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GLUCOSE ANALOGUES ALTER THE RESPONSE OF CHO-KI CELLS TO GAMMA IRRADIATION

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Abstract

Pretreatment of CHO-KI cells with 2-deoxy-D-glucose or L-glucose, two glucose analogues, reduces their survival if subsequently exposed to ^{60}Co irradiation. The reduction in survival is constant irrespective of the time the cells are in contact with the analogues before irradiation and occurred even when cells were irradiated 6 hours after plating, suggesting that cell cycle effects are probably not involved. Interestingly, split-dose recovery was not affected to the expected degree, despite a reduction in the extrapolation number of the primary curve. It is suggested that interference with energy production from glucose is responsible for the reduced capacity of cells to survive the irradiation.

The role of energy metabolism in determining the final response of cells to radiation is receiving increased attention. This follows reports that factors influencing ATP production and utilization can markedly alter the radiobiologic response in a variety of organisms (2, 7, 13, 16).

We have shown previously that lactate affects the radiation response of CHO-KI cells (12, 19). Since there are indications that lactate may be inhibiting glucose breakdown (18), it was decided to inhibit the normal breakdown of glucose using glucose analogues and to monitor the radiation response. The analogues investigated were 2-deoxy-D-glucose (2-DG), an antimetabolite of D-glucose, and L-glucose, an optical isomer. 2-DG has been shown to reduce the survival curve shoulder of respiration deficient yeast mutants (16). L-glucose does not appear to have been investigated.

Fluoro-derivations of 2-DG have already been shown to be promising as chemotherapy agents (3, 4, 8–10) and in tumour imaging (20), making the radiobiology of the substance of obvious interest in the area of cancer therapy.

In the present paper we report the effects of direct inhibition of glucose breakdown using glucose analogues on the survival and recovery of irradiated CHO-KI cells in culture.

Methods

The methods have been described in detail previously (19). Briefly, CHO-KI cells (Flow Laboratories, Scotland) were maintained in Ham's F12 nutrient mixtures supplemented with 10% foetal calf serum (Gibco Biocult Ltd., Scotland). They were routinely subcultured using trypsin (0.2%) and versene (0.02% 1 : 1 v/v).

S-DG (Sigma, London) was obtained as a sterile aqueous solution (5 g in 25 ml). It was diluted with medium to give appropriate concentrations which could be added in a volume of 0.1 ml to the culture medium. The most suitable concentration range following preliminary toxicity studies proved to be 1 to 10 mmol/l. L-glucose (Sigma, London) was obtained in solid form and was dissolved in medium sterilised by membrane filtration and used as previously described to give the same final concentrations. Cells

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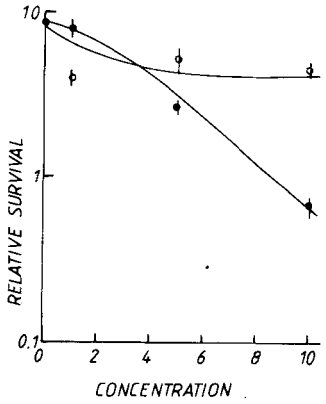

Fig. 1

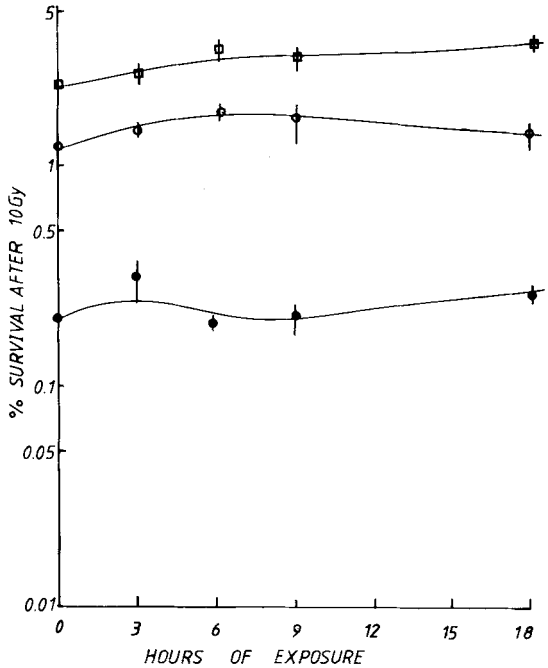

Fig. 2

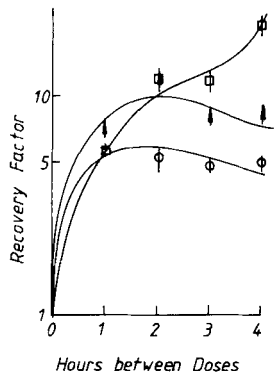

Fig. 3

Fig. 1. The effect of 18 h pre-exposure to increasing concentrations of L-glucose (○) and 2-DG (●) on the relative survival of CHO-KI cells irradiated to 10 Gy.

