SHORT COMMUNICATION

A cytotoxic DNA precursor is taken up selectively by human cancer xenografts

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The failure of chemotherapeutic agents to be highly effective against most human cancers is widely attributed to drug resistance (Curt et al., 1984; Goldie & Coldman, 1985). Resistance mechanisms are various and may result from selective pressures acting on a genetically unstable population. Resistance does not occur in normal renewal tissues and their sensitivity is dose limiting (Goldie & Coldman, 1985). It has recently been suggested that it might be possible to take advantage of the resistance of cancer cells and the sensitivity of normal cells to anti-cancer drugs (Bagshawe, 1986). Hydroxyurea (HU) which inhibits DNA synthesis probably through its action on ribonucleotide reductase (Ackerblom et al., 1981) is relatively ineffective against most solid cancers and resistance readily develops (Ariel, 1970). It was therefore suggested that treatment with inhibitors of DNA synthesis should cause more marked inhibition of DNA synthesis in normal renewal tissues than in resistant cancers. If so, then it might be possible to incorporate precursors of DNA that are cytotoxic, or suitable for scintigraphic imaging, selectively into tumour cell DNA.

It was further suggested (Bagshawe, 1986) that this approach might be explored using the pyrimidine analogues 5-iodo-2'-deoxyuridine (IUdR) and 5-bromo-2'-deoxyuridine (BUdR) which differ from thymidine, the normal pyrimidine base, only by substitution of a halogen for the 5-methyl group. They compete with thymidine for phosphorylation and incorporation into DNA (Prusoff, 1959; Djordjevic & Szybalski, 1960). IUdR is rapidly dehalogenated unless incorporated into DNA but IUdR in DNA is retained until the cell divides or dies. IUdR and BUdR are known radio- and photo-sensitisers (Djordjevic & Szybalski, 1960) and 125I-IUdR is a potent cytotoxic agent (Hofer, 1980).

Preliminary experiments to test the hypothesis were performed in nu/nu mice carrying a human chorionic carcinoma xenograft (CC3) (Figure 1 a-h). Group I (Figure 1a) received only 125I-IUdR and tissues excised 24 h later showed, as have previous studies (Shuhmacher et al., 1974; Hampton & Eidinoff, 1961) that uptake of 125I-IUdR was ~4 times greater in small intestine and colon than in tumour. When Hu was given before 125I-IUdR (group 2, Figure 1b) the total counts for intestinal tissues were substantially reduced but tumour counts were not reduced, indicating differential sensitivity to HU and suggesting that DNA synthesis continued in the tumour when it was suppressed in normal renewal tissues.

Drugs which block thymidine synthesis increase utilisation of extracellular thymidine (Tattersall & Harrap, 1973) or thymidine analogue, probably through the thymidine salvage pathway (Sneider & Potter, 1969). They may reduce the thymidine pool (Tattersall & Harrap, 1973; Taylor et al., 1983) thereby favouring uptake of a thymidine analogue and they may delay dehalogenation of IUdR (Prusoff, 1963). 5-fluoro-2-deoxyuridine and 5-fluorouracil (5FU) increase uptake of IUdR by S phase cells probably through a combination of these mechanisms (Djordjevic & Szybalski, 1960; Benson et al., 1985). We therefore gave 5FU to tumour bearing mice (group 3, Figure 1c) followed by 125I-IUdR and found that mean counts in high uptake tissues (intestine, spleen, bone marrow) increased 3-5 fold compared with those from mice receiving 125I-IUdR alone. Mean tumour counts increased almost 7-fold compared with 125I-IUdR alone.

Methotrexate (MTX), which reduces thymidine synthesis through its anti-folate action, was also given to CC3 bearing mice (Figure 1d). MTX produced a less marked increase in uptake of 125I-IUdR than 5FU in the dosages employed, but tumour uptake was again increased relative to that by normal tissues. We had therefore shown that a fluoropyrimidine and a folate antagonist increased uptake of 125I-IUdR in both tumour and normal renewal tissues.

