Eur J Cancer, Vol. 27, No. 3, pp. 281–284, 1991.
Printed in Great Britain

0277-5379/91 $3.00 + 0.00

Feature Articles

The Cell Membrane as a Target for Cancer Chemotherapy

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PLASMA MEMBRANE AS TARGET FOR ESTABLISHED ANTITUMOUR AGENTS

MOST CURRENT antitumour agents are directed against DNA or designed to interfere with nucleic acid biosynthesis (e.g. alkylating agents, platinum complexes and anthracyclines). The interaction of these drugs with DNA is well established, but the precise mechanism by which these agents inhibit tumour growth is unknown. In fact, these compounds cause both structural and functional alterations of the plasma membrane at therapeutic concentrations and interactions of alkylating agents, platinum complexes and anthracyclines with the plasma membrane. This may contribute to the antitumour activity of these drugs and may be more important than has been appreciated [1–4]. Membrane effects may also offer new avenues for optimisation strategies with these compounds, and allow the development of more specific agents that act on the cell membrane and associated signalling pathways [5, 6].

GROWTH FACTORS AND THEIR RECEPTORS

The effects of antitumour agents on the cell membrane attract increasing attention because of the progress in our understanding of the molecular basis of regulation of cell proliferation in general and malignant growth in particular. Growth factor receptors are exposed at the plasma membrane, and a series of enzymes that play an essential role in growth factor signal transduction are membrane-bound. Thus, the plasma membrane is intimately involved in the control of cell proliferation [7, 8]. Furthermore, transformation by oncogenes is closely related to processes occurring at the plasma membrane level. Oncogenes have been shown to cause: (1) autrocrine production of growth factors, (2) synthesis of growth factor receptors which are active even in the absence of the corresponding ligand, and (3) constitutive activation of elements of growth factor signal transduction [9]. These findings offer new strategies for a more selective antitumour therapy directed against oncogene products or interfering with their function.

The concept of agents that could prevent biological growth factors stimulating their corresponding receptors seems especially promising in small cell lung cancer, in which autocrine production of bombesin is often significant for the growth of these tumours—although this does not seem to be a general feature of this tumour type [8]. Monoclonal antibodies directed against bombesin receptors inhibit growth of human small cell lung carcinoma *in vivo* [10]. The generation of antibodies against growth factors or growth factor receptors has become a special area of tumour immunology (beyond the scope of this review) which focuses on chemotherapeutic strategies directed against membrane targets. Synthetic bombesin antagonists have been prepared [10]. However, the growth inhibitory effect of these agents was not mediated by bombesin receptors. Suramin inhibits the binding of various growth factors, including platelet-derived growth factor (PDGF), transforming growth factor β and fibroblast growth factor [12, 13]. Clinical trials with this compound have been started [14, 15].

Somatostatins seem to represent biological growth factor antagonists [16]. The inhibition by somatostatin of epidermal growth factor (EGF) stimulated growth has been correlated to an activation of phosphotyrosine phosphatase [17]. This interesting concept has led to the development of somatostatin analogues, some of which have been used for clinical trials [15, 17, 18].

TYROSINE KINASES AS TARGETS

Various growth factor receptors, including the receptors for EGF, PDGF and insulin-like growth factor, exhibit protein kinase activity that catalyses the transfer of phosphate from ATP to tyrosine residues of the peptide substrate. This, together with the fact that a variety of oncogene products exhibit tyrosine kinase activity [19], has led to a search for protein tyrosine kinase inhibitors. The flavone quercetin proved effective against protein tyrosine kinase [20]. Although this compound is non-specific, also inhibiting protein kinase C, protein kinase A and other enzymes, its toxicity is low. Quercetin has synergistically enhanced the antitumour activity of cisplatin and may thus become clinically useful [21]. The isoflavone genistein is a much more specific inhibitor of protein tyrosine kinase than quercetin [22]. Genistein's toxicity, however, has so far prevented the use of this agent and its derivatives in the clinic. The situation is similar for erbstatin and its derivatives with anti-protein tyrosine kinase activity [23]. Nevertheless, these drugs represent useful lead substances. These compounds should be effective against a broad variety of tumours, including oestrogen receptor negative mammary tumours with high-affinity EGF receptors.

