Salmonella typhimurium Treated by Radiation as an Approach for Cancer Management

A thesis submitted for the award of the degree of PhD in Biochemistry

By

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ENGLISH ABSTRACT

This study was conducted to evaluate the efficiency of γ and UV-radiation to induce attenuated *Salmonella typhimurium* variants from its wild type strain; *S. typhimurium* (ATCC 14028). And also to investigate the effect of these attenuated variants for their antitumor activity *in- vivo* and *in -vitro.*

Sensitivity of *S. typhimurium* wild type strain towards UV- and γ-radiation was determined and the results show a reduction in its viable count with the increase of the dose of γ radiation exposure and the time of the exposure to UV-radiation as well, cycling dose for γ radiation chosen to be 1kGy and for UV-radiation 120minutes.

Exposure of *S. typhimurium* .WT. to several successive cycles of radiation (20 cycles for γ and 10 cycles for UV-radiation) leads to an increase in its radiation resistance and decreased in its virulence in mice by the magnitude of 10^2.

By testing *S. typhimurium* and its radiation induced variants for cytotoxicity *in vitro*, it was found that a great inhabitation in EAC cells viability count was occurred when *S. typhimurium* .WT. exposed to γ radiation (1and 4 kGy) and to UV radiation for 300mins ,also, when *S. typhimurium* .WT. exposed to 6, 12, 14, 18 cycles of γ radiation and 3 cycles of UV-radiation .

From those previous mentioned variants *S. typhimurium* γ 1kGy, *S. typhimurium* UV-C3, *S. typhimurium* γ-C 14were chosen to be tested for their antitumor activity *in vivo* since they showed least lethality percentage in mice.

One hundred adult male mice weighing 20-25 gm. were used, for this purpose and divided into 10 experimental groups 5 of these groups were mice bearing EACs solid tumor ,after the tumor volume reached $1cm^{3,}$ all groups were treated with *S. typhimurium* .WT. and its variants, tissue histology as well as proliferation, immunological biomarker, cell cycle analysis were assessed.

The attenuation of *S. typhimurium* .WT. variants result in more accumulation and colonization in tumor in the muscular thigh tissue of mice, which in turn cause eradiation in tumor volume and retardation in its growth more than the wild type stain do.

The results indicated that, the treatment with radiation induced variants showed a significant decrease in TNF-α, IL-6and VEGF levels and increase in caspase-3 activity , total leukocyte, and lymphocyte count percentage, but showed a significant decrease in neutrophil count

percentage. Also, there was a significant increase in CD4, CD8, and P53 percentage count, cell cycle analysis showed significant increase in G0, while significant decrease in S and M phase count percentage. Also, the flow cytometry analysis showed increase in apoptotic cell count % (sub G_1). In addition, the histopathological analysis revealed that the treatment of tumor bearing mice with *S. typhimurium* attenuated variants achieve improvement of the muscular thigh tissue compared to EAC tumor bearing mice.

CONTENTS

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English summary

The present study was planned to evaluate the efficiency of UV- and γ–radiation as a tool to induce variant derivatives from *S. typhimurium* . WT. capable to prevent the solid tumor proliferation.

To achieve our goal the protocol divided into 2 sections. First, the exposure and cycling of *S. typhimurium* .WT. strain. Using UV and γ radiation to induce variants strains have different characters and to reduce its pathogenicity in mice *in-vivo*. Second, was testing these UV- and γ–radiation induced variants for their antitumor activity *in -vitro* and *in- vivo*.

To fulfill the target of this study a total of 100 male mice were allocated into groups as the following:

Group 1: Control group; animals left without any treatment.

Group 2: (tumor group); animals bearing solid Ehrlich tumor left without any treatment.

Group 3: (*S. typhimurium* .WT.); animals not bearing solid Ehrlich tumor and injected with 100µl of *S. typhimurium* . WT. at dose of $1x10^4$ cfu (i.p) and 200lµl at same dose 3 times (i.m) 4 days apart.

Group 4: (*S. typhimurium* . WT. + tumor); animals bearing solid Ehrlich tumor and injected with 100µl of *S.*

typhimurium .WT. at dose of $2x10^4$ cfu twice (i.p) and 200lµl at same dose 3 times (i.t) 4 days apart.

Group 5: (*S. typhimurium* UV-C3); animal not bearing solid Ehrlich tumor and injected with 100µl of *S. typhimurium* . cycled by UV-radiation at dose of $2x10^6$ cfu twice (i.p) and 200lµl at same dose 3 times (i.m) 4 days apart.

Group 6: (*S. typhimurium* UV-C3 + tumor); animals bearing solid Ehrlich tumor and injected with 100µl of *S. typhimurium* . UVC3 at dose of $2x10^6$ cfu (i.p) twice and at same dose 200µl (i.t) times 4 days apart.

