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An Overview of Peroxisome Proliferator-Induced Hepatocarcinogenesis

by M. Sambasiva Rao* and Janardan K. Reddy*

Peroxisome proliferators are hepatocarcinogens in rats and mice. Chronic administration of these compounds results in the development of altered areas and neoplastic nodules followed by hepatocellular carcinomas. All three types of hepatic lesions do not express γ-glutamyltranspeptidase, glutathione *s*-transferase-P, and α-fetoprotein and are resistant to iron accumulation after overload. The mechanism by which nongenotoxic peroxisome proliferators induce hepatic tumors is not well understood. It has been proposed that with continuous administration of peroxisome proliferators, liver cells are subjected to persistent oxidative stress resulting from marked proliferation of peroxisomes and a differential increase in the levels of H_2O_2 producing (20- to 30-fold) and degrading (2-fold) enzymes. Free oxygen radicals lead to DNA damage (both directly and through lipid peroxidation) and thus may cause initiation and promotion of the carcinogenic process.

Introduction

Epidemiologic studies have unequivocally established a close association between exposure to some chemicals and development of cancer in humans. The best way to prevent development of tumors is to minimize or, if possible, completely avoid exposure of humans to such chemicals. For identification of carcinogenic chemicals, several short-term *in vitro* and *in vivo* tests have been developed based on the concept that carcinogens lead to alterations in DNA (*1,2*). However, there are some chemicals that elude detection in short-term tests and produce tumors in animals in long-term experiments. These compounds are termed nongenotoxic or nonmutagenic carcinogens. Initially, the idea of induction of tumors by nongenotoxic chemicals was considered a novelty and not well received. Because of the identification of increasing numbers of nongenotoxic carcinogens over the last 15 years, the possibility of induction of tumors by chemicals that do not react with DNA has become a reality and is now well recognized (*3–5*).

Nongenotoxic carcinogens induce tumors in different organs and tissues (*6*). Interestingly, the most often affected organ is the liver, and there are several examples of hepatocarcinogenesis models in rats and mice using dietary manipulation or chemicals of industrial and medicinal value (*5,7–9*). However, the nagging question that remains unanswered is by what mechanism(s) do these various compounds with different chemical and functional properties and with no DNA binding ability induce tumors? In this article an overview of current status of hepatocarcinogenesis induced by peroxisome proliferators, the prototype of nongenotoxic carcinogens, is presented. In addition, we briefly discuss the possible mechanisms involved in peroxisome proliferator-induced carcinogenesis. This subject is of considerable importance because peroxisome proliferators are nongenotoxic and elude detection by the available short-term tests and because, most importantly, the possible health hazards to humans stemming from exposure to peroxisome proliferators either through environmental contamination of plasticizers and herbicides or through therapeutic use of hypolipidemic drugs.

Peroxisome Proliferators

Peroxisomes are single membrane-bound cytoplasmic organelles with peroxidative functions. Peroxisomes are present in all mammalian cells except red blood cells. However, their number, size, and enzyme profile varies between different tissues (*10*). These organelles are found in large numbers in hepatocytes, followed by proximal tubular epithelial cells of the kidney (*7*). Peroxisomes can be readily induced to proliferate in the liver cells and to a lesser extent in the kidney cells by different chemicals which are designated as "peroxisome proliferators" (*11*). Peroxisome proliferators have a minimal or no effect on other tissues with reference to the induction of peroxisomes (*12*). The number of conditions that lead to peroxisome proliferation is rapidly increasing: these conditions include dietary factors, hormones, hypolipidemic compounds, phthalate ester plasticizers, trichloroethylene, and herbicides (*7,13–16*). The chemicals, with divergent structures and phar-

*Department of Pathology, Northwestern University Medical School, Chicago, IL 60611.
Address reprint requests to M. S. Rao, Department of Pathology, Northwestern University Medical School, Chicago, IL 60611.

macokinetic properties, induce similar types of qualitative changes in the liver cells. However, the quantitative response is quite variable, and the degree of peroxisome proliferation is dependent on the species and potency of the compound (*17–20*). The maximum response is observed in rats and mice. The following discussion, therefore, will be confined to these two species. The mechanism by which such a diverse group of chemicals induces such a singular response is not unequivocally established. Currently available data lead to the postulation of the existence of specific recognition molecules in responsive cells (*21,22*).