PHOSPHOLIPID METABOLISM

Several growth factors, including bombesin, thrombin, PDGF and EGF, stimulate a phosphatidyl inositol-4,5-bisphosphate (PIP_2) specific phospholipase C, generating inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 mediates the release of Ca^{2+} from intracellular stores whereas DAG activates protein kinase C. Both the elevation of cytosolic free Ca^{2+} as well as the stimulation of protein kinase C are

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Received 9 Nov. 1990; accepted 23 Nov. 1990.

important mitogenic signals [24]. Inhibitors of phospholipase C should block mitogenic stimulation by growth factors that use this enzyme and should thus be potential antitumour agents. Furthermore, several isozymes of phospholipase C with limited sequence homology are known to exist, which offers the chance of a more selective blockade [21].

Drugs that inhibit phospholipase C include mepacrine [26] and monoalide [27]. However, these agents are not specific for the PIP_2 specific phospholipase C. Neomycin inhibits phosphoinositide metabolism because of its high affinity for these phospholipids [28]. In view of the severe toxicity of the aminoglycosides, attempts to exploit their effect on phosphoinositide metabolism for cancer chemotherapy have not yet been initiated. Doxorubicin activates phosphoinositide turnover, an effect that has been correlated with the antitumour activity of this antibiotic [3].

Ether lipids and alkylphosphocholine are presently the most promising lead substances with an effect on phospholipase C. Ether lipids and hexadecyl phosphocholine depress growth factor induced formation of IP_3 and the resulting Ca^{2+} response [29, 30]. The reduction of IP_3 formation may indeed be caused by inhibition of phospholipase C [31]. The reduction by phospholipid analogues of second messenger formation occurs at the same drug concentrations required for inhibition of tumour cell growth [31]. Phospholipid analogues gave promising results in clinical trials [32–35]. Improvement of specificity on phosphoinositide metabolism by development of inositol analogues is a logical consequence in view of the available data. These developments should also consider as additional targets the various kinases acting on phosphotidylinositol or inositol phosphates.

Studies on these lines have been started. Phosphonate analogues of phosphotidylinositol have been synthesised and shown to inhibit phospholipase C in a dose-dependent manner [36]. 5-deoxymyo-inositol and 5-deoxy-5-fluoro-myo-inositol inhibit phosphorylation of phosphotidylinositol phosphate [37]. Non-hydrolysable phosphothioate analogues of inositol phosphates have also been synthesised [38]. All these compounds may serve as building blocks for specific inhibitors of inositol phosphate metabolism. It should be emphasised, however, that besides the phosphotidylinositol specific lipase C, a phosphotidylcholine specific phospholipase C and probably also a phospholipase D are involved in mitogenic signalling [39]. Whether these enzymes are affected by the available phospholipid analogues remains to be studied.

PROTEIN KINASE C

Phospholipase C generates DAG which in turn activates protein kinase C. The activation of protein kinase C and its concomitant translocation to the plasma membrane are essential steps in mitogenic signalling by a variety of growth factors [40]. Furthermore, protein kinase C is essential for the growth stimulatory action of transforming *ras* oncogenes [41], the most frequently expressed oncogenes in human tumours [42]. Protein kinase C is an important target of ether lipids and alkylphosphocholines [43, 44]. Similar dose-response relations are observed for the growth inhibitory effect of these phospholipid analogues and their activity against protein kinase C [43]. Besides phospholipid-analogues, various other inhibitors of protein kinase C have been identified [45]. However, most of these agents are not specific for protein kinase C. Many, including local anaesthetics and Ca^{2+} calmodulin antagonists, are lipophilic drugs binding non-specifically to the hydrophobic regulatory binding site of the enzyme [45]. The most potent inhibitor of protein kinase C available is staurosporine, a microbial alkaloid acting on the catalytic domain of the enzyme [46]. Again this compound is not strongly specific for protein kinase C, since it also inhibits protein tyrose kinase and cAMP and cGMP dependent protein kinases, although with somewhat higher IC_{50} values.

