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STUDY OF THE RADIOMODIFYING EFFECT OF FOLIC ACID

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1. Introduction

Folic acid (FA), historically linked to the vitamin B12, plays a crucial role in health, being essential for vital biochemical processes. Initially isolated in 1943, its importance was reinforced by studies by Wills, Day, and others, evidencing its relevance in anemia and cell growth. [1]
This water-soluble vitamin, present in many foods, acts as a coenzyme in key metabolic pathways, including nucleotide synthesis and methylation reactions. [2] Folic acid is crucial during pregnancy, influencing the increase of red blood cells, fetal development, and playing key roles in cell division and protein synthesis. [3]
In the oncological context, folic acid is administered as an adjuvant in treatments with the antifolate fluourouracil, demonstrating efficacy in cases of grade III colon cancer [4,5] Its application as a radiomodifier in radiotherapy treatments has been explored, aiming to reduce side effects and protect surrounding tissues. [6] Research indicates that folic acid may act as an exogenous antioxidant, minimizing oxidative stress caused by ionizing radiation. [7]
To be an effective radiomodifier, folic acid must exhibit characteristics such as protection against adverse effects of radiation, general protective effects on non-target organs, low toxicity, simple administration, and pharmacological compatibility. [8]
In summary, folic acid emerges as a tool with therapeutic potential in the oncological context, offering benefits both in the prevention of anemia and in minimizing the adverse effects of radiotherapy. Its role as a radiomodifier opens up promising prospects for improving the efficacy of treatments and protecting healthy tissues.

2. Methodology

The NCTC Clone 929 cell line was cultured in flasks containing Eagle Minimum Essential Medium (MEM) medium supplemented with 10% fetal bovine serum (FBS), 0.1 mM of non-essential amino acids, and 1.0 mM of sodium pyruvate, without the addition of antibiotics. After the formation of a cell monolayer in the greenhouse at 37°C, dispersion was performed using a solution containing 0.05% trypsin and 0.02mM EDTA. Cells were counted, adjusted to appropriate concentrations, and distributed in 96-well microplates, with 0.2 mL of cell suspension in each well. The microplates were incubated for 24 hours at 37°C, with a humid atmosphere containing 5% carbon dioxide (CO2).

2.1 In vitro determination of the cytotoxicity index (IC50 %) of folic acid

An in vitro cytotoxicity study was conducted to evaluate the toxicity potential of folic acid on NCTC Clone 929 cells. The cells were cultured in 96-well microplates at a density of 0.2 mL cells/well. Folic acid was dissolved in different solvents (DMSO, NaOH, NaHCO3 and PBS) and added to cells in increasing concentrations. After 24 hours of incubation, cell viability was evaluated using the MTS staining assay. The IC50% was calculated for each solvent, indicating the concentration of folic acid that inhibits 50% of cell viability. The results revealed folic acid cytotoxicity to the cells at all concentrations tested, with 50% CI of

1.61µM for DMSO, 1.89µM for NaOH, 2.03µM for NaHCO3 and 2.17µM for PBS. It is concluded that folic acid can be safely used with NaHCO3 or PBS, but has the potential for toxicity depending on the concentration and solvent used. The PBS solution showed a better response in the cell analyses in this study due to its PH and its specific cellular characteristics.

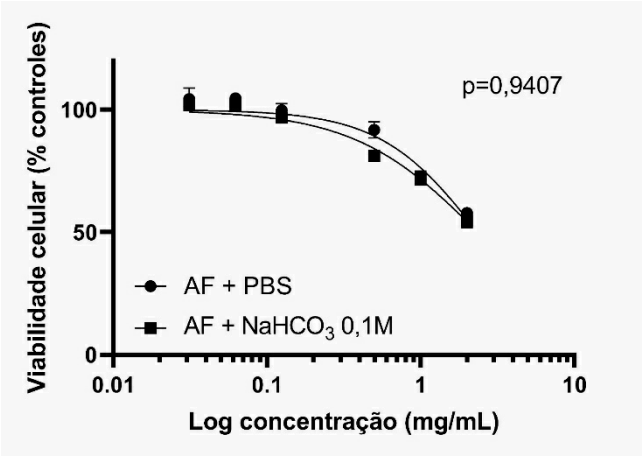

Figure 1 - Cell viability curve obtained in cytotoxicity assays comparing AF+PBS and AF+NaHCO3 0.1M.

2.2 In vitro determination of lethal dose 50% (LD50) of gamma radiation

An in vitro assay was performed to determine the lethal dose 50% (LD50) of gamma radiation to NCTC Clone cells 929.As cells were cultured in 96-well microplates at a density of 1.0 x 104 cells/well and irradiated with doses of 250, 500, 750 and 1000Gy. After irradiation, the cells were incubated for 24 hours. Cell viability was assessed using the MTS supravital dye staining assay. The results showed that the LD50% was 750Gy.
These results suggest that gamma radiation is highly cytotoxic to NCTC Clone 929 cells, requiring a dose of 750Gy to kill 50% of the cells.

