DOSE - EFFECT AND TIME - EFFECT DEPENDENCIES OF TUMOR FORMATION UNDER THE INFLUENCE OF IONIZING RADIATION AND CHEMICAL CARCINOGEN

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Abstract
Sublethal and potentially lethal damages induced by radiation are saved in a cell for a long time till following influence of promoter agent. Evaluation of dose-effect dependencies in the case of pure irradiation and evaluation of these dependencies after adding of carcinogen at different terms after irradiation allows getting additional information on particularities of process of tumor formation under combined action of radiation and chemical carcinogen. Dose-time dependencies of frequency of adenomas appeared under combined effect of radiation and urethane has been studied at different terms of urethane adding after irradiation. It has been shown that the frequency of tumors is determined both by radiation dose and the time interval between irradiation and adding of carcinogen substance. The data obtained make possible to analyze the period of conservation of induced damages and the non-linearity of the process of tumor formation under combined action of radiation and toxic factors on the organism. The latter is to be taken into account when forecasting the radiation risk under the real ecological conditions. Mathematical model has been constructed to describe the number of damages recovered by chemical promoter after a concrete time delay after irradiation.

Introduction
Appraisal of the radiation induced cancer risk in the real ecological conditions is connected with considerable difficulties due to multiple stages of carcinogenesis and additional factors affecting cell gene damaged by radiation. On the cellular stage according to doses dependencies there are several dose ranges described by curves of different types caused by the character of radiation influence on a cell and different transformation mechanisms of the transformed cell into tumor. Cells with low proliferation activity can exist in life even under existence of lethal damages if they are not going into mythos. When in the cells there are concealed damages that are sublethal or potentially lethal, there is transmission of them to further cellular generations without phenomenological appearance up to the moment of the promotional factor affect and manifestation of the damage in the form of stochastic or genetic modifications. Period between the radiation action and appearance of the tumor process can be rather long that is obvious from the periods of latent sizes for radiation induced tumors of different organs and tissues (decades). Therefore while analyzing the probability of post-radiation stochastic consequences progress perhaps the amount of radiation damages is not only and may be not so much more important than the amount of the damaged cells and structures conserved after finished processes of reparation and elimination. From this point of view high dozes cause more serious disturbances and a result of it more expressed rehabilitation processes in contrary to low doses. Hence not excluded that under the influence on the organism irradiated to low dozes by the promotional factor biological consequences of the radiation damages of stochastic origin may appear more often. Actually it is the problem of the combined consequences and factors of the non-radiation origin. One of the methodical approaches in revealing concealed damages may be the additional affect on the irradiated organism by non-radiation agents playing the role of promoters. Appraisal of dose-effect dependence in tumor induction while single irradiation acts and appraisal of the same effect after carcinogen including in different terms after irradiation allows to get additional information about peculiarities of the tumor formation process under combined action of radiation and chemical carcinogen that resulted in the ground to carry out the present work.

Materials and methods

Researches are carried out on mice of Af line at the age of 12 weeks and with the mass of 22-25 gram. Each experimental group consisted of 30 animals. The total irradiation was conducted by the experimental gamma-ray source (^{60}Co). In accordance with the irradiation character the test consisted of 3 series: 1 - total single irradiation in doses: 0,035, 0,1, 0,35 and 1,0 Gray at the dose capacity of 1,0 Gray/hour; 2 – fractional irradiation in the total dose of 0,35 Gray at 0,07 Gray/portion within 5 days at the dose capacity of 1,0 Gray/hour; and the 3d series - irradiation in the total dose of 0,35 Gray at 0,0389 Gray/portion within 9 days at the dose capacity of 0,008 Gray/hour. Urethane was incorporated intraperitoneally to the animals in the form of 10% solution on the basis of 1 milligram/gram of the animal's weight in a day after single and the last fractional irradiation. For intact control the same amount of physiological solution was incorporated. Mice slaughtering were carried out after 5 months after irradiation and urethane incorporation. In the 1st series the urethane was incorporated at the 1st, 3d, 7th, 15th and 30th days after irradiation. Its own control was for each experimental series. After animals slaughtering their lungs were fixing in the 10% formalin solution and were calculating adenoma (1). The amount of the induced adenomas per mouse and mice percentage with adenomas was the measure of the carcinogenic efficiency. Interaction index was calculated in accordance with (2) and was the ratio of the observable result under the combined action to the sum of the results of different affects of irradiation and urethane with correctness to the spontaneous level. Reliability of differences is defined in accordance with Student.

