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Mechanisms in chemical carcinogenesis*

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Abstract

In ever rising frequency, chemical substances are reported to have increased the tumor incidence in animal experiments. Although these compounds belong to a variety of chemical classes, a large number seems to have in common the ability to react with deoxyribonucleic acid (DNA), the carrier of genetic information, after metabolism to chemically reactive intermediates. As opposed to this group of genotoxic carcinogens, non-genotoxic carcinogens act by modulation of one or several out of a number of biochemical and biological steps governing amount and expression of unavoidable DNA lesions towards the formation of a tumor.

Zusammenfassung

Laufend wird über chemische Substanzen berichtet, von denen eine krebsfördernde Wirkung im Tierversuch gezeigt worden ist oder für den Menschen vermutet wird. Solche Substanzen kommen aus den verschiedensten Stoffklassen. Eine grosse Gruppe von organischen, schlecht wasserlöslichen Kanzerogenen wird über chemisch reaktive Zwischenprodukte metabolisiert. Deren Reaktion mit der Erbsubstanz DNS scheint das zentrale Element der Wirkweise von genotoxischen Substanzen zu sein. Da auch endogene, essentielle und unvermeidliche Verbindungen diese Eigenschaft haben, kann ein gewisses Mass an DNS Schäden nicht vermieden werden. Die Folgen werden allerdings dadurch gemildert, dass effiziente Reparatursysteme solche Schäden reparieren können. Die Wirkung nicht-genotoxischer Kanzerogene basiert auf der Modulation unvermeidlicher DNS-Veränderungen. Dies kann auf verschiedenen Stufen geschehen, z.B. durch Erhöhung der DNS-Bindung von anderen, genotoxischen Substanzen, durch Erhöhung der Ausbeute an kritischen DNS-Schäden, oder durch Beschleunigung des langsamen Prozesses der Entwicklung einer transformierten Zelle zu einem Tumor.

*Updated and modified version of the article in German "Mechanismen der Krebserzeugung", Neue Zürcher Zeitung Nr. 173, S. 39, July 29, 1981.

It was not necessary in the last years to read scientific journals to know that an ever increasing number of chemicals has been found to have increased the tumor incidence in animal experiments. In newspapers we were warned of *aflatoxins* on mouldy food; cigarette *smoking* is surely responsible for most lung cancers and is a contributing factor to many other types of cancer; *nitrosamines* form an important class of strong carcinogens which can be generated by nitrosation of amines; some *metal* salts have been discovered as industrial carcinogens and *hormones* were widely discussed very recently. *Asbestos* is an important factor in the induction of tumors from exposure at the work place, and *Saccharin* gave rise to headline news because of some bladder tumors induced in male rats whose diet consisted of 5 percent saccharin for life. In this introductory article I will not discuss the importance of these carcinogens for human health but I would like to present the current view on the mechanism of carcinogenic action of these chemicals. The structural formulas are given in figure 1. For cigarette smoke, benzo(a)pyrene is shown as a representative of the many carcinogens present. For the hormones, the synthetic estrogen diethylstilbestrol was taken.

No common feature can be discerned which could give a hint for some common mechanism of carcinogenic action of these compounds. More meaningful and perhaps more informative might be to look at the fate of these compounds in the animal.