Fig. 2. The effect of varying times of pre-exposure to L-glucose (1 mmol/l) 2-DG (10 mmol/l) on the relative survival of CHO-KI cells irradiated to 10 Gy. L-glucose (○) , 2-DG (●) , control (□).

Fig. 3. The relative recovery of CHO-KI cells irradiated to 7.5 Gy followed by a further 7.5 Gy 0 to 4 h later. 10 mmol/l 2-DG (□) and 1 mmol/l L-glucose (▲) pretreatment for 18. Control (○).

Table 1

The effect of 18 h treatment with L-glucose and 2-DG on the plating efficiency, multiplicity, glucose and lactate metabolism of CHO-KI cells (mean values for 3 separate experiments) measured 24 h after initial plating. Initial glucose and lactate in medium with analogues but no cells were 43.5 μmol/l/5 ml and 4.96 μmol/5 ml, respectively

Concentration	Plating efficiency (%)	24 h multiplicity	Lactate production (μmol/l/5 ml)	Glucose utilisation (μmol/l/5 ml)
Control	75±7.9	2.8±0.3	18.1±0.63	34.3±0.4
1 mmol/l L-glucose	70±6.3 NS	3.1±0.2 NS	18.3±0.7 NS	37.0±0.17 NS
5 mmol/l L-glucose	60±8.2 NS	3.6±0.25 NS	15.8±0.2 $p<0.05$	37.8±0.44 NS
10 mmol/l L-glucose	60±6.9 NS	3.1±0.4 NS	14.3±1.57 $p<0.05$	36.5±0.08 NS
1 mmol/l 2-DG	80±6.4 NS	3.0±0.5 NS	5.73±0.86 $p<0.001$	36.0±1.29 NS
5 mmol/l 2-DG	42±3.8 $p<0.05$	2.8±0.2 NS	5.6±0.16 $p<0.001$	40.3±1.72 $p<0.05$
10 mmol/l 2-DG	42±3.6 $p<0.05$	2.1±0.3 NS	4.63±0.77 $p<0.001$	43.2±1.0 $p<0.05$

were plated in 40 ml flasks (Nunc) containing 5 ml medium at least 2 to 6 hours before addition of the analogues. The cells were irradiated 6 or 24 hours after plating using a ^{60}Co treatment unit delivering 2.0 Gy/min at SSD 60 cm. Three hours after irradiation all cultures received a medium change to remove the analogues. Survival was assessed using the colony formation assay of PUCK & MARCUS (15). Cellular multiplicity was determined at the time of irradiation by counting the number of cells in a hundred randomly selected microcolonies.

Glucose breakdown was assessed by estimating the amount of glucose used and lactate produced in medium samples after 24 hours growth of the cells. Glucose and lactate levels were measured in perchloric acid extracts of medium samples using the method of SCHMIDT (17) for glucose and GUTMANN & WAHLEFELD (6) for lactate.