In the next study (group 5, Figure 1e) 5FU and HU were given together before and during exposure of the mice to 125I-IUdR. There was a reduction in uptake by all tissues compared with group 3 (Figure 1c) which received only 5FU and 125I-IUdR but the reduction in tumour uptake was less marked so that the mean tumour to colon ratio was 0.91. A similar effect occurred when MTX and HU were given (group 6, Figure 1f) but the effect of reducing normal tissue uptake of 125I-IUdR was greater and the mean tumour:colon ratio was 4.5.

Since uptake of IUdR is restricted to cells in S phase or engaged in unscheduled DNA synthesis (Lewensohn et al., 1982), a high proportion of tumour cells is likely to be labelled only by repeated administration. This was studied first by giving HU and 125I-IUdR on each of 3 successive days (Figure 1g). Intestinal tissue counts were not higher than after the same drugs given once (Figure 1b) but counts in the tumour were increased giving a mean tumour:colon ratio of 2.4. When 125I-IUdR was given after HU and 5FU on 3 successive days a mean tumour:colon ratio of 8.1 was obtained. The proportion of total administered dose retained in the tumour was 0.7% g-1 24 h after the last injection of 125I-IUdR.

These studies achieved a selective uptake of 125I-IUdR in a human cancer xenograft in mice. Prolonged retention of 125I-IUdR by the tumours was indicated by the data from groups 7 and 8 (Figure 1g and 1h), and this was consistent with incorporation of 125I-IUdR into DNA. Excretion of 125I was not complete by 24 h so that free 125I or 125I bound non-specifically to protein (Prusoff, 1963) contributed to both tissue and tumour radioactivity when measured by gamma counting the digested tissues.

The intracellular location of 125I-IUdR is particularly relevant to its potential cytotoxicity; within the nucleus it is highly cytotoxic but toxicity is low when 125I-IUdR is confined to the cell membrane (Hofer, 1980). Autoradiographs were therefore obtained. Figure 2 shows autoradi-
Table 1. Tumour and tissue nuclear grain counts 24 h after $^{125}$I-IUdR and tumour/colon ratios by tissue counting and nuclear grain counts

| Group | Agents used in addition to $^{125}$I-IUdR | Tumour/colon by tissue counting | Tumour cell nuclei | Non-neoplastic nuclei | Necrotic areas of tumour/100 $\mu$m $^2$ | Liver | Bone marrow | Colon muscularis mucosa | Tumour/colon by nuclear grain count |
|-------|------------------------------------------|---------------------------------|--------------------|----------------------|----------------------------------------|-------|-------------|------------------------|-----------------------------------|
| 1.    | nil                                      | 0.24                            | 3.68               | 0.27                 | 0.26                                   | 0.12  | —           | 1.54                   | 0.16                             | 2.38                             |
| 2.    | HU                                       | 0.77                            | 3.84               | 0.09                 | 0.18                                   | 0.12  | —           | 0.53                   | 0.17                             | 7.24                             |
| 3.    | SFU                                      | 0.43                            | 28.06              | 0.43                 | 0.05                                   | 0.36  | —           | 4.65                   | 0.15                             | 6.03                             |
| 4.    | MTX                                      | 0.38                            |                    |                      |                                        |       |             |                        |                                  |                                  |
| 5.    | HU, SFU                                  | 0.91                            |                    |                      |                                        |       |             |                        |                                  |                                  |
| 6.    | HU, MTX                                  | 4.5                             | 14.43              | 0.06                 | 0.10                                   | 0.47  | 0.45        | 0.59                   | 0.15                             | 24.4                             |
| 7.    | HU (×3)                                  | 2.4                             | 6.67               | 0.05                 | 0.16                                   | 0.10  | —           | 0.49                   | 0.09                             | 13.47                            |
| 8.    | HU, SFU, MTX (×3)                        | 8.1                             |                    |                      |                                        |       |             |                        |                                  |                                  |
| 9.    | HU, SFU (×3)                             | —                               | 24.67              | 0.05                 | 0.16                                   | —     | 0.61        | 0.94                   | 0.09                             | 26.20                            |

Table 1 Groups 1–8 are described under Figure 1 and group 9 under Figure 2. The tumour:colon tissue count ratio is derived from the data shown in Figure 1. Additional mice were included for autoradiography in groups 1, 2, 3, 6, 7; protocols were identical except that they received a large (15 $\mu$Ci) dose of $^{125}$I-IUdR.

