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Chairman's Summary of Session D

It is clear that the nitroimidazoles possess two biological properties which make them an important class of anti-cancer agent. The first property, in which they mimick the radiation sensitization effect of oxygen is a fast reaction detected within milliseconds of mixing drugs and hypoxic cells and is a property of the parent compound [1]. The results from ongoing clinical trials indicate that improvements in radiotherapy may occur from the use of such radiation sensitizors. Since all the experimental methods are available to measure, for example, the electron affinity of such agents, their pharmacokinetics and tissue distribution, there seems no doubt that agents with low toxicity and optimal sensitizing properties will be discovered in the future. The second biological property of these compounds is a prodrug effect, whereby the agent is selectively reduced in hypoxic cells to reactive species which are cytotoxic. It is clear from the proceedings of this meeting that this important property of the nitroimidazoles is less well understood.

The "reducing" properties of cancers have been commented upon for many years and have been the starting point for the design of many prodrugs which are pharmacodynamically and toxicologically inert but which may be reduced in tumours to highly cytotoxic species. Nitromin for example is the *N*-oxide of nitrogen mustard. Since the spare pair of electrons on the nitrogen atom are involved in bonding it would be expected to be fairly unreactive and have little cytotoxicity. Many years ago it was shown to be particularly effective against large transplanted tumours which probably contained large numbers of hypoxic cells [2]. The possibility that the drug was being activated to nitrogen mustard in hypoxic cells, a proportion of which then diffused to oxygenated cells, was not investigated. However, it is quite clear that this mechanism operates in the nitroimidazole series. Using labelled misonidazole, covalent binding has been shown to occur predominantly in hypoxic cell areas [3]. Activation of 2-nitroimidazoles in hypoxic cells may be associated with cytotoxicity in neighbouring oxygenated cells, since, unlike the normal situation seen with most cytotoxic agents, tumour destruction is first seen close to the areas of necrosis and then extends outwards [1]. Similarly in cell spheroids the 2-nitroimidazoles cause necrosis in the centre, the zone of necrosis again spreading outwards. Given that activation of prodrugs can take place in hypoxic cells, then the nitroimidazoles discussed during this meeting are probably not the best type of cytotoxic agent since their reactive species are monofunctional agents whereas bifunctional agents are as a general rule much more cytotoxic than their monofunctional analogues [4]. In the nitrogen mustard series for example the di-2-chloroethylamines have often potent antitumour activity at low concentrations *in vivo* while the monochloroethylamine analogues are often inactive at maximum tolerated doses. Support of this view comes from data presented at this meeting in which a nitroimidazole containing an alkylating aziridine side chain was shown to be highly cytotoxic [5]. It is of interest that the antitumour agent mitonafide which requires activation of its nitrogroup for optimal antitumour activity is probably acting as a bifunctional agent since the molecule also has intercalating properties and an aliphatic side chain which could possibly be converted to a reactive carbinolamine [6].

It is now important to re-examine the large numbers of prodrugs designed over the years to be activated by reduction to see if they are selectively reduced by hypoxic tumour cells to metabolites which are both highly toxic and diffusible. In the nitrogen mustard series alone a range of N and S-oxides, nitroso and azo derivatives as well as tetrazoliums and quinones have been synthesized, all of which are relatively non-toxic but would form highly toxic products on reduction [7]. .

An intriguing aspect of the meeting has been the nature of the enzyme carrying out the reduction in hypoxic cells. It does not appear to be cytochrome P450 which is probably low in most tumour cells nor xanthine oxidase. There is some evidence that the enzyme may be DT diaphorase also referred to as NAD(P)H quinone oxidoreductase, menadione reductase and vitamin K reductase. This enzyme reduces a variety of structures including quinones, azolinkages, tetrazoliums and nitroso compounds mentioned above. Although the enzyme occurs widely in all tissues [8] it is known to be very high in tumours [9–11], the level of the DT diaphorase m-RNA being increased up to 7-fold in hepatocyte nodules induced by carcinogens [12]. It is of interest that in chemically induced hepatocarcinogenesis the level of the enzyme in early neoplastic foci is so high that it is used to visualise them [13]. One possibility is that DT diaphorase is not very active under normal conditions but is activated in anoxic cells perhaps by the consequences of anoxia including decreased pH. It is known that tyrosine hydroxylase for example is activated by anoxia and low pH [14] while a 2.5-fold increase in the rate of DT diaphorase has been observed when the pH of the reaction mixture was lowered from 7.5 to 6.0 [15]. Since DT diaphorase is inhibited by extremely low concentrations of dicoumarol it would be simple to examine the role of the enzyme in carrying out the activation of nitroimidazoles by seeing if the selectivity toxicity to hypoxic tumour cells was abolished by pretreatment with dicoumarol.

Studies on the nitroimidazoles have thus aroused renewed interest in the designs of prodrugs which may be selectively reduced in hypoxic tumour cells particularly if the active metabolites formed can diffuse into neighbouring oxygenated cells. They might well have considerable antitumour activity in their own right or be effective in combination with agents which act primarily on well oxygenated cells.

Priorities for the future will be the characterization of the enzyme involved in the reduction and the substrates it utilizes. If it turns out to be DT diaphorase, hepatocyte cell lines are available which are high in the enzyme and which could be used to detect whether reductive activation of prodrugs occurs. Although the presence of gut bacteria which also reduce the prodrugs may be a complication *in vivo* this might be overcome by temporary sterilization using antibiotics. Clinical trials could be easily carried out in the case of some cancers, for instance cervical cancer which often contains large numbers of hypoxic cells. Since single agent therapy, usually cisplatin, is the usual chemotherapy then a comparison of cisplatin alone compared with cisplatin plus the hypoxia activated prodrug would soon give some indication of the usefulness of the concept of using prodrugs activated by reduction.

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