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Chairman's Summary of Session C

In this session, a number of widely diverse topics were discussed. Indeed one may describe them as disciplines separated by a common survival curve! Surviving fraction on a logarithmic scale was plotted as a function of X-ray dose, of drug concentration or of acetamine accumulated. Presented in this way they all look much the same, but in fact they are not.

Mitomycin C. On the one hand there was a discussion of the anti-cancer agents mitomycin-C and porfiromycin. These agents kill aerated cells, but are much more effective against hypoxic cells. They have already been used in the clinic in combination with radiation. The strategy is based on complementation, the strength of one balancing the weakness of the other. X-rays are most effective against aerated cells, while mitomycin-C preferentially kills hypoxic cells that are resistant to X-rays. There is no claim of an interaction between the X-rays and the drug; their effects appear to be independent and additive.

The nitroimidazoles as radiosensitizers. By contrast the rationale for the nitroimidazoles is based on a drug–radiation interaction. These compounds were initially introduced as hypoxic cell radiosensitizers, and this has been their principal clinical use to date. Several thousand cancer patients have received misonidazole in conjunction with radiotherapy in the course of clinical trials. Radiosensitization is by a fast free radical process involving the parent compound and bioreduction is not required. This component of radiosensitization is referred to as dose-multiplicative radiosensitization.

When cells are incubated under hypoxic conditions for extended periods of time with misonidazole or many of the other nitroimidazoles, a cytotoxicity becomes apparent. This is specific for hypoxic cell, strongly temperature dependent and varies with the electron affinity of the compound involved. Prolonged preincubation of cells with a nitroimidazole also results in a second component of radiosensitization, often referred to as "slow radiosensitization". This results from the depletion of intracellular protein and non-protein thiols, resulting from the bioreduction of the nitroimidazole. How important this slow component of sensitization is in the human is not known. This aspect of radiosensitizer research has led to an explosion of interest in compounds designed specifically to deplete cellular thiols. Of these, buthionine sulfoximine (BSO) appears to be the most promising, and acts by blocking the first step in the synthesis of glutathione. The notion is that the use of thiol depleters would potentiate the effectiveness of radiosensitizers administered concomitantly.

Chemosensitization. Nitroimidazoles also potentiate some chemotherapy agents, particularly the alkylating agents. In the long run, this may turn out to be the most important application of these compounds. Hypoxia appears to be a prerequisite for chemosensitization, which gives it some specificity for tumors since, in general, they contain a significant proportion of cells at low oxygen tension. At least 3 mechanisms have been suggested to account for the chemosensitization observed experimentally.

(i) Depletion of intracellular thiols.
(ii) Modification of pharmacokinetics.
(iii) Increased crosslinking of DNA.

The relative importance of the various mechanisms varies for different combinations of sensitizer and cytotoxic drug.

Clinical use of sensitizers and the new drugs. The clinical use of sensitizers shows signs of an important renaissance at the present time. The first exciting positive result of a controlled clinical trial, showing a clear advantage for radiotherapy patients receiving Misonidazole, coincides with the introduction of new improved compounds into the clinic. SR-2508 is entering Phase III controlled clinical trials in the United States. This compound is more hydrophilic than Misonidazole, so that it does not readily cross the blood brain barrier. Doses 3 to 5 times higher can be given before a dose-limiting neurotoxicity is seen. In the U.K., Ro-03-8799 has been chosen for testing in clinical trials. This is more electron affinic than Misonidazole and tends to concentrate in tumor cells so that it should prove to be much superior in the clinic. Perhaps the most revolutionary of the new drugs is RSU-1069. This is a 2-nitroimidazole, having an aziridine ring at the end of the side chain which acts as a mono-functional alkylating agent. It is a potent radio and chemosensitizer, but also highly cytotoxic. The next stage in development will be to synthesize a series of analogues of this lead compounds, with various substitutions in the aziridine ring, with the aim of reducing toxicity while preserving the excellent sensitizing properties.

The anti-bacterial activity of metronidazole. In the killing of bacteria by metronidazole, the DNA is clearly the target and the bioreduction of the parent compound under hypoxic conditions is a necessary prerequisite. The precise nature of the metabolite of metronidazole responsible for cell lethality is not clear. The proportion of cells killed correlates with the accumulation of acetimide, and this is the way in which data are commonly plotted and compared.

Some *E. coli* mutants are exquisitely sensitive to metronidazole when they lack certain repair systems. These are, generally, the result of point mutations. By contrast, deletion mutants are not sensitive because, while they may lack repair systems, they also lack the reductase necessary to reduce metronidazole to its active form.

Potential carcinogenicity of nitroheterocyclics. Not only is the metabolite of metronidazole responsible for cell lethality not known, but it is also not known for sure whether the same or a different metabolite is responsible for the carcinogenic potential of the drug. It has been shown that metronidazole can produce tumors in experimental animals. The other nitroimidazoles in clinical use have not been as extensively tested in animals, but they have been screened and compared using *in vitro* oncogenic transformation assays. It is clear that the various compounds are widely different in their ability to produce transformants and that these differences can be accounted for to some extent by the structure of the side chain. It is also clear that the number of transformants produced is increased greatly under hypoxic conditions where the drugs undergo bioreduction.

On the method of bioreduction. Bioreduction of nitroheterocyclic and quinoid chemotherapy drugs involves a common enzymatic pathway (i.e. NADPH-cytochrome *c* reductase). Other enzymes may also reduce drugs, but their relative contribution to the overall reduction process is unknown. The reduction of nitro compounds by bacteria involves iron–sulphur protein complexes named ferridoxins. The reduction of nitroimidazoles and quinoid drugs under aerobic conditions produces radical intermediates that are reactive with oxygen. The reaction with oxygen produces peroxide and the original drug molecule. This process of electron transfer has been referred to as "futile cycling" and is not toxic to cells unless glutathione is

depleted. Depletion of glutathione inhibits glutathione peroxidase, the major enzyme involved in peroxide and organic peroxide reduction. The most important source of electrons for the reduction process comes from the metabolism of glucose via the hexose monophosphate shunt. Under anaerobic conditions metronidazole, misonidazole and mitomycin-C are more toxic. The nitro compounds accept additional electrons to produce alkylating species that react with nonprotein and protein thiols as well as with RNA and DNA. One electron reduction of mitomycin-C results in decomposition of the parent molecule into an alkylating agent. The role of glucose as the ultimate electron donor in the reduction process was stressed. Future strategies may involve increasing tumor glucose, in order to ensure the reduction of the drug to the alkylating species. This may be necessary for tumors identified as being extremely hypoglycemic. The mechanisms involved in the killing of hypoxic cells by the nitroimidazoles are at the present time unknown, although alkylation of DNA and inhibition of key glycolytic enzymes may be contributing factors. As mentioned earlier, glutathione depletion enhances the cytotoxic effects, while exogenous GSH or cysteamine protects cells against the toxic effects.

In summary biochemical modulation of tumor tissue *in vivo* might be achieved by glutathione depletion in combination with glucose administration. Glucose would ensure maximal reduction of the drug under hypoxic conditions. Glutathione depletion would make the tumor tissue more vulnerable to lower drug concentrations.

Conclusion. It was evident from the session that a vast body of experimental data exists on the biochemistry and biological properties of the nitroheterocyclics. It was equally evident that a great deal more remains to be done—enough to keep us all busy for many years. The exchange of ideas between the various groups was productive and useful. We may be three disciplines divided by a common survival curve, but we have much in common and much to learn from one another.

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