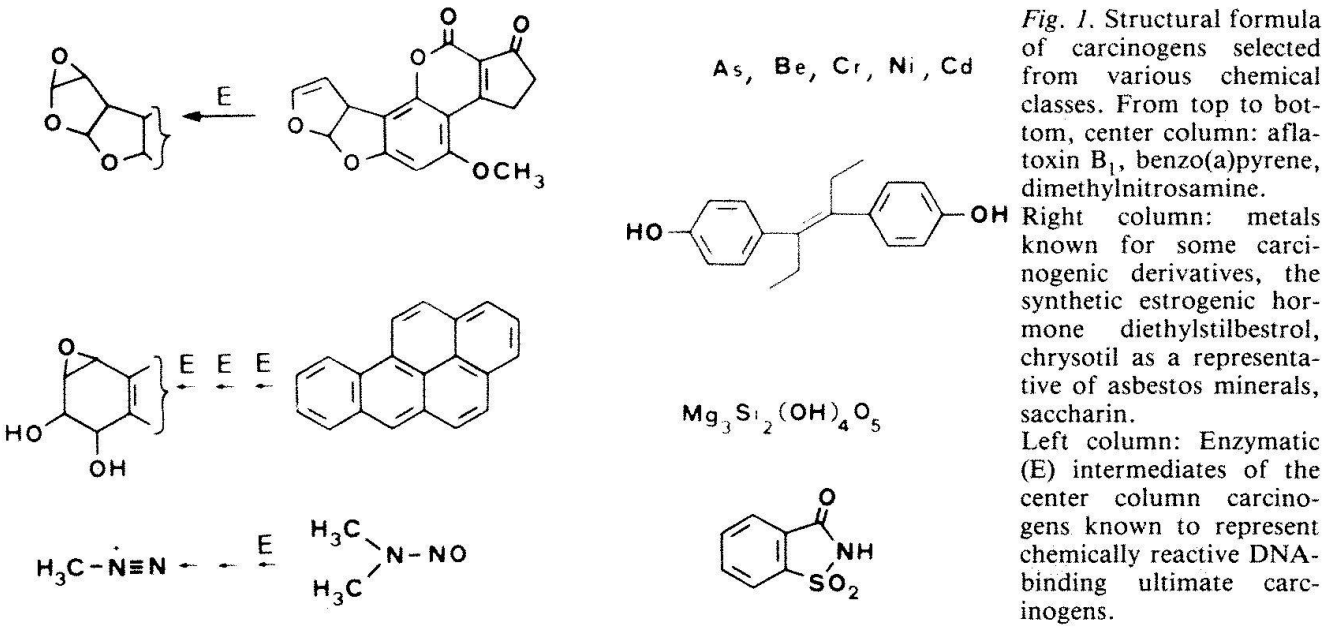

Fig. 1. Structural formula of carcinogens selected from various chemical classes. From top to bottom, center column: aflatoxin B_1, benzo(a)pyrene, dimethylnitrosamine. Right column: metals known for some carcinogenic derivatives, the synthetic estrogenic hormone diethylstilbestrol, chrysotil as a representative of asbestos minerals, saccharin. Left column: Enzymatic (E) intermediates of the center column carcinogens known to represent chemically reactive DNA-binding ultimate carcinogens.

Genotoxic carcinogens

Chemically reactive metabolites

One important aspect in the metabolism of many organic carcinogens is the fact that an excretion of the water-insoluble compounds is possible only after introduction of hydroxyl groups and subsequent conjugation with water-soluble molecules. In the course of these enzymatic oxidation processes, chemically reactive metabolites are formed, such as epoxides or diazonium ions as shown on the left hand side of figure 1. We have therefore found, at least for three of our standard carcinogens, the common feature of *electrophilic intermediates* which are also called ultimate carcinogens. These metabolites are unavoidably formed in a process that should finally lead to the excretion of the foreign compounds. By far the largest part is indeed rapidly inactivated by processes shown in figure 2, by rearrangements or enzymatic and non-enzymatic reactions with small molecules, and only a minute but biologically important fraction escapes and reacts with macromolecules, some of which are critical with respect to a triggering of the process of tumor formation.

DNA as critical reactant

There are many indications that most tumors have grown from one single cell. A heritable change must therefore have occurred in this cell. This is most directly achieved by some critical mutation in the genes (DNA). During cell division, DNA is replicated and evenly distributed among the two daughter cells. If a carcinogen is bound to the DNA, the copy process can be disturbed so that one daughter strand carries a wrong piece of information (fig. 3). This is called mutation and the mechanism of action of the carcinogen was by *genotoxicity*. By far not all carcinogens chemically bound to

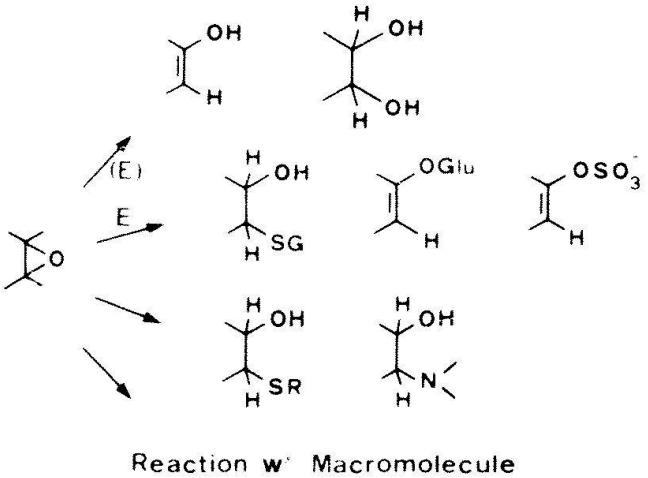

Fig. 2. Chemical and enzymatic (E) reactions involved in the further metabolism of reactive intermediates, such as epoxides. 1st row: rearrangement, addition of water. 2nd row: enzymatic conjugation reactions with glutathione (GS), glucuronide (Glu), sulphate. 3rd row: non-enzymatic reaction with low molecular nucleophiles, containing thiol and amino groups. 4th row: reaction with macromolecules, such as protein and nucleic acids.

