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Short report

Tumour initiators, promoters, and complete carcinogens

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That the induction of cancer generally includes many stages is now well established. Three clear stages are:

Initiation which is caused by mutagens.

Promotion which often entails the production of free radicals and the induction of "benign" lesions.

Malignant transformation ("tumour progression") by which the first lesions are changed into malignant cancers; this like the first stage may be caused by mutagens.

Under most environmental conditions low levels of initiators and promoters are present, so that either type of agent will induce some tumours. The suggestion that "if a substance is carcinogenic, but not mutagenic, then it is most probably a tumour promoter"[1] seems a reasonable assumption. With the definition of a carcinogen as an agent that increases either the incidence or rate of appearance of cancer, then the suggested definition of promoters may be too wide, as inhibitors of DNA repair or of immune response might increase the incidence of cancer by mechanisms not generally considered to be "tumour promotion." On the other hand, the claim of Ames and McCann that "carcinogens are mutagens"[2] is too broad, as some tumour promoters such as saccharin and phenobarbitone are carcinogens but not mutagens. In practice there is some overlap; either promoters or initiators will both produce tumours if administered in large doses. In the conventional measurement of tumour promoting activity on mouse skin a "sub-threshold" application of 7–12 dimethylbenz(a)anthracene is used as initiator. 7–12 Dimethylbenz(a)anthracene is a complete carcinogen, which is considered potent because it induces tumour more rapidly than other polycyclic hydrocarbons. Berenblum and Shubik stated, "the minimal dose-response is a measure of initiating action, while the average latent period is a measure of promoting action. Dibenz(a,h)anthracene is undoubtedly a potent initiator but a weak promoter."[3] This indicates that dibenz(a,h)anthracene could be a better agent than 7–12 dimethylbenz(a)anthracene as an initiator in tests for tumour promoting activity on mouse skin.

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Initiators are mutagens

The stage of initiation is generally induced by mutagens. The initiators may, like alkylating agents, be themselves chemically reactive or they may be substances that are transformed into chemically reactive agents by metabolic processes. The active agents that are mutagenic react with nucleic acid and so cause mutations. The active mutagenic forms of polycyclic hydrocarbons are epoxide derivatives and the reactive products of aromatic amines are hydroxylamine derivatives. Some polycyclic hydrocarbons and some aromatic amines, however, are complete carcinogens.

Free radicals

Tumour promoters are not mutagenic and need not react with nucleic acid. Evidence is accumulating that some tumour promoters are active by forming free radicals in tissues and particularly at cell membranes. Lyons and Spence detected free radicals in carcinogenic exhaust fumes, cigarette smoke, and chimney smoke.[4] The exposure of mice to free radicals produced by a radio frequency discharge did not increase the incidence of tumours.[5] Whether the tumour promoters that are surface active agents produce free radicals is not known.

The suggestion of Risse that *x* rays produce hydroxyl ions in water was made in 1929, about the time in which Muller showed that radiation was mutagenic. It now appears that radiation is more effective in tumour promotion than initiation.[6] This agrees with the concept that free radicals cause tumour promotion. Support of this came from the observations of Slaga *et al* that benzoyl peroxide and other peroxides are tumour promoters and that antioxidants inhibit promotion,[7] and of Troll *et al* that potent tumour promoters increase the formation of superoxide ions in tissues.[8]

Compounds that are complete carcinogens have

initiating and mutagenic activity due to the formation of chemically reactive molecules such as epoxide or hydroxylamine derivatives. The tumour promoting capacity could be due to formation of radicals through different metabolic pathways. Thus in addition to the mutagenic dihydroxydiol epoxy derivative of benzo(a)pyrene a 6-oxy-benzo(a)pyrene radical is formed.[9] This free radical would act as tumour promoter and so explain why benzo(a)pyrene is a complete carcinogen.

2-Naphthylamine is an acknowledged human carcinogen. It is not a potent compound, as large doses and long periods are needed to induce tumours in animals, but it is a complete carcinogen. The initiating activity of 2-naphthylamine appears to be due to the product of N-hydroxylation—2-naphthyl hydroxylamine, which is mutagenic[10] and carcinogenic.[11]

Another metabolite is 2-amino-l-naphthol which is carcinogenic[12] on injection or bladder implantation[13] and forms a free radical that is produced on incubating 2-naphthylamine with microsomes.[9] Among the animal species tested, dogs are the most sensitive to the carcinogenic action of 2-naphthylamine in the bladder and excrete relatively more 2-amino-l-naphthol than do other mammals.[14] This could be due to the amino-naphthol radical acting as a tumour promoter in the bladder.

Other complete carcinogens behave in similar ways. 4-Nitroquinoline *N* oxide reacts with nucleic acid after reduction to 4-hydroxylaminoquinoline *N* oxide, but it also readily forms a free radical on incubation with liver homogenates.[9] Other complete carcinogens that react with nucleic acid and also produce free radicals include *N*-methyl-*N*-nitro-*N*-nitroso guanidine, 2-acetylaminofluorene, 3-methyl-*N* methylaminoazobenzene and Mitomycin C.

Epidemiological and laboratory investigations have shown that asbestos acts as a tumour promoter. Experiments carried out in Africa, Canada, and Scotland have shown that the addition of asbestos to respiring tissue preparations in vitro increases the production of free radicals. Thus asbestos appears to have a similar mechanism of action as the potent tumour promoters such as the phorbol esters.

Sensitivity of infants

Some tumour promoters, such as healing after injury, increase cell division. Rapid cell division is associated with tumour promotion. It is for this reason that fetuses and young animals are more sensitive to carcinogenic action. Tumour initiators might therefore be expected to act as complete carcinogens in neonatal or young animals. This is exemplified by the recent work in which 4-amino-azobenzene induced hepatomas in young mice.[15] The authors suggest that this compound may have strong initiating activity for adult rat liver but little tumour promoting activity. These considerations provide some rational basis for the high susceptibility of the young to carcinogenic hazards.

Malignant transformation

Shubik showed that the skin tumours produced by a simple application of an initiator and repeated treatment with croton oil were mainly benign warts, whereas continuous treatment with a carcinogen induced some malignant tumours.[16] This indicated that initiation and malignant transformation are both produced by mutagenic agents. This has recently been confirmed when it was shown that "malignant conversion of mouse skin tumours is increased by tumour initiators and unaffected by tumour promoters."[17] These observations indicate that benign tumours in patients treated with mutagenic drugs would be converted to malignant lesions.[18] A practical consequence of this is that patients with papillomas or other non-malignant tumours should not be treated with mutagenic chemotherapeutic agents. Many of the agents used to treat cancer are themselves carcinogenic and mutagenic.

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