3. Results and Discussion

3.1 In vitro determination of lethal dose 50% (LD50) of gamma radiation

The graph depicted below shows the results of the cytotoxicity experiment in NCTC Clone 929 cells irradiated with doses of 250, 500, 750 and 1000Gy. Relative cytotoxicity values indicate a LD50% curve compared to the control. LD50% was estimated at 1081Gy, with a coefficient of determination (R^2) of 0.9980, showing an excellent correlation between the irradiation doses and the cytotoxic response observed.

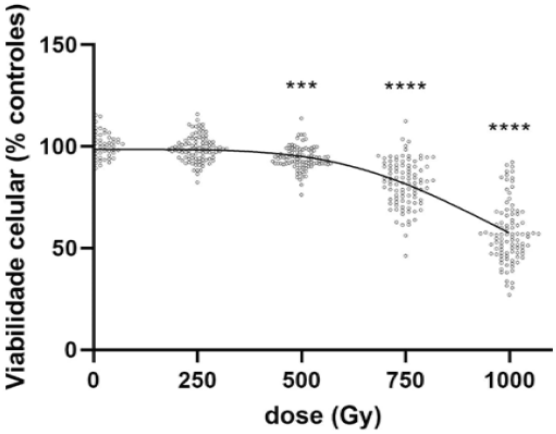

Figure 2 - **Gamma radiation cytotoxicity and LD50% determination.**

Ionizing radiation induces cell death in a dose-proportional manner, following a pattern adjusted to the linear-quadratic function, a second-order polynomial, as established by previous studies. [14,15] The mathematical relationship of this dynamic is detailed in the formula $N = N\_0-aD+bD^2$. This mathematical model supports the understanding of cytotoxicity proportional to the radiation dose.

3.2 In vitro determination of the cytotoxicity index (IC50 %) of folic acid

Folic acid in aqueous solution plays a significant role in radioinduced cytotoxicity by influencing the cellular response to ionizing radiation, as demonstrated by the adjustments of cytotoxicity data in irradiated NCTC Clone 929 cells, evidenced in the graph below. [9] The presence of folic acid revealed notable disparities between treated and untreated crops, being more relevant at lower doses, indicating a change in the influence of the linear coefficient (α) at more energetic doses ($p = 0.0307$).
The α coefficient is related to the induction of Locally Multiply Damaged Sites (LMDS), resulting in cell death by a single hit killing event, associated with the efficacy of radiotherapy in tumors, especially at lower doses. [10, 11] Folic acid, even at higher doses than therapeutic, exhibited a radiomodifying effect, increasing radiation toxicity in experiments with lower doses.

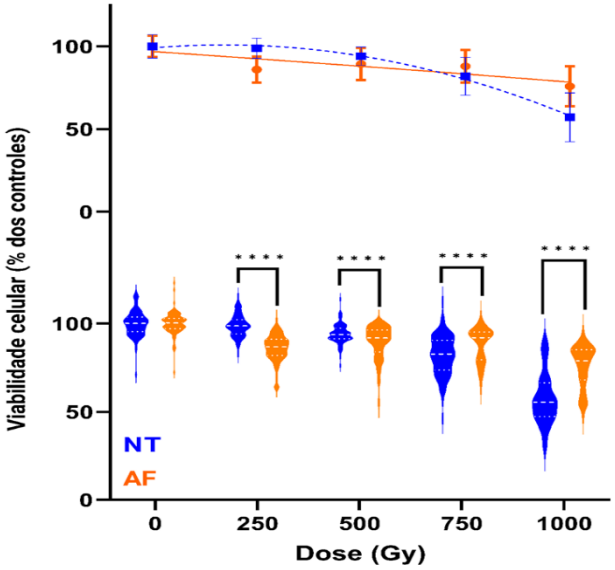

Figure 3 - **Cell viability after irradiation.**

The graph above presents cell viability data in treated or untreated cultures, irradiated, and supplemented with folic acid. Lower viability was observed at the doses of 250 and 500Gy, while at the doses of 750 and 1000Gy, the relationship was reversed, highlighting the complex effect of folic acid at different doses of radiation. [12] Folic acid supplementation is associated with reduction of apoptotic fraction at low doses, while deficiency is related to chromosomal instability. The complexity lies in the fact that intracellular folate can both sensitize and protect at different therapeutic doses. [13]
The analysis of the radioinduced cytotoxicity experiments, expressed in the graph above, highlights the dual effect of folic acid, sensitizing cells at lower doses and protecting at higher doses, highlighting the complexity of its role as a radiomodifier in cancer treatments.