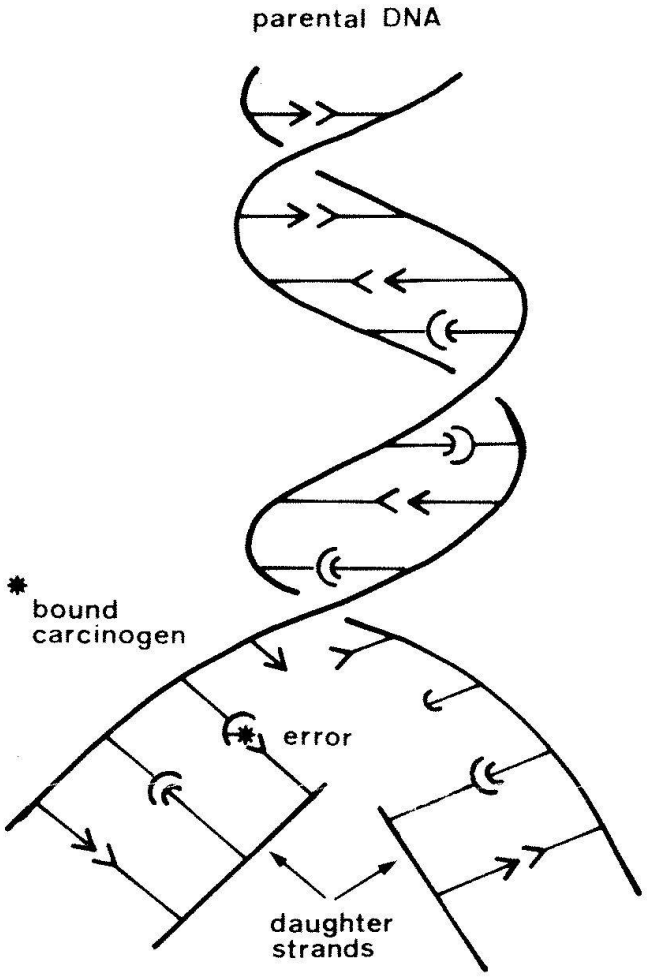

Fig. 3. Schematic representation of the deoxyribonucleic acid (DNA) genes, the heritage of a cell, during replication before cell division. The bound carcinogen molecule (*) can disturb the replication process in a way as to direct a wrong coupling partner to be introduced into the daughter strand. If this wrong piece of information lies in a critical gene, the cell might be initiated to become a cancer cell.

DNA produce a mutation. A number of processes are known for the *repair of the DNA*, and it is astonishing that microorganisms and phylogenetically lower animal species possessed such repair mechanisms long before the synthetic chemistry invented new genotoxic compounds.

Unavoidable DNA damage

We must therefore assume that DNA damage is as old as life and that some unavoidable genotoxicity resulted in a strong evolutionary pressure to develop efficient DNA repair systems. Among these unavoidable sources of DNA damage is radiation, both cosmic and terrestric gamma rays as well as UV. A variety of endogenous or essential compounds are degraded by the same routes of oxidation known for the genotoxic carcinogens. Many genotoxic carcinogens are produced in the process of cooking or frying and are not completely avoidable, and the formation of carcinogenic nitrosamines can take place in the acidic milieu of the stomach by nitrosation of ubiquitous amines in the presence of nitrite generated from bacterial reduction of nitrate. In addition, many genotoxic agents are of natural origin. Besides the well-known mycotoxins, such as aflatoxins, there are pyrrolizidines, widely distributed alkaloids in plants. Safrole and estragole are components of many spices, gyromitrin is a carcinogenic hydrazone derivative isolated from the false morel mushroom Gyromitra esculenta, and the next years will see the discovery of many more carcinogens of natural origin. There can therefore be no doubt that a certain level of DNA damage cannot be avoided.

Now that we have shown that the first three of our standard carcinogens act by genotoxicity, let us discuss the remaining. It has been shown with the carcinogenic *metal* ions that their presence during the replication of DNA decreases the fidelity in the synthesis of an exact copy for the daughter strands. We could therefore call this mechanism of action an *indirect genotoxicity*. What about the others?

Non-genotoxic carcinogens

In order to answer this question it might be helpful to summarize at this point the general knowledge of the process of tumor formation (fig. 4). We have already discussed the processes leading to the DNA binding of genotoxic carcinogens. The resulting, potentially critical DNA lesion can lead to the transformation of the cell to a tumor cell able to progress to a tumor. These final steps are often summarized under the term "promotion" a process which characteristically requires a substantial fraction of the animal's life time, whereas the earlier steps leading to the critical DNA lesion can take place in a few days.

