A Study on the Shoulder of Cell Survival Curve in Relation With Repair Capacity of Cells After Being Irradiated

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Abstract

Aim:- to show that not all survival curves without shoulder are not able to repair or have lost the ability for the accumulation of sublethal damage.

Background:- the shoulder of the survival curve is considered as a region of accumulation of sublethal damage also as an indicator for cell capacity to repair. The size can be influenced by the change of the slope of the linear portion of the survival curve.

Results:- we have shown that a survival curve with shoulder size of 1.5 Gy can be a straight line when the slope of the exponential part is changed so the same region of the low dose of the same survival fraction can show shoulder at one time and with no shoulder at another.

Conclusion:- we have shown that in some cases cell survival curve could lose its shoulder without the necessity of losing its capacity for repair.

Introduction

The assessment of cell survival after being exposed to radiation is usually assessed by single cell cloning, the procedure was first developed by Puck and Marcus (1956) (1).Since then the single cell survival assessment was the most popular tool to measure the effectiveness of the cell survival modifying agents such as radiation, drugs and heat. Survival curves were the most important procedure available to give an idea about the effectiveness of the treating agent.

This procedure has been criticized as it does not give results with high degrees of precision especially for low radiation dose and high survival fraction(2), this is in addition to other reasons such as the uncertainty of the number of the seeded cells and it's obey to the statistical variation. However, an increasing number of experiments can significantly reduce error.

The introduction of a new procedure, the Dynamic Microscope Image Processing Scanner (DMIPS) (3) has increased the measurement precision and enabled the scientist to measure the survival fraction for very low doses as low as 0.2 Gy (4).

Survival curves show different shapes according to the cell type whether it is bacterial or mammalian cells or depending on the LET of the radiation or when the repair is disabled 5, 6. The most important difference is whether the survival curve is shouldered or a straight line. The appearance of shoulder in the survival curve has a special importance in cell survival post irradiation, in the design of the size of dose fractionation in radiotherapy, the estimation of repair post irradiation (7).

As the shoulder appeared of a vital importance, its measurement is also very important, it is therefore divided into two different segments according to the shape of that part of the curve. The first belongs to the initial part of the survival curve which represents the shoulder as an indicator for the accumulation of sublethal damage also for repair. The assessment of the accumulation of sublethal damage through the measurement of the shoulder size, i.e. ,D_q (the quasi threshold dose). The other is the exponential part which represents the cell death (7).

The usual measurement procedure could give different values for the size of the shoulder, i.e., the bending part of the survival curve in cases where the slope of the exponential part is changing with no or small change in the size of the bending part of the curve-the shoulder-(the region of accumulation of sublethal damage). This situation may occur when testing some drugs or using low dose rate in comparison with the high dose rate where the effect can influence primarily the slope of the survival curve rather than the shoulder (8), or when comparing between two different cell lines where the major difference between them is in the radio sensitivity, i.e., the slope of the survival curve D_0. The back extrapolation intersection with the line starting from 100% survival parallel to the x-axis would give a measurement for the shoulder influenced by the exponential part of the curve rather than independent measurement of the shoulder. Accordingly, (Dq) the shoulder size is influenced by both the low dose survival fraction value, i.e., the shoulder region *per se* and the slope of the linear part of the survival curve (D_0).

The procedure for the shoulder measurement may actually be considered as a combined value originated from the shoulder and the linear part of the survival curve may be better called the (should-linear) value

In the present work we have shown that the survival curve can be shouldered at one time and straight line at another time in spite of the survival fraction in the low dose region is the same for both curves. We think that a more conclusive criterion for shoulder interpretation should be established to assess the amount of sublethal damage inflicted in a cell population.

Theoretical investigation

The explanation of survival curves is of a vital importance in the explanation of the radiation effect on the living cells and its theoretical curve fitting leading to the prediction of the radiation effects on the cell survival. It follows that the shoulder size measurement is very important in the assessment of cell survival post irradiation. This is because that the shoulder is related with the cell recovery from the radiation injury. The idea behind this explanation is that as the curve is bending at the low dose region it gives higher survival than if it were a straight line and the effect is attributed to the cell repair from subleathal damage leading to an increase in the cell survival in comparison with the curve without shoulder (straight line) fig. 1. Several explanations were given to the survival curve some of them are similar others are different or modified such as the dual action theory by Kellerer and Rossi 9, in which the shoulder is explained on the basis of the production of sublesions and when they are close enough they will interact and cause a lethal damage. A similar explanation was given by Chadwik and Leenhouts 10 the (α,ß) theory, a stochastic model has also been introduced to explain the cell death after exposure to radiation 11.

In order to explain the survival curve it is divided into two parts, the shoulder and the exponential (linear) part, in which the former represents the accumulation of sublethal and repair while the latter represents the exponential cell kill.