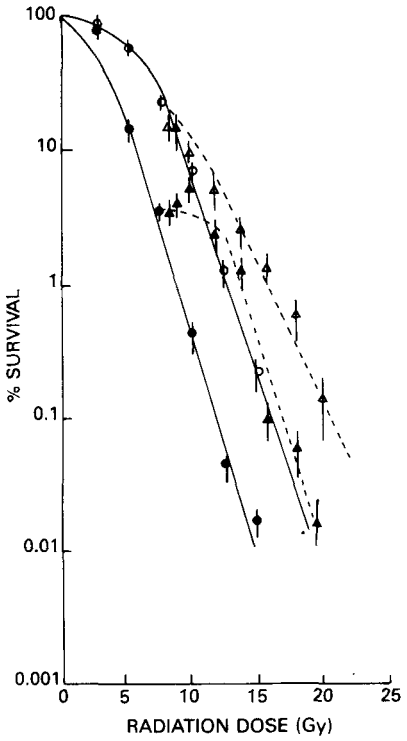

Fig. 4

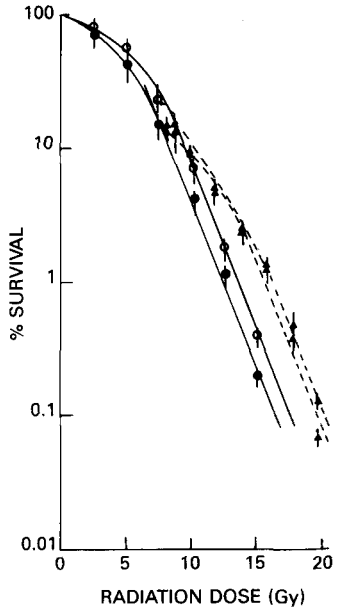

Fig. 5

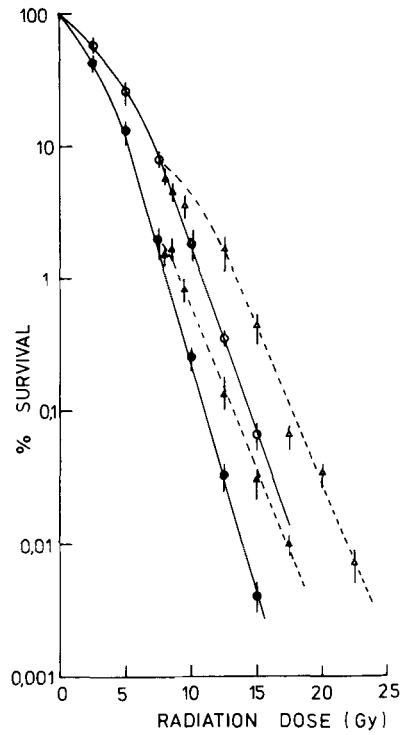

Fig. 6

Fig. 4. Primary and secondary survival curves for CHO-KI cells pretreated for 18 h before irradiation with 10 mmol/l 2-DG. Primary curve: Control (○) , 2-DG (●). Secondary curve: Control (△), 2-DG (▲).

Fig. 5. Primary and secondary survival curves for CHO-KI cells pretreated for 18 h before irradiation with 1 mmol/l L-glucose. Primary curve: Control (○), L-glucose (●). Secondary curve: Control (△), L-glucose (▲).

Fig. 6. Primary and secondary survival curves for CHO-KI cells plated 6 h before irradiation, 10 mmol/l 2-DG was added 2 h before irradiation. Primary curve: Control (○), 2-DG (●). Secondary curve: Control (△), 2-DG (▲).

Table 2

Survival curve parameters 'n' and 'Do' for CHO-KI cells set up 24 h before irradiation and pretreated for 18 h with glucose analogues and then given single doses of 0 to 15 Gy or a first dose of 7.5 Gy followed 3 h later by a second dose of 0.5 to 15 Gy. 'n' values have been corrected for average cellular multiplicity (see Table 1)

	Control	10 mmol/l 2-DG	1 mmol/l L-glucose
Primary curve, n	5.5±0.4	4.3±0.12	3.7±0.2
Primary curve, Do	1.8±0.1	1.5±0.2	1.8±0.1
Secondary curve, n	2.1±0.18	7.5±0.4	3.0±0.25
Secondary curve, Do	2.0±0.2	2.0±0.1	2.0±0.2

Results

Table 1 shows the effect of increasing levels of 2-DG and L-glucose on plating efficiency, glucose breakdown and lactate production of cells plated 24 hours before irradiation and exposed to the analogues for 18 hours. Both 2-DG and L-glucose inhibit glucose breakdown and lactate production, 2-DG being more effective. The higher concentrations of 2-DG reduce the plating efficiency by about 50 per cent but there is only a slight effect on multiplicity. L-glucose has a small but not statistically significant effect on plating efficiency and no effect on multiplicity. Where cells were plated 6 hours before irradiation plating efficiency was unaffected by 2 hours exposure to 10 mmol/l 2-DG and multiplicity was 1 in both control and treated cultures.