Recently, however, derivatives of staurosporine [47, 48] have been synthesised that have remarkable protein kinase C specificity. One of these compounds also has antitumour activity *in vivo* [48]. Another strategy for the development of inhibitors of this enzyme originates from the observation that tamoxifen, a triphenylethylene, has activity against protein kinase C, which may be responsible for its antitumour effect in oestrogen receptor negative tumours [49, 50]. As a consequence of these studies triphenylacrylonitriles, which can be classified into two subgroups, one acting on the regulatory domain of the enzyme and another interfering with the catalytic site, have been synthesised [51].

The bryostatins are macrocyclic lactones isolated from marine bryozoans [52]. They resemble phorbol esters in their high affinity for protein kinase C and, like phorbol esters, they activate this enzyme [53]. The initial activation is then followed by down-modulation. However, in contrast to phorbol esters, they do not act as tumour promotors [54]. Bryostatins have antitumour activity [55, 56]. Studies to optimise bryostatins by systemic derivatisation procedures have begun [57].

Protein kinase C comprises a family of closely related enzymes; eight isozymes have been discovered [40]. Several laboratories are investigating whether differences in the sensitivity of the various isoenzymes to different inhibitors can be detected. If such differences are found, this could lead to tissue or tumour selective antagonists of protein kinase C. Inhibitors of this enzyme synergistically enhance the antitumour activity of cisplatin *in vitro* as well as *in vivo* [21, 43]. All protein kinase C inhibitors studied so far have this property. This observation may lead to the first application of compounds active against protein kinase C in combination chemotherapy.

Evidence is accumulating that protein kinase C activity is correlated to pleiotropic drug resistance [58]. This seems to be the result of an activation of the *mdr*-1 gene product gp170 mediated by protein kinase C [58]. Thus, inhibitors of this enzyme seem to be suitable candidates to overcome drug resistance. Preliminary results from our laboratory with staurosporine-etoposide combinations in etoposide-resistant cells are promising.

PLEIOTROPIC DRUG RESISTANCE

The *mdr*-1 gene product gp170 is an interesting target for tumour chemotherapy. This membrane protein acts as a non-specific pump decreasing the intracellular concentration of several antitumour agents so that toxic levels cannot be achieved [59]. Several compounds, including verapamil, chlorpromazine and cyclosporin, partly reverse the multidrug resistance (MDR) phenotype. This effect seems to be due to a competitive inhibition of drug efflux [60]. However, the dosages that have to be administered to reach effective concentrations *in vivo* lead to severe side-effects. Therefore, programmes for a rational design of MDR modulators have been started. Derivatives of Ca^{2+} antagonists that no longer bind to Ca^{2+} channels appear especially attractive [61, 62].

Ca^{2+} CHANNELS

Cytosolic free Ca^{2+} increases after stimulation by most growth factors [24]. In many cases, stimulation of quiescent cells with a growth factor leads to an immediate, transitory Ca^{2+} response, which is caused by mobilisation of Ca^{2+} from intracellular stores. This intracellular Ca^{2+} release is then followed by an additional influx of extracellular Ca^{2+}. The corresponding Ca^{2+} channels are different from the well-studied voltage-operated calcium channels (VOC) in excitable cells. For growth factors, the channels are usually activated by second messengers. Ca^{2+} influx by these poorly defined channels has been termed receptor mediated calcium entry (RMCE) [63]. These channels are attractive targets for growth inhibitory agents. At concentrations one to three orders of magnitude higher than those required to inhibit voltage-dependent Ca^{2+} entry, several Ca^{2+} antagonists acting on VOCs also depress Ca^{2+} influx via RMCE [63]. At these concentrations, Ca^{2+} antagonists acting upon VOCs have antitumour activity *in vivo* [64]. However, these effects do not seem to be caused by a blockade of Ca^{2+} entry [64]. Obviously, specific antagonists of RMCE are required. Recently, a compound that is claimed to inhibit selectively RMCE, 1-β-[3-(*p*-methoxyphenyl)-propyloxy]-*p*-methoxyphenetyl-1H-imidazole hydrochloride, has been presented [63]. This may lead to a new class of antitumour agents acting on RMCE.