Group 7: (*S. typhimurium* γ-1kGy); animals not bearing solid Ehrlich tumor and injected with 100µl of *S. typhimurium* γ-1kGy at dose of $2x10^6$ cfu twice (i.p) and 200lµl at same dose 3 times (i.m) 4 days apart.

Group 8: (*S. typhimurium* γ-1kGy + tumor); animals bearing solid Ehrlich tumor and injected with 100µl of *S. typhimurium* γ-1kGy at dose of $2x10^6$ cfu twice (i.p) and 200µl at the same dose (i.t) 3 times 4 days apart.

Group 9: (*S. typhimurium* γ-C14); animals not bearing solid Ehrlich tumor and injected with 100µl of *S. typhimurium* γ-C14 at dose of $2x10^6$ cfu twice (i.p) and 200lµl at same dose 3 times (i.m) 4 days apart.

Group 10: (*S. typhimurium* γ-C14 + tumor); animal bearing solid Ehrlich tumor and injected with 100µl of *S.*

typhimurium γ-C14 at dose of $2x10^6$ cfu twice (i.t) and 200 µl of the same dose (i.p) 3 times 4 days apart.

The results indicated that the viable count of *S. typhimurium* WT. was decreased gradually by the increment of the exposure dose of γ radiation also. The same attitude when *S. typhimurium* .WT. exposed to different periods of time to UV-radiation. The UV irradiation survival curve showed a small, but significant resistance to UV. This resistance was also developed concomitantly with these observed for gamma irradiation.

To induce variants that differ from the wild type strain in their physiological characters, *S. typhimurium* .WT. was exposed to 20 successive cycles of γ radiation at cyclic dose of 1kGy.

Induction of radiation resistance in *S. typhimurium* .WT. strain may accompanied by changing in its physical characters, from this point of view, *S. typhimurium* .WT. was exposed to 10 successive cycles of UV-radiation at cyclic period of time equal 120 mins .

Exposure of *S. typhimurium* .WT. to several successive cycles of γ-radiation and UV-radiation increased its radiation resistance and decreased its virulence in mice by magnitude of 10^2. It was found that treatment of EAC cells with *S. typhimurium* .WT. exposed to different doses of γ-radiation

and different period of time for UV radiation induced 56% mortality for *S. typhimurium* γ-1kGy and 70% mortality for *S. typhimurium* UV 300mins respectively.

On the other hand the cycling of *S. typhimurium* .WT. by using γ and UV radiation improved the inhabitation effect on EAC cells line since, *S. typhimurium* γ- C6, *S. typhimurium* γ-C12, *S. typhimurium* γ-C14 and *S. typhimurium* γ-C 18 give a higher mortality percent of (86, 85, 88, 90) respectively and *S. typhimurium* UV-C3 give 86 mortality percent.

The cytotoxic effect of *S. typhimurium* .WT., *S. typhimurium* UV-C3, *S. typhimurium* γ-1kGy and and *S. typhimurium* γ– C14was increased in liver carcinoma cell line (HepG2) and show cytotoxic activity IC_{50} $1x10^9$ cells/ml respectively.

Our data showed that the cytotoxic activity IC_{50} in colon carcinoma cell line (HCT-116) was 19.8 x 10^6 cells/ml for *S. typhimurium* . WT., 13.3 x 10^6 cells/ml for *S. typhimurium* UV-C3 , 14 x 10^6 cells/ml for *S. typhimurium* γ-1kGy, 12.5 x 10^6 cells/ml for *S. typhimurium* γ-C14.

` Also, the cytotoxic activity IC_{50} in breast carcinoma cell line (MFC7) was recorded for *S. typhimurium* .WT. (18 x 10^6) for *S. typhimurium* UV-C3 ($14.6x10^6$ cells/ml), for *S. typhimurium* γ-C14 (12.4 x 10^6 cells/ml).

It was found that the increment of *S. typhimurium* .WT. and its variant concentration injected in mice were accompanied with the increase in mice mortality percentage, the least percentage of mortality was recorded, for *S. typhimurium* γ-C14, *S. typhimurium* γ-1kGy and *S. typhimurium* UV-C3, which is mean that, the previous induced variants were less virulent to mice than *S. typhimurium* wild type strain.

Mice bearing tumor treated with *S. typhimurium* variants (*S. typhimurium* UV-C3, *S. typhimurium* γ-1kGy showed marked reduction in the tumor volume as compared to the corresponding treated mice with the wild type strain.

In addition, serum IL-6, TNF-α level and tumor tissue VEGF concentration in tumor bearing mice which treated by *S. typhimurium* induced variants were significantly decreased ($p \leq 0.001$), compared to the control and tumor groups.

In the present study, the treatment of tumor bearing mice with *S. typhimurium,* WT. *S. typhimurium.* UV-3, *S. typhimurium.* γ-1kGy caused considerable increase in caspase-3 activity and the measured hematological parameters; in total leukocyte and lymphocyte and a decrease in neutrophil count percentage . Flow cytometry analysis of T lymphocytes revealed a significant increase in CD4 and CD8 percentage.