Fig. 1 shows the relative survival of cells treated with L-glucose and 2-DG (1, 5, and 10 mmol/l) 18 hours before irradiation to 10 Gy. The results show that at all levels of L-glucose tested there is a small but statistically significant ($p<0.05$) radiosensitising effect over 9 replicate points (approximately 40% reduction in survival). With increasing concentrations of 2-DG the radiation survival is progressively decreased.

To examine the variation in radiation response with duration of exposure, cultures seeded with appropriate cell numbers 24 hours before irradiation were treated with 0.1 ml ordinary medium or medi-

Table 3

Recovery factors for CHO-KI cells treated with glucose analogues for 18 or 3 h before irradiation. The substance was removed 3 h after irradiation by a medium change. The recovery factor is calculated as the survival following a dose given as 2 fractions over the survival from the same total dose given as a single fraction

Total dose (initial) dose 7.5 Gy)	Control		2-DG (10 mmol/l)		L-glucose (1 mmol/l)	
	18 h	3 h	18 h	3 h	18 h	3 h
10.0	1.8	2.2	12	6.25	3.5	1.4
12.5	2.4	6.7	20	8.7	4.5	3.0
15.0	2.8	11.5	20	10.0	5.5	5.4

Table 4

Survival curve parameters 'n' and 'Do' for CHO-KI cells set up 24 h before irradiation and pretreated for 3 h with glucose analogues and then given single doses of 0 to 15 Gy or a first dose of 7.5 Gy followed 3 h later by a second dose of 0.5 to 15 Gy. 'n' values have been corrected for average cellular multiplicity (see Table 1)

	Control	10 mmol/l 2-DG	1 mmol/l L-glucose
Primary curve, 'n'	14.0±0.4	7.4±0.3	7.4±0.1
Primary curve, 'Do'	1.6±0.2	1.25±0.1	1.7±0.3
Secondary curve, 'n'	19.0±0.5	8.0±0.2	5.0±0.7
Secondary curve, 'Do'	1.8±0.2	2.0±0.3	1.8±0.2
Average cellular multiplicity	2.8±0.3	2.7±0.2	2.8±0.2

Table 5

Survival curve parameters 'n' and 'Do' for CHO-KI cells set up 6 h before irradiation and pretreated for 2 h with 2-DG and then given single doses of 0 to 15 Gy or a first dose of 7.5 Gy followed 3 h later by a second dose of 0.5 to 15 Gy. 'n' values have been corrected for average cellular multiplicity (see Table 1)

Primary curve, 'n'	8.0±0.09	5.1±0.06
Primary curve, 'Do'	1.6±0.1	1.2±0.2
Secondary curve, 'n'	3.0±0.25	1.4±0.15
Secondary curve, 'Do'	2.0±0.2	1.6±0.15
Average cellular multiplicity	1	1

Table 6

Recovery factors for CHO-KI cells set up 6 h before irradiation and treated with 2-DG for 2 h before irradiation. The substance was removed 3 h after irradiation by a medium change. The recovery factor is calculated as the survival following a dose given as 2 fractions over the survival from the same total dose given as a single fraction

Total dose (Gy)	Control	10 mmol/l 2-DG
10.0	1.8	2.3
12.5	4.7	4.8
15.0	6.5	7.5

um containing 1 mmol/l L-glucose, or 10 mmol/l 2-DG at various times before irradiation to 10 Gy. The analogues were removed by a medium change 3 hours after irradiation. It can be seen (Fig. 2) that varying the time of exposure did not alter the degree of radiosensitisation induced by L-glucose. 2-DG also had a consistent radiosensitising effect.

Recovery experiments were performed on 24-hour-old cultures exposed to 2-DG or L-glucose for 18 hours before irradiation. Fig. 3 shows the relative recovery following a split dose of radiation (7.5 Gy initially, followed 1–4 hours later by a second dose of 7.5 Gy). The results have been adjusted so that in all cases the combined dose (15 Gy) 0 hour value is 1, to allow the recovery factors to be read directly from the graph. It can be seen that treatment with L-glucose produces a consistent increase in the recovery factor from 5 in the control to 10 in the treated cultures, while with 2-DG the recovery factor increases from an initial value of 1 to a maximum of 20 after a 4 hour gap between radiation doses.