CONCLUSION

Phospholipid analogues are a new class of antitumour agents that inhibit tumour growth by an interference with plasma membrane functions. These compounds have already entered the clinic. They represent the first generation of antitumour agents directed against the plasma membrane. These drugs will be followed by a second generation, targeted against well-defined structures of the plasma membrane that are essential in growth control, including growth factor receptors and enzymes involved in growth factor signal transduction. These rationally designed compounds are being developed in many laboratories around the world. There is a high probability that these efforts will soon lead to new, clinically useful antitumour agents.

1. Hickman JA, Scanlon K, Tritton TR. The cell surface as a target for cancer chemotherapy. *Trends Pharmacol Sci* 1983, **5**, 15–19.
2. Tritton TR, Hickmann JA. Cell membranes as a chemotherapeutic target. In: Muggia F, ed. *Experimental and Clinical Progress in Cancer Chemotherapy*. Boston, Martinus Nijhoff, 1985, 81–131.
3. Tritton TR, Hickman JA. How to kill cancer cells: membranes and cell signaling as targets in cancer chemotherapy. *Cancer Cells* 1990, **2**, 95–105.
4. Grunicke H, Doppler W, Hofmann J, *et al.* Plasma membrane as target of alkylating agents. *Adv Enzyme Regul* 1986, **24**, 247–261.
5. AACR, EORTC, and BACR Special Conference in Cancer Research. The cell membrane and cell signals as targets in cancer chemotherapy. *Cancer Res* 1990, **50**, 2203–2211.
6. Workman P. The cell membrane and cell signals: new targets for novel anticancer drugs. *Ann Oncol* 1990, **1**, 100–111. (See enclosure to **1** (5) for corrected version.)
7. Lyons RM, Moses HL. Transforming growth factors and the regulation of cell proliferation. *Eur J Biochem* 1990, **187**, 467–473.
8. Heldin CH, Westermark B. Growth factors as transforming proteins. *Eur J Biochem* 1989, **184**, 487–496.
9. Weinberg RA. The action of oncogenes in the cytoplasm and nucleus. *Science* 1985, **230**, 770–776.
10. Cuttina F, Carney DN, Mulshine J, *et al.* Bombesin-like peptides on function as autocrine growth factors in human small-cell lung cancer. *Nature* 1985, **316**, 823–826.
11. Layton JE, Scanlon DB, Soveny C, Morstyn G. Effects of bombesin antagonists on the growth of small cell lung cancer cells *in vitro*. *Cancer Res* 1988, **48**, 4783–4789.
12. Garret JS, Coughlin SR, Niman HL, Tremble PM, Giels GM, Williams LT. Blockade of autocrine stimulation in simian sarcoma virus-transformed cells reverses down-regulation of platelet-derived growth factor receptors. *Proc Natl Acad Sci USA* 1984, **81**, 7466–7460.
13. Huang SS, Huang JS. Rapid turnover of the platelet-derived growth factor receptor in cis-transformed cells and reversal by suramin. *J Biol Chem* 1988, **263**, 12608–12618.
14. Van Oosterom AT, De Smedt EA, Denis LJ, de Bruijn EA, Mahler C. Suramin for Prostatic Cancer: a Phase I/II Study in Advanced Extensively Pretreated Disease. *Eur J Cancer* 1990, **26**, 422.
15. Abstracts 2. Int Symp on Hormonal Manipulation of Cancer: Peptides, Growth Factors and New (Anti)Steroidal Agents, Rotterdam April 9–11, 1990. *Eur J Cancer* 1990, **26**, 143–197.
16. Schally AV. Oncological applications of somatostatin analogues. *Cancer Res* 1988, **48**, 6977–6985.
17. Liebow C, Reilly C, Serrano M, Schally AV. Somatostatin analogues inhibit growth of pancreatic cancer by stimulating tyrosine phosphatase. *Proc Natl Acad Sci USA* 1989, **86**, 2003–2007.