Since we must assume that our DNA is constantly damaged to some unavoidable extent

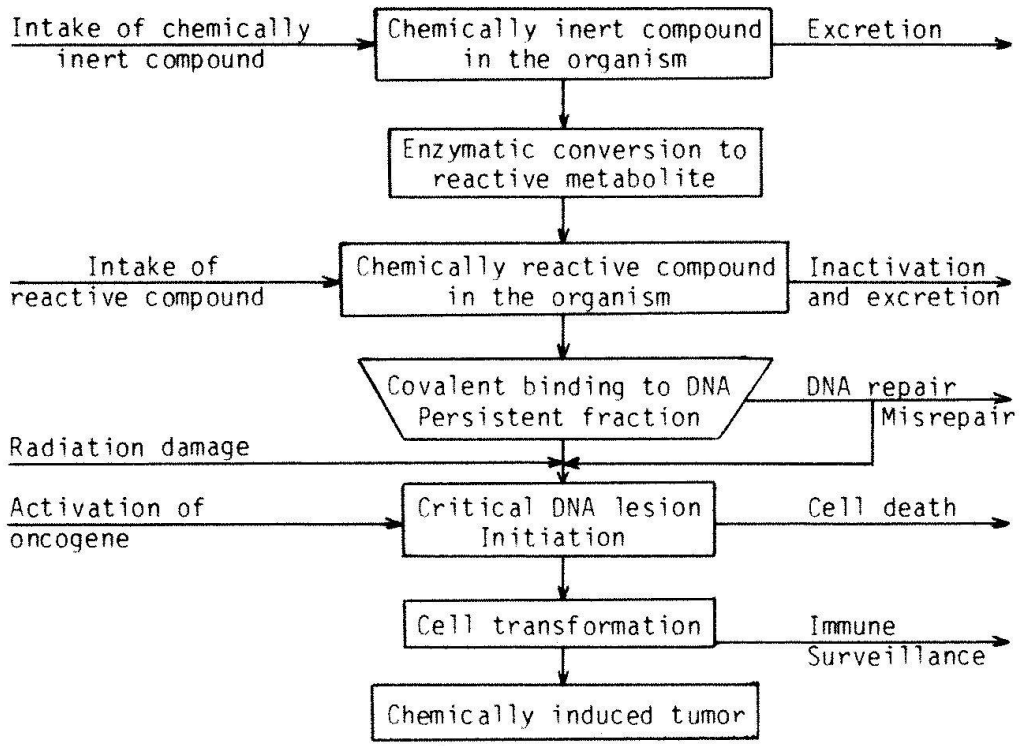

Fig. 4. Schematic representation of the sequence of events governing the process of tumor induction by chemicals (vertical axis). The horizontal arrows pointing to the right summarize some of the reactions protecting the host from the carcinogenic stimulus.

we can deduce that those compounds also increase the tumor incidence which increase the amount of DNA damage set by genotoxic substances or help to promote the frequency of cell transformation to a tumor cell and the progression to a tumor. In the flow chart shown in figure 4, this means that all chemicals which have an influence on the speed of any of the reactions will alter the final tumor incidence. Cocarcinogenesis is achieved by accelerating the vertical processes or slowing the horizontal rescue processes (to the right hand side), anticarcinogenesis is achieved by the opposite activity. For the sake of an easy classification, the site of modulation can roughly be subdivided into three parts.

Modulation of DNA binding

The first group of non-genotoxic carcinogens leads to an increase of the DNA damage by genotoxic carcinogens. One example for this type of activity might be the generation of nitrosamines from amines and nitrite in the stomach. Since this reaction is dependent on the pH, hyperacidity of the stomach might well be a modulatory factor, and a physiological basis for an eventual relation of stress factors with gastro-intestinal tumors might be envisaged.

Many studies deal with the influence of drug-metabolizing enzyme activities on DNA damage. In all the studies so far reported, a pretreatment of a laboratory rodent with an enzyme-inducing agent has resulted in a slight decrease of DNA binding by a subsequent dose of a standard carcinogen, such as benzo(a)pyrene. Because of the complexity of activating and inactivating processes, enzymatic and non-enzymatic, which govern the concentration of reactive intermediates, this finding should not, however, be taken as representative for other carcinogens. Situations will certainly arise where the induction of enzyme activities will result in a higher level of DNA binding exerted by genotoxic carcinogens.