To estimate recovery at other combinations of doses, primary and secondary survival curves were examined for 24-hour-old cultures exposed to 2-DG (Fig. 4) or L-glucose (Fig. 5) for 18 hours before irradiation. The survival curve parameters 'n' and 'Do' calculated from linear regression plots and corrected for multiplicity where it occurred are shown in Table 2. It can be seen that following 18 hours exposure to the analogues the reduction in survival

is due mainly to a change in the initial shoulder region of the primary curve. But the secondary curves for cultures given a conditioning dose of 7.5 Gy followed 3 hours later by doses of 0.5 to 15 Gy show an unusual effect, i.e. recovery between doses is equal to or greater than that obtained in the control. The relative recovery factors at some dose combinations are shown in Table 3.

The above experiments were repeated using short term exposure to the analogues as follows: (a) Cells were set up 24 hours before irradiation and exposed to the analogues for 3 hours before the first dose. The analogues were removed by a medium change 3 hours after the second dose, or (b) cells were set up 6 hours before the first dose and the analogues were removed as in (a).

The results (Tables 3–6, Fig. 6) indicate that following short term exposure a greater reduction in the primary survival curve shoulder is seen and the unusual increase in split dose recovery seen after 18 hours exposure is absent.

Discussion

Radiation survival curve data for both glucose analogues show some very interesting effects. Pre-exposure of CHO-KI cells to both 2-DG and L-glucose reduce their survival following a dose of 10 Gy. Exposure of the cells to the analogues produced effects on the radiologic response, even in the absence of effects on plating efficiency and under conditions where the cellular multiplicity was either the same as the control or was not a factor. This would suggest that neither cytotoxic effects nor multiplicity effects were the predominant causes of the reduced survival. The constancy of the effect with duration of exposure makes cell cycle involvement unlikely.

Within the concentration range examined (1–10 mmol/l) L-glucose was found to reduce the survival consistently by 40 per cent while 2-DG had a concentration dependent effect ranging from a reduction of 30 per cent to a reduction of 75 per cent in survival, after correction for any effects on the control plating efficiency. Attempts to characterise the effect further by examining the radiation survival curves produced some unexpected results. Following both long and short term exposure to 10 mmol/l 2-DG, a clear reduction in the size of the survival curve shoulder was obtained. Similar but less significant effects were obtained with L-glucose. This type of effect is usually associated with correspondingly reduced levels of recovery between a split dose of irradiation (5, 16). When the secondary survival curves were examined, however, it was discovered that the normal one to one relationship between primary and secondary curve shoulders did not apply in all instances. Although the expected relationship held following short pre-exposure to the analogues (2–3 h), prolonged pre-exposure led to an increase in the amount of split dose recovery compared with control cultures irradiated in the absence of the analogues. These results could be demonstrated over the whole survival curve and are reflected in the increased recovery factors at each dose point. It is interesting that when the time course of split dose recovery was examined where a gap of one to 4 hours separated the doses L-glucose showed a constant elevation in recovery while the recovery with 2-DG increased as the length of time between doses increased. This suggests perhaps that in the case of the latter analogue a time dependent process is initiated by the initial radiation dose.

The nature of the mechanism or mechanisms is unknown but both substances are analogues of D-glucose, the main energy source available to these cells (14). As the cells rely heavily on anaerobic glycolysis of glucose to lactate for their energy supply (11, 18, 19), it could be expected that the mechanisms would involve altered energy metabolism. Altered energy metabolism certainly occurs with 2-DG, which is shown to inhibit glucose breakdown and lactate production severely. L-glucose has a far less severe effect on glucose breakdown and this correlates well with the less marked radiobiologic effects. The difference in the effects of the two analogues on both the radiation response and glucose metabolism could provide further evidence for the suggestion that energy metabolism limits repair (2).

These results may contribute to the discussion concerning the nature of the shoulder of the mammalian survival curve (1, 2) since it is difficult to reconcile the results presented here with the Elkind type recovery model which requires a positive 1 : 1 correlation between the size of the shoulder and the extent of split dose recovery. Our results would tend to favour a repair type model.

To conclude, the results show that the glucose analogues examined alter the radiation response of cultured CHO-KI cells. While the unexpected recovery effects possibly limit their therapeutic value,

the results may be important in the elucidation of radiation mechanisms since it can be argued that they show that there is no clear cut association between the shoulder of the primary curve and Elkind type recovery. This would allow the shoulder and recovery to be thought of as processes that could be independently modulated.

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