Biological Effects of Peroxisome Proliferators in Liver

Administration of peroxisome proliferators leads to predictable morphological and biochemical changes that can be characterized as early "adaptive" and late "carcinogenic" effects. During the adaptive phase there is hepatomegaly associated with peroxisome proliferation and induction of peroxisomal, endoplasmic reticulum, and cytosolic enzymes (*23*). Increase in liver weight and size is apparent within a few days of administration of peroxisome proliferator, reaching a steady state by 2 weeks. Hepatomegaly is secondary to hyperplasia and hypertrophy of hepatocytes (*7*). Hyperplasia of hepatocytes is short-lived, reaching maximum in a week followed by progressive decrease (*24,25*), whereas hypertrophy persists as long as the xenobiotic treatment is continued. Hypertrophy of hepatocytes is due to a marked increase in the cytoplasmic volume resulting mainly from proliferation of peroxisomes and to a lesser extent from smooth endoplasmic reticulum (*26*). Induction of peroxisomal enzymes begins within a few hours after administration of the peroxisome proliferator (*27*). However, the levels of increase of different enzymes is markedly variable. Catalase and urate oxidase activities are increased about 2-fold, whereas fatty acid β-oxidation enzyme system is increased 20- to 30-fold. Such a variation is dependent on the differential regulation of genes encoding peroxisomal enzymes (*11,27*). Peroxisome proliferators, in addition, also stimulate (laurate hydroxylase, epoxide hydrolase, carnitine palmitoil transferease) or inhibit (glutathione peroxidase, superoxide dismutase) the synthesis of other enzymes (*23,28,29*). Persistence of these alterations in the levels of various enzymes appears to contribute significantly to the development of tumors in the liver.

Hepatocarcinogenicity of Peroxisome Proliferators

The original description of the carcinogenic effect of nafenopin in mice by Reddy et al. in 1976 (*30*) was followed by several reports establishing several peroxisome proliferators as complete hepatocarcinogens in rats and mice (*7,20,31–38*). Chronic administration of peroxisome proliferators results in the appearance of liver lesions in a sequential fashion. Altered areas (AA) appear first, followed by neoplastic nodules (NN,) and finally hepatocellular carcinomas (HCC) develop. Morphological and phenotypic properties of these lesions are well characterized (*39–41*). In AA and NN there is increased cellularity, crowding of nuclei, and prominence of nucleoli. The cytoplasm of cells in AA and NN is eosinophilic and granular. HCC are usually well differentiated with a trabecular pattern. In 20 to 40% of the animals, HCC metastasize to lungs. AA, NN, and HCC induced by peroxisome proliferators do not express γ-glutamyltranspeptidase, glutathione *s*-transferase-P, and α-fetoprotein and are resistant to iron accumulation after overload. The lack of expression of γ-glutamyl transpeptidase and glutathione *s*-transferase-P is not due to drug toxicity or the presence of inactive protein, but is due to failure of derepression of genes encoding for these enzymes (*42*). The failure of γ-glutamyl transpeptidase expression is irreversible and cannot be altered by the administration of genotoxic carcinogens (*43*). Similar enzyme patterns are also reported in lesions initiated by diethylnitrosamine and promoted by peroxisome proliferators (*35,37*). In addition, HCC are low in the activities of drug metabolizing and detoxifying enzymes and epoxide hydrolase (*44*).