4. Conclusions

This study examined the effects of gamma radiation and folic acid on NCTC Clone 929 cells. The results include the determination of LD50% of irradiated cells (1081Gy) and IC50% of folic acid in aqueous solution (2.366mg/mL). The presence of folic acid demonstrated radiomodifying effect, amplifying radiation toxicity at lower doses and mitigating it at higher doses.

The key findings are as follows:

1. DL50% of NCTC Clone 929 cells is 1081Gy.
2. The 50% CI of folic acid in aqueous solution is 2.366mg/mL.
3. Folic acid, in aqueous solution, acts as a radiomodifier, adjusting the toxicity of radiation at different doses.
4. Future studies are needed to elucidate the molecular mechanisms underlying the radiomodifying effect of folic acid, in order to optimize its application.
5. Additional trials are recommended to evaluate the efficacy of folic acid as a radiomodifying agent in cancer cells, animal models, and in vivo clinical trials.
6. The results strengthen the prospect of folic acid as a valuable tool in the treatment of cancer in

combination with radiotherapy, laying a solid foundation for future research and the development of refined strategies for its use as a radiomodifying agent.

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References

[1]. VANNUCHI, H.; MONTEIRO, T. H. Funções Plenamente Reconhecidas de Nutrientes. **INTERNATIONAL LIFE SCIENCES INSTITUTE DO BRASIL**, São Paulo, v. X, p. 3-15, Fevereiro 2010. ISSN ISBN: 978-85-86126-23-9.

[2]. MARCHIORO, A. A.; SÁ-NAKANISHI, A. B. D.; CAMPANERUT, P. A. Z. IMPORTÂNCIA DO ÁCIDO FÓLICO. **UNINGÁ Review**, Maringá, n. 1, p. 64-70, Janeiro 2010.

[3]. SANTOS, L. M. P.; PEREIRA, M. Z. Efeito da fortificação com ácido fólico na redução dos defeitos do tubo neural. **Cad. Saúde Pública**, Rio de Janeiro, v. 23, n. 1, p. 17-24, Janeiro 2007.

[4]. BALUZ, K.; CARMO, M. D. G. T. D.; ROSAS, G. O papel do ácido fólico na prevenção e na terapêutica oncológica: revisão. **Revista Brasileira de Cancerologia**, v. 48, p. 597-607, 2002.

[5]. CHOI, S.-W.; MASON, J. B. Folate and Carcinogenesis: An Integrated Scheme.**American Society for Nutritional Sciences**, Massachusetts, v. 130, n. 2, p. 129-132, 2000.

[6]. SOUZA, C. A. et al. Terapêutica citoprotetora em pacientes tratados com quimio e/ou radioterapia anti neoplásica. **Revista Brasileira de Hematologia e Hemoterapia**, v. 22, n. 2, p. 123-128, Junho 2000.

[7]. WEISS, J. F.; LANDAUER, M. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. **Toxicology**, v. 189, p. 1-20, Julho 2003.

[8]. MAISIN, J. R.; LECTURE, B. A. A. A. Chemical radioprotection: past, present and future prospects. **International Journal of Radiation Biology**, v. 73, n. 4, p. 443-450, 1998.

[9]. FARKAS, K. et al. PeerJ. **Insights on early mutational events in SARS-CoV-2 virus reveal founder effects across geographical regions**, 2020. 1-14.

[10]. BOUCHER, D.; TESTARD, ; AVERBECK, D. Low levels of clustered oxidative DNA damage induced at low and high LET irradiation in mammalian cells. **Radiation and Environmental Biophysics**, 2006. 267-276.

[11]. CHAPMAN, J. D. Single-hit mechanism of tumour cell killing by radiation. **International Journal of Radiation Biology**, 2003. 71-81.

[12]. PADULA, G.; PONZINIBBIO, M. V.; SEOANE, A. I. Possible radioprotective effect of folic acid supplementation on low dose ionizing radiation-induced genomic instability in vitro. **Indian Journal of Experimental Biology**, 2016. 537-43.

[13]. KHOSHGARD, K. et al. Radiosensitization effect of folate-conjugated gold nanoparticles on HeLa cancer cells under orthovoltage superficial radiotherapy techniques. **Physics in Medicine & Biology**, 2014. 2249-2263.

[14]. KELLERER, A. M.; ROSSI, H. H. RBE and the primary mechanism of radiation action. **Radiation Research**, 1971. 15-34.

[15]. CHADWICK , K. H.; LEENHOUTS, H. P. Physics in Medicine & Biology.**A molecular theory of cell survival**, 1973. 78-87.