With the measurement of the shoulder in the conventional method, i.e., the intersection of the back extrapolation of the exponential part with the line parallel to the x-axis starting from the 100% survival value. The measured shoulder size is influenced by the slope of the linear part and this can be clearly seen in the theoretical representation fig. 2. In this figure one can notice that the survival at the low dose region 2Gy is the same for both curves A and B. while the difference between the two curves is only the change in the slope of curve B.

As we have discussed earlier that the size of the shoulder can be affected by the linear portion of the survival curve in which it gives a different explanation for the damage accumulation and repair occurred in the cells fig. 2.

The important idea in signifying the shoulder importance is the bending shape which means that less cell killing than if it were a straight line fig 1. For this reason when the shoulder region becomes a straight line influenced by the change of the slope of the linear part will be interpreted differently as cells are not able to repair.

In an experimental work it is well known that the use of radiation with low dose rate can influence the slope of the survival curve fig. 3. In cases like this, the shouldered survival curve will be transformed to a straight line. This will make the curve at the same survival fraction at the low dose region, a straight line at one time and a shouldered at another which can give two different explanations for the same survival fraction at the same low dose region.

Results

Figure (1) shows the effect of the shoulder on the value of the survival fraction of cells exposed to a sparsely ionizing radiation. It can clearly be seen that the survival value at 0.01 approximately corresponds to dose = 4.4 Gy and = 6.9 Gy for the straight line and the shouldered survival curve, respectively. This gives that the shouldered curve is 36.2% higher than the straight line, i.e. ,the exponential part of the curve.

The size of the shoulder in fig. 2 A has a shoulder value approximately 1.5 Gy this is measured by the recommended way i.e. the intersection between the back extrapolation and the line parallel to the x-axis taken from 100% survival point. On the other hand, fig. (2)

B has no shoulder, i.e., a straight line. This result can show clearly the change in the shoulder with the change of the slope of the linear part of the curve in spite of the same survival at the 2Gy -the low dose-region for both curves. This can be explained as a loss of capacity for repair while the disappearance of the shoulder is caused by the higher survival at the high dose region which is caused by higher repair. One example similar to this hypothetical curve is the work on Hela cells testing the effect of the dose rate (8), fig. 3 is the survival curve for Hela cells in which a small shoulder is seen on it. The figure shows different survival curves taken for several dose rates. It can be clearly seen that the shoulder size is reduced gradually as the slope of the survival curve is reduced until a straight line is reached. These results can show the influence of the slope on the shoulder size.

Discussion

The bending part at the low dose region of the survival curve is considered as the region of the accumulation of the sublethal damage and/or the cell capacity for repair. As the dose progresses, the degree of curvature decreases and it will end up with a straight line or very close to it.

As mentioned earlier that the shoulder means that there is repair. For this reason we are interested in the measurement of the nonlinear part of the survival curve. This will lead to the conclusion that the actual measurement of the shoulder, i.e., the region of repair is the measurement of the bending part solely. As we have shown earlier (results) that the change in the slope of the linear part of the survival curve can change the value of the shoulder especially when the survival curve has a small shoulder. For the first glance the interpretation of the survival curves in fig. 2 is that, for curve A, has the ability to repair the sublethal damage because it has a shoulder while curve B hasn't such ability because curve B is a straight line. Such conclusion is unacceptable because if curve B, for example, in fig. 2 shows the same survival at the low dose region as curve A but as the slope changes we can see clearly that one curve A has a shoulder of 1.5 Gy and the other B without shoulder. The important question is that, in the linear curve B, is there repair in the same region of the shoulder as in curve A or does it accumulate damage or repair to the same extent of the shouldered curve A .The answer to such a question is debatable if we do not have a previous knowledge on the cells response to a challenge dose i.e. an ordinary survival curve and cells are without treatment. For example the low dose effect fig. (3) the straight line can be explained as an increased repair because we know that the low dose rate can allow cells for more repair and the change in the slope of the linear part of the curve is caused by increased repair, but what about curves with a straight line in which we do not know about the rate of repair. One of very important aspects of this issue is the explanation of the induced radio resistance. Always when there is an induced radioresistance accompanied with an increase in radiosensitivity in the shoulder region. This is an example which we are not sure about the extent of cell repair in the shoulder region and its interaction with the subsequent dose.

One suggestion for the interpretation of lose of shoulder in such cases is the comparison between the slope of the shouldered curve with the straight line curve. If the slope of the curve without shoulder is less steep than the shouldered curve then the loss of shoulder is because of repair and the cells have not lost the capacity for repair. This is valid when the comparison for one cell line having two different treatments but may not be applicable for two different cell lines in which we are not sure about their comparative response to the challenge dose.