Initiation Versus Promotion by Peroxisome Proliferators

An issue that is often raised by some in regard to peroxisome proliferator-induced hepatocarcinogenesis is whether these compounds are true initiators or promoters, simply promoting the spontaneously initiated lesions. This concern is mainly based on two findings: lack of genotoxicity of peroxisome proliferators and the long latency period involved in the development of tumors. It is clear that there are several chemicals that react with DNA and lead to alterations (genotoxic carcinogens). However, one has to keep in mind that chemicals that are nongenotoxic, yet carcinogenic, can lead to DNA alterations through their biological effects. Even under normal physiological conditions there are cellular processes that are inherently mutagenic (*45*). The biological effects of nongenotoxic carcinogens can simply amplify the normally operative mutagenic mechanisms and overwhelm the protective defense mechanisms. This is particularly relevant in the case of peroxisome proliferators as they markedly enhance the activity of H_2O_2-generating enzymes.

The latency period for the development of HCC ranges from 50 to 120 weeks and is dependent on the type and dose of peroxisome proliferator used (*32,39*). With potent peroxisome proliferators such as ciprofibrate and Wy-14,643, tumors develop as early as 50 weeks. With weak peroxisome proliferators such as phthalate esters, tumors develop after 90 weeks. The extended period of time required for the appearance of tumors is not peculiar to peroxisome proliferators. Even with genotoxic carcinogens, the incidence and latency

period depends on the potency and the dose of the chemical (*46*). Carcinogenic potency between different genotoxic chemicals is variable by several orders of magnitude (*47,48*).

The results of a recent study clearly establish the role of peroxisome proliferators as initiators. Administration of ciprofibrate to 24-week-old and 1-year-old rats for 60 weeks resulted in an identical tumor incidence and number of tumors per liver (*49*). These findings negate the assumption that peroxisome proliferators are selective tumor promoters because of the absence of increased tumor incidence in 1-year-old rats. If peroxisome proliferators are only promoters of spontaneous lesions, a higher tumor incidence should have been observed in old rats because of expected increase in presumably spontaneously initiated lesions when compared to younger animals (*50*).

Mechanism of Carcinogenesis by Peroxisome Proliferators

Because of the nongenotoxic nature of peroxisome proliferators, it has been postulated that their carcinogenic effect may be dependent on two major biological effects, i.e., cell proliferation and peroxisome proliferation (*7,51,52*). Since the hepatic cell hyperplasia is present only during early adaptive state and decreases thereafter, it is unlikely to have a significant effect on the development of tumors at a much later time (*24,25*). In addition, the type of cell proliferation caused by peroxisome proliferators is a mitogenic response, where the cells are less susceptible to carcinogens, unlike cells dividing as a compensatory response (*53*).

The experimental evidence inculpating the role of peroxisome proliferation in hepatocarcinogenesis is certainly more convincing. Persistent peroxisome proliferation associated with a marked increase in H_2O_2 generating enzymes and decrease in free radical scavenging enzymes leads to oxidative stress (*7,27,28,54*). Increases in the levels of H_2O_2 and hydroxyl radicals in liver cells in peroxisome proliferator-treated rats and mice have been documented (*55,56*). Free oxygen radicals lead to alterations in DNA, lipid peroxidation in membranes, and critical sulfhydryl bonds in proteins (*57,58*). H_2O_2 was shown to induce DNA strand breaks in hepatocytes maintained in culture (*59*). Chronic administration of a peroxisome proliferator was also shown to lead to increased levels of 8-hydroxydeoxyguanosine, an indicator of free radical damage (*60,61*). Additional DNA alterations caused by peroxisome proliferators include reduction in the levels of I-compounds in the liver DNA (*61*). Decreased levels of I-compounds may play a significant role in the initiation phase of carcinogenesis.

With peroxisome proliferators there is a good correlation between the potency of peroxisome proliferation and hepatocarcinogenicity. Because of this strong association, and in the absence of any other reliable short-term tests, it is prudent to screen nongenotoxic chemicals for their ability to induce peroxisome proliferation either in *in vivo* or in cell culture systems.

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