Co-mutagenicity

The fixation of the primary DNA damage, the binding of a genotoxic carcinogen, in the form of a heritable mutation is a central event in chemical carcinogenesis and is subject to a number of important modulatory influences. After DNA binding by a genotoxic carcinogen a competition between DNA repair and DNA replication starts. All stimulation of cell division reduces the time allotted for repair and an increased fre-

quency of mutations can result. Cell division is an absolute requirement for growth and for the proper functioning of a number of tissues and there are endogenous stimulants for this type of response, such as *hormones*. The regenerative processes which are elicited after exposure to cytotoxic compounds or from local irritation could explain the carcinogenicity of implants or of insoluble *asbestos* fibers. It is interesting in this respect that lung tumor incidence is greatly enhanced for cigarette smokers exposed to asbestos. It is possible that the DNA damage exerted by the genotoxic constituents of cigarette smoke can be repaired less effectively if the cell division rate is increased in the presence of an asbestos fiber.

Promotional activity

For a discussion of the last steps and the respective modulations, some background knowledge is required: It is not as yet generally known what type of lesion is required to convey to a cell the attitude of a cancer cell. In some special cases there is indication for a differentiation back to some type of multipotent ancestor cell. In other systems, the activation of some oncogene has been shown to induce the transformation of the cell. An intriguing characteristic of the chemical induction of a tumor is the fact that a considerable fraction of the life is required in most situations. During this latency period of up to 20 years in man, of many months in the rat, there seems to be a requirement for the continuous challenge of the initiated cell by something called "promoter". The most potent promoters have been isolated from plants, and the classical constituent is a phorbol ester, a diterpene derivative isolated from the oil of croton seeds. This compound has been found to bring about a significant tumor incidence if painted repeatedly on the skin of mice after one single topical application of benzo(a)pyrene as genotoxic carcinogen. Although there is only insufficient evidence that tumor promoters are not by themselves genotoxic, such a mode of action seems unlikely. Tumor promoters have been found to induce a variety of biochemical and biological responses but it is not known which one is causally related to the effectiveness in cancer induction.

The search for a reliable short-term test on promoting activity of a compound is therefore very active these days, and there are reports that *saccharin* has been found to exhibit qualitatively similar effects like typical promoters. For this last compound selected from our headline news, we were able to exclude a DNA binding activity and it is possible that saccharin is extremely weakly active as a modulator of the final but long stages of tumor promotion and progression. There is good epidemiological evidence that fat consumption is correlated with the risk of cancer, especially of the colon and the breast. Animal experiments have shown that dietary fat can indeed increase the tumor incidence if given continuously after a single dose of a genotoxic carcinogen. In addition to this promotional type of activity, a genotoxicity of fat itself cannot yet be ruled out because it is well known that polyunsaturated fatty acids readily form chemically reactive derivatives, such as peroxides. Furthermore, there is evidence that the intestinal bacterial flora can play an important role in the generation of genotoxic carcinogens from non-carcinogens, e.g. by reduction of nitroarenes to carcinogenic aromatic amines. Since the diet ultimately determines the composition of the bacterial flora with respect to strain and number, it might well be that a fatty diet predisposes the host to carry potentially dangerous intestinal bacteria.

Multiple modulatory activities

The situation is therefore not as simple as I have depicted it in the introductory paragraphs. Just like fat, many carcinogens do not only act on one single level. Cigarette smoke is another well studied and illustrative example. It is well known that it contains a number of genotoxic organic chemicals, enzyme-inducing agents and also carcinogenic metals such as cadmium. The nitroxides and aldehydes present in smoke are cytotoxic and irritate the mucous membranes of the respiratory tract. The resulting synergism might be the reason why as much as about thirty percent of all tumors in man, non-smokers included, can be traced back to cigarette smoking.

Toxicological implications

On the basis of the above analysis, we can conclude that endogenous factors are operative on all levels of tumor induction so that cancer will never be completely avoidable. Evaluation of the epidemiological data available suggest that this unavoidable tumor incidence will lie somewhere between 10 and 30 percent, assuming that the life expectancy remains unchanged. In order to achieve this low risk for ourselves, it would be most profitable to avoid all those exogenous factors that act strongly on many levels. Above all, *cigarette smoking must be stopped.* Then, it seems that a *reduction of fat consumption* and an *increased uptake of undigestable fibers,* possibly in the form of vegetables and fruits rich in vitamins, will have a beneficial effect. And finally, it will be beneficial to reduce the uptake of known and unknown carcinogens with the diet by *eating little of everything.* These simple rules will much more effectively result in a decreased cancer incidence in the general population than any other cancer policy.

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