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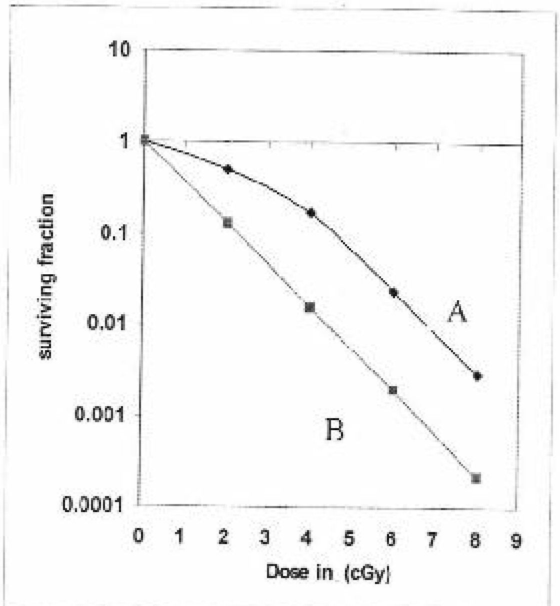

Fig. (1)Two survival curves of the same slope (D_0) showing different survival at any given dose. One shouldered (A) and the other without shoulder (B)

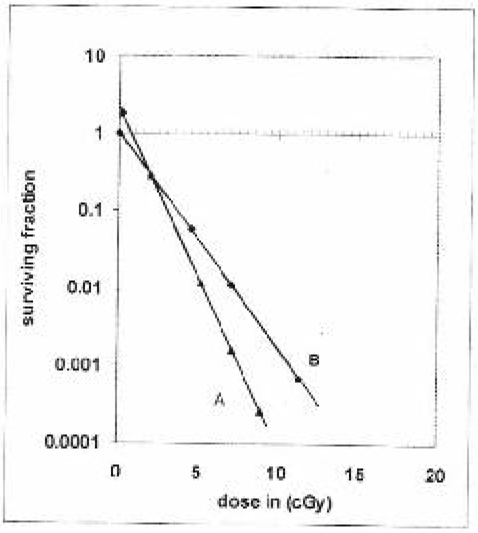

Fig. (2)The effect of the change in slope (D_0) on the value of the shoulder. Curve A has a shoulder size of one Gy while B is straight line with no shoulder. Note that both curves have the same surviving fraction at the shoulder region.

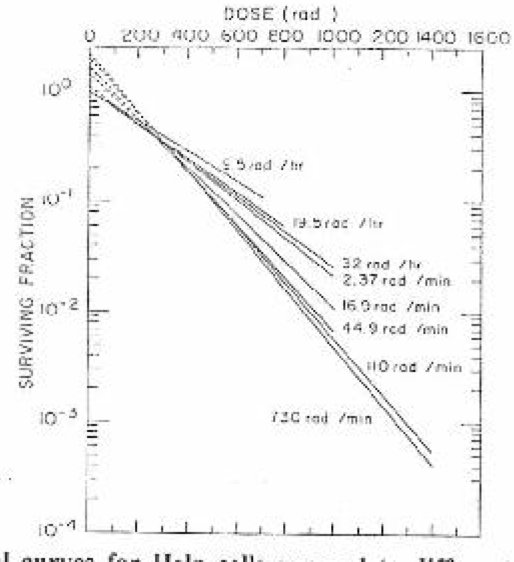

Fig. (3) Survival curves for Hela cells exposed to different rates showing a change in the slope and the disappearance of shoulder at dose rate of 2.37 cGy /min (rad /min),from Hall EJ (8)

دراسة قياس منطقة الكتف في منحنيات معيشة الخلايا وعلاقتها بقابلية إصلاح الضرر الناجم بعد تشعيعها

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الخلاصة

الهدف:- إن الهدف من البحث هو انه ليس كل منحني لمعيشة الخلايا بدون كتف(shoulder) ،هو دلاله على فقدان الخلايا لاصلاح نفسها من الضرر الناتج عن الاشعاع.

أساس البحث:- إن جزء الكتف (shoulder) من المنحنى لمعيشة الخلايا بعد تشعيعها يعتبر كدلاله على قابلية الخلايا على إصلاح نفسها من الضرر الناتج عن الإشعاع وان كبر الكتف هو قياس لذلك. إن كبر الكتف من الممكن أن يتأثر بميل الجزء المستقيم من المنحني مما يؤثر على القياس.

النتائج:- لقد بينا بأن منحني معيشة الخلايا الذي يتضمن كتف (shoulder) بكبر 1,5 كراي من الممكن أن يكون مستقيما فيما إذا لو تغير انحدار الجزء المستقيم من المنحني.

الاستنتاج:- إن منحني معيشة الخلايا قد يفقد الكتف (shoulder) بدون الضرورة إلى فقدان الخلايا قابليتها على إصلاح الضرر الناجم عن الإشعاع