Results

Analysis of the dose-time dependence of the adenoma genesis frequency under combined action of radiation with chemical carcinogen incorporated at different terms after irradiation revealed that tumor frequency is defined not only by radiation dose but also by period of time between irradiation and carcinogen incorporation. The dose of 0,035 Gray did not cause increase in tumor genesis frequency. After further increase in irradiation dose 3-times increase in tumor frequency was observed as compared to the testing level. But in this connection there was no statistically considerable differences between the groups up to 10-times dose difference: 0,1 and 1,0 Gray as well, and the tumor frequency in the group with irradiation of 0,35 Gray was lower than at the dose of 0,1 Gray. Increase in the irradiation dose in 10-times from 0,1 to 1,0 Gray raised tumor frequencies only by 14 %. It concerns the results in the mice amount with adenomas in the groups equally: the minimum value (24,1 %) in the group at the irradiation of 0,35 Gray and the maximum value (80,0 %) at the irradiation of 1,0 Gray. Mice percentage with adenomas in the groups at the irradiation of 0,1 and 1,0 Gray practically did not differ between each other. The findings do not coincide with the strong criteria of the dose-effect linear dependence and are more complicated. As it was indicated (3) at the doses of more than 1,0 Gray the curve co-ordinates mostly with square dependence on dose, at the dose of 0,3 Gray the amount of the transformed cells is directly proportional to the dose, i.e. - linear dependence, and in the dose interval between 0,3 and 1,0 Gray frequency of the transformed cells genesis do not change with the dose increase. As for (4) it can be explained that single track effects domain at low doses and multiple track effects are considerable in high doses due to which in the dose dependence a quadratic member appears influence of which increases together with the dose capacity increase. The result at low doses increases first of all due to increase in the amount of cells with which there is irradiation interaction. After further increase in the dose there is increase in the amount of the cells experienced more than one hit and at the increased capacity of the dose there is more possibility that further hit to the target cell will be earlier before the first hit rehabilitation is over. Urethane incorporation induced tumor genesis at all mice of the testing group. Tumor genesis for the irradiated animals depended on urethane incorporation term after irradiation and on increase in the dose. Perhaps differences are caused by different intensity of the rehabilitation processes on the post-radiation period. Assessment criterion of the tumor genesis process under the combined action except the absolute tumor amount per mouse is the interaction index - K(в), that can respect the process of antagonism, additivity and synergism. Undoubtedly the maximum is the synergetic effect that reflect not only availability of several mechanisms and multiple changes in the cell during its transformation process but also the amount of the non-repaired damages after the first agent action. Synergetic quantification is the effect of the biological consequence excess of the combined action of radiation and non-radiation factors as compared to the result sum of its separate action, i.e. its value exceeds 1,0. Synergetic maximum if it exists can considerably vary in accordance with the experiment conditions, order of the affecting factor influence and other reasons. At the irradiation dose of 0,035 Gray tumor frequency did not differ for sure from the urethane control at the combined action. Further increase in the irradiation dose resulted in the tumor frequency increase. However period of time after the irradiation influenced mostly on the

effect expressed extent. Thus, in the irradiation group of 0,035 Gray this index changed from the additivity in the 1st day to the synergism by the 30th. As for the group with irradiation of 0,35 Gray maximum tumor cases were induced at the urethane incorporation in the 1st day after irradiation and for doses of 0,1 and 1,0 Gray – at the incorporation in the 7-15 days. This proves that the duration of conservation of the radiation induced damages exceeding their supposed rehabilitation period. Damaging and rehabilitation processes at mammals of different types have different duration and as for mice irradiated to low dose capacity processes last 15-30 days (5). Therefore the revealed results of the synergetic interaction in different terms after irradiation proves the availability of non-repaired concealed radiation damages and different cells sensitivity to carcinogen action in the post-radiation period. Numerous investigations on the post-radiation rehabilitation problem do not give an unambiguous answer to the question of the radiation damages rehabilitation process duration and of the speed with which it proceeds. Irradiation can increase blastomogenic effect of the chemical factors even when the action of the last ones proceeds after one third of the lifetime. Promotion of tumor formation process at the irradiated animals was shown under the influence of sex hormones on the example of the breast tumor, adenoids carcinoma and osteosarcoma under somatropine, thyroid gland cancer under methylthiouracil, liver hepatite under carbon tetrachloride (2, 3, 6, 7). Ullrich and Ethier (8) observed the expressed synergetic effect under the combined action of dimethylbenzantrathen and irradiation at low doses (0,24 and 0,5 Gray). Frequency of the breast tumor revealed in the test considerably exceeded the expected values and at high doses the effect was additive. Mijata (9), in particular, summarized people observation being affected after nuclear explosion and marked that most people health rehabilitated after 9 years but organism reaction for stressful influence changed to the side of chronic disorder. The findings reflect not only preservation duration of the radiation-induced damages but also non-linearity of the tumor genesis processes under the additional influence of the carcinogen on the irradiated organism. It follows from K(в) values average measures of which increased from 1,05 to 1,24, 1,04, 1,65 correspondingly. However at the Кв average increase their absolute values considerably vary in dependence with the period of time after irradiation and differ for example in the group with irradiation of 0,35 Gray in 5 times at incorporation of the urethane on the 1st and 30th day after irradiation. As for minimum dose K(в) started to increase after 7 days and culminated to the 30th day. As for doses of 0,1 and 0,35 Gray, vice versa, the most effective was the initial post-radiation period. The maximum dose of 1 Gray for all terms characterized by synergism with the maximum effect on the 7th day.

