better understanding of the biological foundations of the RT treatments that are currently being imposed.

Acknowledgments

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SUPPLEMENTARY MATERIAL

Table SI. Patient information. Patients are grouped according to the type of treatment they underwent CRT, HRT or SBRT. Data are shown for: Age (years); tumor Stage and Anatomopathology classification; patient Follow-up time after treatment (days); irradiation procedure in Number of fractions, Dose by fraction and Total dose (Gy); and if the patient had any type of Toxicity derived from the treatment (dermatological, respiratory, neurological or gastrointestinal), classified according to CTCAE.

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