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Effect of some carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbons on gap junction intercellular communication in hepatoma cell cultures

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Abstract

One of the systems that regulate tissue homeostasis is gap junction intercellular communication (GJIC). It is accepted that the down-regulation of GJIC is linked to the tumor-promoting properties of carcinogens. In this study, the effect of some carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbons (PAH) on GJIC was investigated. It was found that in hepatoma cell culture (Hep G2) carcinogenic PAH inhibited GJIC after 24 h exposure by 75–100% depending on the PAH structure. The inhibition effect on GJIC is reversible because removing the PAH by changing of culture medium restores the GJIC. The non-carcinogenic PAH do not significantly influence GJIC. α-Naphthoflavone, an inhibitor of PAH metabolism, has no effect on inhibition of GJIC by carcinogenic PAH. 2,3,7,8-Tetrachloro-*p*-dibenzodioxin, an aryl hydrocarbon (Ah) receptor ligand, inhibits GJIC by about 50% only after 48 h exposure. To clarify the role of formation of PAH metabolites and interaction with Ah receptor on inhibition of GJIC, we determined the effect of benzo/a/pyrene on hepatoma G27 cells in which neither mRNA of CYP1A1 nor Ah receptor was determined. As in Hep G2 cells, benzo/a/pyrene, unlike non-carcinogenic benzo/e/pyrene, inhibits GJIC. We conclude that in the studied hepatoma cells carcinogenic PAH inhibit GJIC directly (that is, not via their metabolites) and this effect is not associated with Ah receptor interaction.

Keywords: Gap junction intercellular communication; Hepatoma cells; Polycyclic aromatic hydrocarbons

Introduction

Polycyclic aromatic hydrocarbons (PAH) are one of the major factors of environmental pollution. They are found in combustion products of different types of fuels, in nutritional products, in tobacco smoke, and in other sources. High carcinogenic activity of PAH has been demonstrated with experimental animals. PAH have been shown to induce a wide spectrum of tumors including hepatocellular carcinomas in a rodent model following either chronic or acute administration (Chramostova et al., 2004; Rodriguez et al., 1997). Carcinogenic PAH like benzo/a/pyrene (BP) under physiological conditions are inert and do not interact with DNA, RNA, and other macromolecules. However, highly reactive epoxides and phenols are formed during oxidation of PAH in the monooxygenase enzymatic system. It has been long recognized that the formation of DNA adducts is an important step in PAH-induced

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carcinogenic, mutagenic, and toxic effects. Therefore, studies on the biological effects of PAH have been mainly focused on their cellular metabolism. Besides genotoxic action, PAH have also been shown to elicit a host of non-genotoxic effects such as activation of genes involved in xenobiotic metabolism like isoforms of cytochrome P450, glutathione-S-transferase, UDP-glucuronyl-transferase, and others (induction effect).

Interaction of the initial, non-metabolized PAH molecule with aryl hydrocarbon (Ah) receptor that is present in the cytoplasm is the first step of signal transduction during induction (see reviews (Denison et al., 2002; Sogawa and Fuji-Kuriyama, 1997)). After ligand binding, the Ah receptor dimerizes with the ARNT protein and this complex is a transcriptional factor that binds to specific enhancer sequences (called xenobiotic responsible element, XRE) present within the promoter region of PAH-inducible genes. Thus, it was demonstrated that there is an effect of PAH that is mediated by the initial, chemically inert molecules of the compound.

Carcinogenesis is a multi-step process, at least two stages of action of chemical compounds being distinguished in it: initiation and promotion. Certain compounds have been found to be initiators, while some others possess only promoting functions. Initiation is mediated by the interaction of an electrophilic metabolite with DNA leading to changes in DNA structure followed by mutation. The principal promoter function consists of "switching-off" of the initiated cells from the regulatory control of adjacent normal cells, thus providing conditions for preferential growth of the initiated cells. The "promotion" stage is also referred to as epigenetic, since it is not associated with modification of the genetic apparatus and is induced by non-genotoxic compounds (Kobliakov, 1998; Rakitsky et al., 2002).

In the past more attention was given to the genotoxic nature of carcinogens but little has been done to study the epigenetic effects. Recently, however, the epigenomic component of environmental carcinogens has been suggested to be very important in human carcinogenesis due to altering gene expression that leads to an imbalance of cell proliferation, differentiation and apoptosis (Thilly, 2003; Trosko and Upham, 2005).

Change in gap junction intercellular communication (GJIC) is one of the major factors of promotion (Trosko, 2001; Yamasaki, 1990). Gap junctions are intercellular channels in the cytoplasmic membranes formed by proteins (connexins) that connect the cytoplasm of the neighboring cells. Molecules with a molecular weight up to 1.2 kDa can pass through these channels and thus coordinate the functions of the cells. Various forms of connexins exist; their expression depends on the cell type. The ability to abolish and to restore functioning of GJIC represents one of the systems of intercellular regulation within tissues (Yamasaki, 1990). Proliferation stimuli decrease the level of GJIC in cell culture independently of their nature (growth factors, chemical compounds) (Warn-Cramer et al., 1998; Yamasaki, 1990). Functioning of GJIC is decreased in the majority of tumors in comparison to normal homologous tissues; in those tumors where the level of GJIC between tumor cells is high, GJIC is absent between tumor cells and the cells of adjacent normal tissue (for reviews see Krutovskikh and Yamasaki, 1997; Ruch et al., 2001; Yamasaki, 1990). Thus, tumors with preserved functioning of GJIC between the cells inside the tumor remain insensitive to the regulatory influence of normal cells mediated by GJIC.

"Complete" carcinogens, i.e., compounds that induce tumors without the need for additional exposure, are able to stimulate both the initiation and promotion stages. Some PAH are found to be complete carcinogens. The initiation process for PAH is relatively well studied; it is accepted that this stage of tumor development involves the interaction of DNA molecules with diol epoxides (Jerina et al., 1991) that are formed during PAH oxidation catalyzed by cytochrome P450 in the monooxygenase enzymatic system. It is not yet established how and in what form PAH mediate the promotion stage. The effect on GJIC is often considered to be a marker of promotion.

During the process of carcinogenesis in the liver so-called "oval cells" appear. These cells constitute a heterogeneous population in which some cells might function as bipotent stem cells (Lowes et al., 2003; Sigal et al., 1992; Trosko and Chang, 1989) giving rise to hepatocytes and biliary cells and might act as progenitors for hepatocellular carcinomas (Knight et al., 2000).

In this work, we studied the effect of some PAH with different carcinogenic potential on GJIC in hepatoma cell cultures of two types, one in which CYP1 isoforms and the Ah receptor exist (Hep G2) and the other one in which mRNA expression of either CYP1 isoforms or the Ah receptor could not be found (G27). We found that carcinogenic PAH with high carcinogenic activity, unlike phenanthrene and benz/e/pyrene, were able to inhibit GJIC in both cell types studied in the same manner. This result demonstrates that inhibition of GJIC by PAH is caused by the unchanged PAH molecules (rather than by their metabolites) and apparently does not depend on interaction with the Ah receptor.

Materials and methods

Materials

BP, benzo/e/pyrene (BeP), benz/a/anthracene (BA), dibenz/ah/anthracene (DB(ah)A), 7,12-dimethyl-BA (DMBA), 3-methylcholanthrene (MC) and phenanthrene were obtained from Fluka or Ferak. 6-Methyl-BP was