Modeling

Attempt of biological results mathematical modeling of the combined action under the hypothesis method (10) turned to be successful only for one dose (0,35 Gray). According to (10),

$N_\Sigma = N_1 + PN_1$ (**M**1), where N_Σ - total value of damages; N_1 - amount of damages from the direct affect of the hazard factor (in our case – this is radiation); P - share of "sub-damages", that do not reveal under the single radiation influence but reveal with addition of the second hazard factor (here – urethane). Model (**M**1) is stationary. If the sub-damages under single factor (radiation) isolated influence do not reveal then they should relax in the direction of the undamaged condition as times goes by. We will consider this process purely probable with constant relative speed (as in the case of the radioactive decay); i.e. the constant relative share of the sub-damages rehabilitates in unit of time. Then: N_0 - amount of the damages from radiation; PN_0 - amount of the sub-damages from radiation; $PN_0 \exp(- t /\tau)$ - decreasing of sub-damages as times goes by t at the constant value " sub-damages lifetime" τ. Then, at any time moment after irradiation under the urethane incorporation we reveal the amount of damages $N(t) = N_0 + PN_0\exp(- t /\tau)$ (**M**2). Here the second part of the sum is the amount of the sub-damages at the moment t, revealed as damages thanks to incorporation of the second hazard factor (urethane). On the basis of the experimental data we find average measures of the parameters P and τ, corresponding to the experimental data with minimum error ε at the whole range of values.

$P = 5{,}8 \pm 0{,}6$ ($\varepsilon \approx 10\%$), $\tau = 16 \pm 3$ days ($\varepsilon \approx 20\%$). Hence, as for the dose of 0,35 Gray we get the following dependence of damages on time of the urethane incorporation after irradiation $N(t) = 1{,}3 + 7{,}4 \exp(- t /16)$ (**M**3). Diagram (figure is not included due to space limitation) constructed in accordance with (**M**3), quite well corresponds to the experimental values. This indicates the specific adequacy of the model (**M**2) with the experimental data in case of a monotonic dependence character of the amount of damages on time.