18. Scambia G, Panici PB, Baiocchi G, Perrone L, Jacobelli S, Manusco S. Antiproliferative effects of somatostatin and the somatostatin analog SMS 201–995 on three human breast cancer cell lines. *J Cancer Res Clin Oncol* 1988, **114**, 306–308.
19. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. *Cell* 1990, **61**, 203–212.
20. Glossmann H, Presek P, Eigenbrodt E. Quercetin inhibits tyrosine phosphorylation by the cyclic nucleotide independent, transforming protein kinase, $pp60^{src}$. *Arch Pharmacol* 1981, **317**, 100–102.
21. Hofmann J, Doppler W, Jakob A, *et al.* Enhancement of the antiproliferative effect of cis-diamminedichloroplatinum(II) and nitrogen-mustard by inhibitors of protein kinase C. *Int J Cancer* 1988, **42**, 382–388.
22. Akiyama T, Ishida J, Nakagawa S, *et al.* Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* 1987, **262**, 5592–5595.
23. Gazit A, Yaigh P, Gilon D, Levitzky A. Tryphostins I: synthesis and biological activity of protein tyrosine kinase inhibitors. *J Med Chem* 1989, **32**, 2344–2352.
24. Berridge MJ, Irvina RF. Inositolphosphates and cell signaling. *Nature* 1989, **341**, 197–205.
25. Fain JN. Regulation of phosphoinositide-specific phospholipase C. *Biochem Biophys Acta* 1990, **1053**, 81–88.
26. Best L, Sener A, Mathias PCF, Malaisse WJ. Inhibition by mepacrinc and p-bromophen-acylbromide of phosphoinositide hydrolysis, glucose oxidation, calcium uptake and insulin release in rat pancreatic islets. *Biochem Pharmacol* 1984, **33**, 2657–2662.
27. Bennett CF, Mong S, Wu HLW, Clark MA, Wheeler L, Cooke ST. Inhibition of phosphoinositide-specific phospholipase C by manoalide. *Mol Pharmacol* 1987, **32**, 587–593.
28. Prentki J, Deeney T, Matschinsky FM, Joseph SK. Neomycin: A specific drug to study the inositol-phospholipid signaling system? *FEBS Lett* 1986, **197**, 285–288.
29. Seewald MJ, Olsen RA, Sehgal I, Melder DC, Modest EJ, Powis G. Inhibition of growth factor-dependent inositol phosphate Ca^{2+} signaling by antitumor ether lipid analogues. *Cancer Res* 1990, **50**, 4458–4463.
30. Grunicke H, Hofmann J, Oberhuber H, Uberall F, Zaknun J, Voegeli R, Hilgard P. Hexadecylphosphocholine inhibits protein kinase-C and depresses the inositol phosphate response in NIH 3T3 Fibroblasts. *J Cancer Res Clin Oncol* 1990, **116**, 889.
31. Uberall F, Oberhuber H, Maly K, Zaknun, J, Demuth L, Grunicke HH. Hexadecylphosphocholine inhibits inositol phosphate Formation and protein kinase C activity. *Cancer Res* (in press).
32. Berdel WE, Fink K, Egger B, Reichert A, Munder PG, Rastetter J. Inhibition by alkyl-lysophospholipids of tendiated thyridine uptake in cells of human malignant urologic tumors. *J Natl Cancer Inst* 1981, **66**, 813–817.
33. Berdel WE, Fromm M, Fink K, Pahlke W, Bicker U, Reichert A, Rastetter J. Cytotoxicity of thioether-lysophospholipids in leukemias and tumors of human origin. *Cancer Res* 1983, **43**, 5538–5543.
34. Hilgard P, Stekar J, Voegeli R, *et al.* Characterization of the Antitumor Activity of Hexadecylphosphocholine (D 18506). *Eur J Cancer Clin Oncol* 1988, **24**, 1457–1461.
35. Hilgard P, Stekar J, Sindemann H, Peukert M, Unger CJ. Antineoplastic activity of alkyl-phosphocholines *in vivo*. *Cancer Res Clin Oncol* 1990, **116**, 889.
36. Shashidhar MS, Volwerk JJ, Keana JFU, Griffith OH. Inhibition

