THE MECHANISM OF RADIOSENSITISATION BY IODOACETAMIDE

D. L. Dewey and B. D. Michael

British Empire Cancer Campaign,
Research Unit in Radiobiology,
Mount Vernon Hospital,
Northwood, Middlesex, England.

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Many compounds containing one or more SH groups protect bacteria and other organisms from the damaging effects of ionizing radiations. Iodoacetamide is a poison of SH groups frequently used to inhibit enzymes. When iodoacetamide is added to bacteria in sub-lethal concentrations the bacteria become very sensitive to damage by X-rays (Dean and Alexander, 1962). In some bacteria this sensitisation is dramatic; for example, in the case of Micrococcus radiodurans, if iodoacetamide is not added, over a hundred times the X-ray dose is needed to produce the same fraction of cell killing. We have found that the sensitisation is not due to iodoacetamide reacting with SH but is a result of damage by an irradiation product of iodoacetamide.

If the normal radioresistance of M. radiodurans is due to a repair mechanism then it seems likely that iodoacetamide might interfere with some part of this mechanism. If this is so, the effect of iodoacetamide should be demonstrable when it is added immediately after irradiation and before appreciable repair has had time to take place.

An attempt to demonstrate post-irradiation sensitisation by iodoacetamide was made in the following experiments.

EXPERIMENTS

The source of radiation was a 1.8 MeV linear accelerator designed for high beam current which was capable of giving the required dose of 3 to 4 Kr virtually instantaneously (in 2 x 10^{-6}secs). The irradiation cells were made entirely of Perspex, the electron entry window being 1 mm. thick to produce sufficient build-up. The interior of the cell was cylindrical, the diameter being 1 cm. and at right angles to the electron beam. The internal depth along the beam was 3 mm. 0.1 ml. of bacterial suspension was placed in the cell. An all-metal syringe was arranged above the cell so that 0.05 ml. of 10 mM iodoacetamide could be injected through a shortened no. 0 needle entering the cell via a hole of 1.5 mm. diameter. The jet was directed to one side of the centre of the cell to produce a swirling motion which improved the mixing. Injection was effected by dropping a weight of 0.5 Kg. from a height of 0.5 m. onto the syringe plunger which was allowed to travel 1.2 mm. The 0.05 ml. of solution was thus injected into the cell in 4 x 10^{-4}sec. at approximately 300m./Sec. (about the speed of sound in air). While the weight was falling it made several electrical contacts. These were used together with an electronic time delay unit to trigger the electron pulse at any required instant before, during or after the injection. Contacts were also used to display the injection period and the relative time position of the electron pulse on a double beam oscilloscope and the traces were recorded photographically for each shot. Time resolution was limited by the injection time (4 x 10^{-4}sec.); the time required for intimate mixing which depends on the geometry

of the cell is of the same order (Chance, B., Eisenhardt, R. H., Gibson, Q. H. and Lonberg-Holm, K. K. 1964).

The dose actually delivered in each pulse was monitored by a charge collector surrounding the cell. This was calibrated by dose measurements made using ferrous sulphate solution inside the cell. The dose variation across the cell was ±5% and the depth dose variation was ±15%.

RESULTS

In practice the apparatus was reliable to 3×10^{-3} sec. and showed conclusively that iodoacetamide added 3×10^{-3} sec. before irradiation showed the full sensitisation observed in long term experiments. Iodoacetamide added 3×10^{-3} sec. or longer, after irradiation was completely without effect.

An important difference between these two results is that in the first, the iodoacetamide and bacteria are both irradiated while in the second, the iodoacetamide was out of the electron beam and was also shielded from scattered radiation. In a second series of experiments the iodoacetamide and bacteria were interchanged. 0.1 ml. of 5mM iodoacetamide was placed in the irradiation chamber and given a dose of 3 k rads. At various times afterwards, bacteria which had not been irradiated were added. These results clearly showed that iodoacetamide was made toxic by irradiation and that this irradiated iodoacetamide solution could account for the entire radiosensitisation. The fact that cysteine removes the radiosensitizing action of iodoacetamide when added to it before irradiation is explained by chemical interaction of the two substances. In one experiment iodoacetamide was added to the bacteria before irradiation and 0.1M cysteine after irradiation. We

found that provided the cysteine was added within 10^{-2}sec. virtually complete protection was obtained. Substantial protection was also obtained for times up to 10^{-1}sec.

For measuring the time course of irradiated iodoacetamide toxicity, the drug only need be irradiated and not the bacteria. It was, therefore, no longer necessary to use the radioresistant organism M.radiodurans.

Most of the experiments were repeated using Serratia marcescens as test organism, and substantially the same results were observed including post-irradiation protection by cysteine. In pure water solution, irradiated iodoacetamide remained active for over 10^{2}sec, but decayed rapidly in the

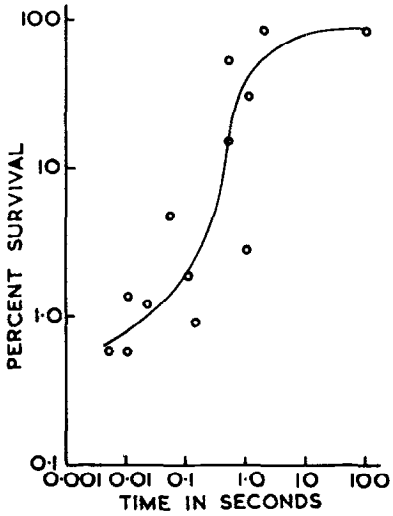

Fig. 1

The survival of unirradiated Serratia marcescens added to irradiated iodoacetamide. The bacteria were injected in 10^{-2} seconds into the iodoacetamide solution at the time delays shown, after the latter had been given a 1.7 K rad dose in a single 2 microsecond electron pulse from a linear accelerator. Radiation killed cells were added to the iodoacetamide before each experiment to simulate the effect of live bacteria.

presence of bacteria. The latter results were obtained by adding heavily irradiated bacteria to the iodoacetamide before irradiation, and testing for survival of unirradiated bacteria added after irradiation. The time course of this reaction is shown in Fig. 1; it will be seen that the reaction is 90% complete in about 1 sec. Most of the scatter can be attributed to unavoidable variations in the test dose delivered by the single pulse technique. The same concentration of potassium iodide irradiated in the same way failed to kill any bacteria, although considerable sensitisation was obtained by adding potassium iodide before irradiation. This indicates that iodide and iodoacetamide radiosensitise by different mechanisms.

REFERENCES

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