Discussions

Difficulty of the tumor genesis process appraisal under combined action of radiation and chemical carcinogen is not limited by duration of the time period between their interactions. Genesis of the radiation-induced tumor depends not only on the dose but also on its time distribution - the dose capacity. Genetic complexity of the multiple stages cellular processes does not exclude different dose capacity value of quality for different tissues and different types of tumor. This defined quite wide range of values of the dose capacity index in the tumor induction under the equivalent irradiation doses. At present there is considerable amount of works proving that efficiency of the ionizing radiation with low energy efficiency concerning the reduction of lifetime and cancer induction are 2-10 times lower than at a single-pulse irradiation (7, 8, 11, 12). Index of the dose and the dose capacity efficiency can change within 2-20 times in accordance with the criterion of the tumor output for different organs and tissues (11, 12). In these conditions the time factor is realized by the fact that the damage appeared after the first irradiation manages to rehabilitate before the second damage is generated. As the starting dose amount was taken 0,35 Gray as the dose caused the maximum effect in the tumor genesis in earlier terms after the urethane incorporation. If the single irradiation resulted in statistically trustworthy increase in the adenomas quantity per mouse then the dose fractionating with kept capacity of the single irradiation (1 Gray/hour), as well as the dose fractionating with the reduced dose capacity up to 0,0389 Gray/hour, reduced tumor frequency that did not differ statistically from the control frequency. In the other way the fractionating and the reduced dose capacity affected the tumor genesis under the urethane incorporation. If after the single-pulse irradiation and the urethane incorporation the adenomas quantity per mouse in the combined action increased in 16 times and K(в) was 1,74, then at the fractionating and the dose capacity preservation these values were correspondingly 42 and 5,87. Reduction in the dose capacity vice versa decreased K(в) up to 1,44, and the tumor frequency per mouse in the combined action exceeds the testing group value only in 5 times. K(в) in this group was 4 times lower than under the fractionating irradiation but the higher dose capacity. Regarding the carcinogenic danger of low doses of irradiation there are opposite views: possibility of the cancer induction at the doses lower than 1 cGray (13) contrary to the view about not dangerous affect of low doses of irradiation (14). It is due to the analysis of different basic data in the estimations of the dose-effect dependence. Data about the purely radiation carcinogenesis are gained mostly in the experiment mainly at the high doses affect. In the real conditions when the ionizing radiation is one of the acting agents different physical, chemical and biological factors can modify the expressiveness of the radiation carcinogenesis. Thus the assessment model of the purely radiation risk in the real conditions is not complete, because it does not take into account possible influence of the additional factors on the final process development – promotional action of the non-radiation factors on the realization of the radiation induced damages of the cell genome. Comparative appraisal of the irradiation contribution into the tumor genesis and duration of the radiation damages preservation at incorporation of the urethane in the equal doses in different terms was analyzed on the basis of the relation index of the adenomas frequency per mouse in the combined action of the irradiation and the urethane. First of all it should be noted that the dose of 1,0 Gray is some how a “bounder”, division of the quantitative affect displaying. At the correlation of the doses up to 0,35 Gray it follows that low doses are less efficient on the first days of the urethane incorporation after irradiation (K(в) is lower than 1). For further terms of the post-radiation period at the doses of 0,035-0,35 Gray more concealed non-repaired damages are kept in the organism than at the higher doses. The latter follows from the relation of the adenoma frequency per mouse at the lower doses of irradiation to the higher ones goes larger 1,0; and for dose range of 0,035-0,35 Gray it is 2,6 after 30 days. It is obvious from this that during the supposed rehabilitation period of the radiation damages at lower doses big amount of the concealed damages are conserved and they are able to manifest the radiation damages at the additional affect of the chemical carcinogen. It can be explained by this fact that affect of the radiation factor on the tumor genesis process at lower doses can be bigger per a dose unit as compared to higher doses, because there is no much destruction of cells in the first case, the reparation processes are less stimulated by the destruction of cells and the change of the irradiated cell sensitivity to other hazard factors affect (15). At no low extent it can be caused by the carcinogens synthesis at low doses affect and genesis of non-repaired genetic damages (16). Comparing lower doses to 1,0 Gray in all (exclusion is only correlation of 0,35/1,0 Gray after 1 day) it is lower than 1,0.

Conclusions

From the findings discrepancy of the observation the extrapolation concerning the tumor induction in the dose range more than 1,0 Gray for low doses of irradiation seems to be incorrect. What is more

important it seems to be incorrect to use these dependencies when predicting radiation risk in the real ecological conditions when the carcinogenesis process is the result of not only radiation affect but also the additional affect of different factors of non-radiation origin playing the role of promoters. Perhaps in accordance with these reasons the program of the US Environmental protection agency (based on the single-factor dependence) the main aim of which was the reduction in cancer diseases by regulation of carcinogens content in the environment failed. This stimulated the oncologists of the USA to move the accent from the exogenous factors to the endogenous factors in the cancer ettiopathogenesis (17, 18). Appraisal narrowing of the risk up to the single-factor dependence (radiation) and tendency to reveal the specific contribution of the radiation factor in the real ecological conditions under which irradiation is one of the acting agents is explained first of all by the technical possibility of the analysis and of reception of some digital values. However, the attempt to extrapolate the findings for the current ecological conditions is connected with considerable difficulties that can mislead or show failure of the made calculations that was vividly revealed in discrepancies between the predictions and the real children diseases with thyroid gland cancer in Belarus after the Chernobyl accident. In the view of this the necessity of the work-out of new methodological principles of the sanitary and ecological evaluation of the environmental quality under the conditions of complex affect of the ionizing radiation and factors of the non-radiation origin on the organism is obvious (19). The research results in this problem will be given in the further messages.

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