of phosphatidylinositol-specific phospholipase C by phosphonate substrate analogues. *Biochem Biophys Acta* 1990, **1042**, 410–412.
37. Moyer JD, Reizes O, Ahir S, Jiang C, Malinkowsky N, Baker DC. Substrate properties of analogs of myo-inositol. *Mol Pharmacol* 1988, **33**, 683–689.
38. Nahorski SR, Potter BVL. Molecular recognition of inositol polyphosphates by intracellular receptors and metabolic enzymes. *TIBS* 1989, **10**, 139–144.
39. Billah MM, Anthes JC. The regulation and cellular functions of phosphatidylcholine hydrolysis. *Biochem J* 1990, **269**, 281–291.
40. Kikkawa U, Kishimoto A, Nishizuka Y. The protein kinase C family. Heterogeneity and its implications. *Annu Rev Biochem* 1989, **58**, 31–34.
41. Marshall CJ, Lloyd AC, Morris JDH, Paterson H, Price B, Hall A. Signal transduction by $p21^{ras}$. *Int J Cancer* 1989, Suppl. 4, 29–31.
42. Barbacid M. *Ras* genes. *Annu Rev Biochem* 1987, **56**, 779–827.
43. Grunicke H, Hofmann J, Maly K, *et al.* The phospholipid- and calcium-dependent protein kinase as a target in tumor chemotherapy. *Adv Enzyme Regul* 1989, **28**, 201–216.
44. Gascher A, Dale IL. Protein kinase C- a novel target for rational anticancer drug design? *Anticancer Drug Design* 1989, **4**, 93–105.
45. Turner RS, Kuo JF. Phospholipid-sensitive Ca^{2+}-dependent protein kinase (protein kinase C): the enzyme, substrates and regulation. In: Kuo JF, ed. *Phospholipids and Cellular Regulation*. Boca Raton, CRC Press, 1985, **2**, 75–110.
46. Tamaoki T, Nomoto H, Takhashi I, Kato Y, Morimoto M, Tomita F. Staurosporine, a potent inhibitor of phospholipid/Ca^{2+} dependent protein kinase. *Biochem Biophys Res Commun* 1986, **135**, 397–402.
47. Davis PD, Hill CH, Keech E, *et al.* Potent selective inhibitors of protein kinase C. *FEBS Lett* 1989, **259**, 61–63.
48. Meyer T, Regenass U, Fabbro D, *et al.* A derivative of staurosporine (CGP 41251) shows selectivity for protein kinase C inhibition and *in vitro* anti proliferative as well as *in vivo* anti-tumor activity. *Int J Cancer* 1989, **43**, 851–856.
49. O'Brian CA, Liskamp R, Solomon DH, Weinstein IB. Inhibition of protein kinase C by tamoxifen. *Cancer Res* 1985, **45**, 2462–2465.
50. Su HD, Mazzei GJ, Vogler WR, Kuo JF. Effect of tamoxifen, a nonsteroidal antiestrogen, on phospholipid/calcium-dependent protein kinase and phosphorylation of its endogenous substrate proteins from the rat brain and ovary. *Biochem Pharmacol* 1985, **34**, 3649–3653.
51. Bignon E, Ogita K, Kishimoto A, *et al.* Modes of inhibition of protein kinase C by triphenylacrylonitrile antiestrogens. *Biochem Biophys Res Commun* 1989, **163**, 1377–1383.
52. Pettit GR, Herald CL, Doubek DL, Herald DL, Arnold E, Clardy J. Isolation and structure of bryostatin 1. *J Am Chem Soc* 1982, **104**, 6846–6848.
53. Smith JA, Smith L, Pettit GR. Bryostatins: potent, new mitogens that mimic phorbol ester tumor promoters. *Biochem Biophys Res Commun* 1985, **132**, 939–945.
54. Hennings H, Blumberg PM, Petit GR, Herald CL, Shores R, Ypsa SH. Bryostatin 1, an activator of protein kinase C, inhibits tumor promotion by phorbol esters in SENCAR mouse skin. *Carcinogenesis* 1987, **8**, 1342–1346.
55. Pettit GR, Day JF, Hartwell JL, Wood HG. Antineoplastic components of marine animals. *Nature* 1970, **227**, 962–963.
56. Dale IL, Gescher A. Effects of activators of protein kinase C, including bryostatins 1 and 2, on the growth of A549 human lung carcinoma cells. *Int J Cancer* 1989, **43**, 158–163.
57. Wender PA, Cribbs CM, Koehler KF, *et al.* Modeling of the bryostatins to the phorbol ester pharmacophore on protein kinase C. *Proc Natl Acad Sci USA* 1988, **85**, 7197–7201.
58. Chambers TC, McAvoy EM, Jacobs JW, Eilon G. Protein kinase C phosphorylates P-glycoprotein in multidrug resistant human KB carcinoma cells. *J Biol Chem* 1990, **265**, 7679–7686.
59. Endicott JA, Ling V. The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu Rev Biochem* 1989, **58**, 137–171.
60. Aikiyama S, Cornwell MM, Kuwano M, Pastan I, Gottesman MM. Most drugs that reverse multidrug resistance also inhibit photoaffinity labeling of P-glycoprotein by a vinblastine analog. *Mol Pharmacol* 1988, **33**, 144–147.
61. Elliason JF, Ramuz H, Haufmann F. Human multi-drug-resistant cancer cells exhibit a high degree of selectivity for stereoisomers of verapamil and quinidine. *Int J Cancer* 1990, **46**, 113–117.
62. Kamiwatari M, Nagata Y, Kikuchi H, *et al.* Correlation between reversing of multidrug resistance and inhibiting of ^3H azidopine photolabeling of P-glycoprotein by newly synthesized dihydropyridine analogues in a human cell line. *Cancer Res* 1989, **49**, 3190–3195.
63. Rink TJ. Receptor-mediated calcium entry. *FEBS Lett* 1990, **268**, 381–385.
64. Schmidt WF, Huber KR, Ettinger RS, Neuberg RW. Antiproliferative effect of verapamil alone on brain tumor cells *in vitro*. *Cancer Res* 1988, **48**, 3617–3621.