To perform nuclear grain counts the sections of tumour and colon tissue were examined under ×100 oil immersion objective. An eyepiece graticule comprising 23 points contained within a circle and divided into 4 quadrants (Graticules Ltd., Tonbridge, Kent) was used to select tumour nuclei for counting. Where a point overlay or touched a nucleus the grains within the nucleus were counted. Where a point did not fall directly over or touch the edge of the nucleus, the nucleus of the first cell on an imaginary straight line to the left was used. Where this line crossed the perimeter of the graticule no point counting could be carried out. Different fields were examined until a total of >400 nuclei had been counted. The number of grains confined within an area bounded by the nuclear membrane were counted. Grains outside the nuclear membrane were not included. Nuclear grain counts were performed on sections of colon by examining at least 10 crypts cut longitudinally so that the distribution of grains within crypts could be assessed. Starting at the mid-point of the base of the crypt and ascending on either side to the tip, nuclear grains were counted. Mean grain densities per nucleus were calculated from at least 400 nuclei.
Graphs of tumour (group 9) and colon (group 9 and group 3 for comparison). The mean nuclear grain count ratio for tumour:colon was 26.2 in group 9. Compared with total tissue counts nuclear grain counts consistently suggested a more favourable distribution of $^{125}\text{I}-\text{IUDR}$ (Table I). In the 5 groups studied by both techniques the mean nuclear grain count ratios were 5.4–14.0-fold higher than the respective tissue count ratios. Cumulative frequencies of nuclear grain counts were determined for colon and tumour and these also confirmed that the number of labelled colonic nuclei and the number of grains within them were reduced by the addition of HU to $^{125}\text{I}-\text{IUDR}$ but the corresponding numbers in tumour nuclei were little changed.

Limited studies using cytosine arabinoside (CA) in place of HU and studies on a human colorectal carcinoma xenograft have given comparable results to those described here with the CC3 tumour but with slow growing tumours $^{125}\text{I}-\text{IUDR}$ uptake is likely to be lower.

No attempt was made in these early studies to ensure the CC3 tumour was resistant to HU nor to optimise the dosage and timing of the drugs used. Nevertheless, the tumour: normal tissue distribution of $^{125}\text{I}-\text{IUDR}$ has been modified in this xenograft model to one that is potentially favourable for diagnostic and therapeutic purposes by blocking normal tissue uptake with HU and, in addition, giving agents which increase $^{125}\text{I}-\text{IUDR}$ uptake by uninhibited cells. Further enhancement of tumour uptake of $^{125}\text{I}-\text{IUDR}$ may be possible by inhibiting thymidine kinase with 5'-aminothymidine (Fischer et al., 1986). The role of HU or CA in this approach is a reversal of their normal role as anti-cancer agents.

Refinement of the present technique might provide a basis for estimating cell deaths as they occur spontaneously, or as a result of therapy in animal tumour models and perhaps in man. Immunoscintigraphic methods are used clinically to identify sites of drug resistant tumour (Bagshawe, 1985; $^{131}\text{I}-\text{IUDR}$ or $^{125}\text{I}-\text{IUDR}$ may prove superior to antibody directed isotopes for some tumours because retention of radioactivity in blood, liver and lungs is relatively low.

Since IUDR and BUdR are known to act as radiosensitisers and photosensitisers their selective uptake by tumour cells may prove advantageous. Their possible role as sensitisers to alkylating agents (Prusoff, 1963) or other chemotherapeutic drugs requires re-investigation. $^{121}\text{I}$, $^{123}\text{I}$ and $^{125}\text{I}$ are characterised by Auger electron emission which greatly enhances their therapeutic potential (Hofer 1980, Lemotte & Little, 1984). The penalty for achieving a selective uptake of a thymidine analogue into tumours is a period of inhibition of DNA synthesis and consequent cell loss from normal tissues. The ultimate equation is the therapeutic:toxicity ratio which reflects total cell losses in tumour and normal tissues resulting from the drugs used to achieve the selective distribution of the analogue as well as the analogue itself.

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