

Hypothetical Two-Step Initiation of Experimental Carcinogenesis by Polycyclic Aromatic Hydrocarbons and Aminoazo Dyes

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Abstract: A new hypothesis is discussed, which describes the *initiation* of the carcinogenesis through polycyclic aromatic hydrocarbons (PAHs) and aminoazo dyes (AZOs) as a two-step process: the oncogenic proteins of the *ras* or *ras*-like oncogenes activated by mutation ("initiation A") co-operate with the complexes in the plasma membrane formed during the "initiation B" stage from the parent compounds of the PAHs or AZOs with cholesterol and apolipoprotein A-I. The final result of this co-operation, or the "complete initiation", is an *irreversibly* modified membrane architecture with negative consequences for growth control.

Keywords: Initiation of carcinogenesis, polycyclic aromatic hydrocarbons, aminoazo dyes.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) and aminoazo dyes (AZOs) exercise several functions as "complete carcinogens" in the multistep process of carcinogenesis [1, 2]:

INITIATION → CONVERSION → PROMOTION → PROGRESSION

Step by step, growth-inhibiting factors are degraded and cell growth is promoted during carcinogenesis, until a tumor is ultimately formed. The still almost unanimous opinion that exclusively genotoxic and cytotoxic metabolites of the PAHs and AZOs are actively involved in this multi-step process, and that the parent compounds remain totally inert, is in need of revision. The parent compounds of PAHs and AZOs can also be directly involved in the process of carcinogenesis, since in the presence of slight amounts of apolipoprotein A-I (apoA-I) - a principal constituent of high-density lipoprotein (HDL) - they form adsorption complexes with cholesterol and can thus change specific functions of the plasma membrane and its components [3, 4]. The stability of these, presumably epitaxial complexes correlates closely with the carcinogenic efficacy of the PAHs or AZOs they contain, and is strengthened by the hydrophobicity in the intermolecular interactions in the complex, but weakened by the aqueous solubility of the carcinogens [4]. The correlation with the strength of the carcinogenic efficacy leads to the conclusion that the parent compounds of the PAHs and AZOs play an essential role in the mechanism of carcinogenesis. Up to now the actual mechanisms underlying this correlation have not been explained. The following ideas should stimulate the discussion.

The results of the "complete carcinogenicity assays" (e.g. PAH as *initiator* and *promoter*) are in agreement with the "assays for tumor initiation" (e.g., PAH as *initiator* and croton oil or 12-O-tetradecanoylphorbol-13-acetate (TPA) as *promoter*) [5, 6]. This relationship can only be interpreted to mean that the relative carcinogenic efficacy of the aromates is largely independent from the type of convertogenic promoter. In other words, the relative efficacy is already manifested in these assays during the *initiation* of the tumorigenesis. The parent compounds of the carcinogenic PAHs and AZOs are consequently initiators.

The results of a multitude of animal experiments conducted in the past decades with PAHs and AZOs create the impression that there are two different types of "initiation": one *initiation* as source of the nonautonomous *benign* tumors [e.g. papillomas (skin), nodules (liver)], and another type of *initiation* as source of autonomous *malignant* tumors (e.g. carcinomas). Primarily nonautonomous tumors develop in the "assay for tumor initiation" in mouse skin, whereas primarily autonomous tumors develop in the "complete carcinogenesis assay" [5, 6].

HYPOTHESIS

Two sub-steps A and B can be distinguished in the process of *initiation* through PAHs and AZOs (Table 1):

Initiation A

Electrophilic metabolites [7] or radical cations [8] of the PAHs or AZOs form a covalent bond to the bases of the DNA. Result: point mutations of the DNA and hence also the activation of proto-oncogenes to oncogenes, e.g. "*ras*-like" oncogenes [9]. Under the repeated influence of a convertogenic promoter, e.g. TPA (skin) [1] or phenobarbital (liver) [2], *benign* nonautonomous tumors (papillomas, nodules) develop from these mutated cells.

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Initiation B

Molecules of the parent compounds of PAHs and AZOs can form hydrophobic complexes with apoA-I and cholesterol in the plasma membrane and thus change the membrane architecture [3, 4]. Such induced changes in membrane fluidity or microviscosity could modulate the distribution and activity of membrane proteins (e.g. receptors) which are critical to the regulation of cellular proliferation. The formation of complexes in the plasma membrane is only a *reversible* event, however, and on its own presumably without long-term effect. In contrast, a fully new situation arises when *initiation A* co-operates with *initiation B* at the plasma membrane (see below).

Hypothetical Co-operation: *Initiation A* + *Initiation B*

The formation of the complexes (*initiation B*) presumably also has *irreversible* consequences if oncogenes are activated in the same cell and whose products (oncogenic proteins) are active at the same location of the plasma membrane where the complexes are found. The oncogenic proteins can presumably stabilize the resulting changes in architecture during the *initiation B* process by binding to or reacting with essential components. Autonomous tumors ultimately develop under the repeated influence of a suitable promoter from these preneoplastic cells arising from a co-operation of *initiation A* with *initiation B* (Table 1).