Eur J Cancer, Vol. 27, No. 3, pp. 284–289, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Meeting Report: Occupational Exposures in Insecticide Application and Some Pesticides

INTRODUCTION

THE LATEST in the series of *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* is the product of the deliberations of scientists from 19 countries (see list of participants at the end of this article), who met in Lyon, France, on 16–23 October 1990. They evaluated the evidence on the carcinogenicity of 17 pesticides, and for occupational exposures in insecticide application [1] using the standardised wording and groupings established within the *Monographs* [2]; the degrees of evidence for carcinogenicity for the different exposures were evaluated as outlined in Table 1.

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Received 10 Dec. 1990; accepted 12 Dec. 1990.

BACKGROUND

Pesticides are now used throughout the world—to varying degrees, depending on the dominating crops in a country, its stage of economic development, climatic conditions and the prevalence of pests. Crops are affected by pests and by competition from weeds; the trend towards large-scale monoculture of new plant species and cultivars, in which natural, indigenous insect predators play no role, has increased the problem. Crop losses due to pests can range from 10% in developed countries up to as much as 75% in developing countries. Furthermore, pesticides are in widespread use in urban areas for the control of disease vectors. Thus, worldwide consumption of pesticides in the mid-1980s was about 3 million tonnes; of these, herbicides accounted for 46%, insecticides for 31% and fungicides for 18%.