DISCUSSION

The assumption that activated oncogenes (here *initiation A*) exclusively initiate *benign* nonautonomous tumors when on their own is not a new idea. Boukamp *et al.* [10] published an equivalent hypothesis, also supported by results obtained after transfection and infection studies in mouse keratinocytes *in vitro* and *in vivo* [11, 12]. What is new here,

however, is the hypothesis that another step is required for the "*complete initiation*" by PAHs and AZOs, brought into action by the parent compounds of PAHs or AZOs and resulting in a changed architecture and function of the plasma membrane. Perhaps the co-operation of *initiation A* with *initiation B* with respect to their consequences is comparable to the longer known co-operation of *ras* or "*ras-like*" oncogenes with *myc* or "*myc-like*" oncogenes in an *in vitro* culture: "*myc* enables *ras* transformants to ignore or override the inhibitory influences of normal neighboring cells" [9].

Possible initiators of an *initiation B* and its relative effectiveness can be determined in simple *in vitro* short-term tests for PAHs and AZOs [4]. Up to now the tests have shown that all PAHs and AZOs with carcinogenic effect - without exception (!) - can be initiators of class B, with a relative effectiveness which correlates with their carcinogenic potency. Furthermore, there are a few PAHs with false positive test results, such as for instance 3,9-dimethylbenz[a]anthracene, triphenylene and perylene [4]. These three non-carcinogenic PAHs are relatively strong complexing agents with cholesterol and apoA-I. Therefore according to the hypothesis presented here, one could expect them to be strong initiators of class B. In an "assay for tumor initiation" (e.g. DMBA + TPA) they should be able to raise the number of the autonomous tumors at the expense of the nonautonomous tumors. The fact that this property of the respective compounds has not yet been detected can probably be explained by the fact that they are already completely inactive as initiators of class A in a usual carcinogenicity assay, and thus a collaboration of *initiation B* with *initiation A* is impossible under these conditions.

Theoretically, only one single PAH or AZO molecule is required for "*initiation A*", i.e. for the mutation of a proto-oncogene. In contrast, it is certain that a larger number of molecules is necessary for "*initiation B*", i.e. for the formation of crystalline complexes with cholesterol. This means

Table 1. Hypothetical Two-step Model of "Initiation" in the Process of Experimental Carcinogenesis by PAHs (skin) and AZOs (Liver)

Step 1	Step 2	Step 3	Step 4	Result	
-----	INITIATION B	CONVERSION	PROMOTION	No Tumors	
INITIATION A	-----	CONVERSION	PROMOTION	Nonautonomous Tumors: Papillomas (skin) Nodules (liver)	Regression
INITIATION A	INITIATION B	CONVERSION	PROMOTION	Persistent Tumors	Progression → CANCER
Complete Initiation		Complete Promotion			
INITIATION A: Point mutations by "ultimate carcinogens" and hence also the activation of proto-oncogenes to oncogenes [9]. Result: Among other things, the activation of oncogenes whose products are active at the plasma membrane. INITIATION B: Formation of PAH/cholesterol/apoA-I - or AZO/cholesterol/apoA-I complexes in the plasma membrane [3, 4]. Result: Reversible changes in the membrane architecture and its functions. <u>Hypothesis</u> : INITIATION A can co-operate with INITIATION B at the plasma membrane. See text. Result: An irreversibly modified membrane architecture with negative consequences for growth control. CONVERSION and PROMOTION see: Marks and Fürstenberger [1]; Schwarz [2].					

that the concentration of PAHs or AZOs at the place of action must surpass a minimum level to be able to induce a "complete initiation". The consequences of a "complete initiation" would have to be expressed *in vitro* in an irreversibly changed behavior of the plasma membrane. It would be conceivable, for instance, that there was a reaction of the cell membrane with an agglutinin [13] and especially a loss of the "contact inhibition" of cell replication [14]. Both characteristics are an expression of a cell transformation which would not have occurred solely by an "initiation A".

The "complete initiation" could modulate the distribution and activity of membrane proteins (e.g. receptors) which are critical to the regulation of cellular proliferation. For instance, the communication of the cell with its immediate vicinity (matrix, cells) could be impaired. Another attractive idea is that actin filaments detach from the adhesion plaques of the plasma membrane [15]. In fact, the actin filaments in transformed cells are fully disorganized [15]. The free actin filaments could lead to disturbances in the cell division, which would result in chromosomal abnormalities and aneuploidy. The chromosomal abnormalities in these cells may inactivate tumor suppressor genes and/or activate additional oncogenes. Such mechanisms remain to be investigated.

CONCLUSION

The previously known course of initiation (here called "initiation A"), i.e. the activation of oncogenes [1, 16], is not challenged by this new hypothesis. But "initiation A" must be supplemented by an "initiation B", that is by a change in architecture of the plasma membrane. The final result of a "complete initiation" after a co-operation between "initiation A" and "initiation B" is a preneoplastic cell with an *irreversibly* modified membrane architecture with negative consequences for growth control. The hypothetical two-step model of *initiation* discussed here applies initially only for PAHs and AZOs. Concrete indications that this two-step model can be transferred in similar form to other complete carcinogens is currently under investigation.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENT

None declared.

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Received: February 19, 2012

Revised: March 29, 2012

Accepted: March 30, 2012

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