Non-genotoxic hepatocarcinogenesis in vitro: the FaO hepatoma line responds to peroxisome proliferators and retains the ability to undergo apoptosis

Alison C. Bayly¹, Nicola J. French², Caroline Dive¹ and Ruth A. Roberts²,*

¹Molecular Pharmacology and Toxicology Laboratory, School of Biological Sciences, University of Manchester, Manchester M13 9PT, England
²Cell Biology Group, ICI Central Toxicology Laboratory, Alderley Park, Cheshire SK10 4TJ, England
*Author for correspondence

SUMMARY

A range of hepatoma cell lines (RH₁, HTC, FaO, 7800C₁ and MH₁C₁), has been studied with the aim of establishing an in vitro model to investigate the molecular mechanisms of hepatocarcinogenicity induced by the peroxisome proliferator class of non-genotoxic carcinogens. In view of speculation that peroxisome proliferators suppress hepatocyte apoptosis in vivo, we have placed particular emphasis on evaluating whether hepatoma cell lines retain the ability to undergo apoptotic cell death.

Expression of the liver-specific differentiation marker albumin and the peroxisome proliferator-activated receptor (PPAR) was highest in the Reuber hepatoma cell line, FaO. This cell line also demonstrated the most marked response to the peroxisome proliferator nafenopin with a 2.2-fold induction of the microsomal enzyme cytochrome p450IVa1. This response was found to display intercellular heterogeneity by immunocytochemistry. Thus, the FaO cell line maintained characteristics of hepatocytes, both in vivo and in vitro, in terms of expression of constitutive and inducible markers. However, none of the cell lines tested mirrored the hyperplastic response of hepatocytes to nafenopin, since no increase in cell growth kinetics was observed on addition of nafenopin to the growth medium.

The mode of cell death in confluent FaO cultures was characterised as apoptosis, by fluorescence microscopy and agarose gel electrophoresis of extracted DNA. Cells detaching from confluent FaO cultures exhibited chromatin condensation and DNA fragmentation patterns characteristic of cells undergoing apoptotic death. Interestingly, no apoptosis was seen in monolayer cells, suggesting that apoptosis in vitro is associated with cell shrinkage and detachment similar to that documented for the liver in vivo. The FaO cell line may be a useful tool to investigate the hypothesis that the suppression of apoptosis plays a crucial role in peroxisome proliferator-induced hepatocarcinogenesis.

Key words: peroxisome proliferator, non-genotoxic carcinogenesis, hepatocarcinogenesis, hepatoma cell lines, apoptosis

INTRODUCTION

Non-genotoxic carcinogens can cause tumours in experimental animals despite their apparent lack of genotoxic activity or potential (reviewed by Ashby and Tenant, 1991). The hypolipidaemic drug, nafenopin, is one of the peroxisome proliferator class of non-genotoxic carcinogens (Staubli et al., 1977), which cause an increase in the size and number of liver peroxisomes in rodents (reviewed by Reddy and Lalwani, 1983). Peroxisome proliferation is accompanied by liver enlargement and elevated transcription of genes encoding the microsomal enzyme p450IVa1 and the peroxisomal enzymes of β-oxidation, which include acyl-CoA oxidase and peroxisomal bifunctional enzyme (Lazarow and De Duve, 1976; Orton and Parker, 1982). The induction of these enzymes is commonly used as a marker for the biological effects of peroxisome proliferators.

It is well documented that chronic administration of peroxisome proliferators to rodents results in the formation of liver tumours (Reddy et al., 1980; Warren et al., 1980). Whereas the mechanisms of hepatocarcinogenesis have not been established, a number of theories have been proposed (reviewed by Green, 1991; Moody et al., 1991). Elevated levels of hydrogen peroxide produced as a result of peroxisome proliferation may initiate tumorigenesis by oxidative DNA damage (Reddy and Rao, 1989; Kasai et al., 1989; Lake et al., 1990), while peroxisome proliferators may also act as liver tumour promoters by stimulation of replicative DNA synthesis (Marsmann et al. 1988; Yeldandi et al.,...
1989; Kraupp-Grasl et al., 1991). Furthermore, the peroxisome proliferator-activated nuclear hormone receptor (PPAR) has been implicated in the mechanism of action of these carcinogens (Issemann and Green, 1990; Tugwood et al., 1992). In addition, peroxisome proliferators may act to inhibit the normal processes of apoptosis (also termed programmed cell death) in the liver (Schulte-Hermann et al., 1991). Withdrawal of peroxisome proliferators from the hyperplastic liver results in a rapid reduction in organ weight, caused by both a decrease in cell size and a reduction of cell numbers. There is evidence to suggest that this cell loss may occur via apoptosis (Bursch et al., 1984; reviewed by Bursch et al., 1992). However, conclusive evidence in support of this theory is proving elusive, since apoptosis is difficult to measure in vivo because of the short half-life of apoptotic cells (Bursch et al., 1990) and the small number of apoptotic cells (1-3%) present at any one time (Bursch et al., 1986). It also remains to be elucidated to what extent the molecular markers of apoptosis established in the immature rat thymocyte (Wyllie, 1980) are applicable to other tissues.

Present knowledge of the effects of peroxisome proliferators has been derived from both in vivo and in vitro studies using primary hepatocyte cultures (Bieri et al., 1984, 1990). However, primary cultures have limited use for detailed analysis of the molecular mechanisms of peroxisome proliferator-induced hepatocarcinogenicity. This is because viability and differentiated functions, such as the presence of liver-specific markers like albumin and the maintenance of a response to peroxisome proliferators, are maintained for only 2-3 days (Richardson et al., 1969). Generation of these primary cells is also time consuming. While cell lines may share some of these problems in part, they have the advantage of being readily available and may permit a greater diversity of experimentation. The identification of cell lines that retain a response to peroxisome proliferators would be a significant step forward in modelling the mechanism of action of the peroxisome proliferator class of non-genotoxic carcinogens.

The majority of liver cell lines that are available are derived from hepatomas and appear to maintain few functions characteristic of differentiated hepatocytes. Examples of such lines in common use are the hepatoma cell lines HTC, derived from the Morris hepatoma 7228C (Thompson et al., 1966), and JB1 (Manson et al., 1981). In contrast, the ‘minimum deviation’ hepatoma cell lines 7800C1 and MH1C1 from Morris hepatomas 7800C and 7795, respectively, and FaO from the Reuber H35-derived cell line H41IEC3, have been shown to secrete albumin and maintain a number of other hepatocyte-specific differentiation markers (Richardson et al., 1969; Wiebel et al., 1984; Snodgrass and Lin, 1987). 7800C1 has also been found to show some response to fatty acid peroxisome proliferators (Spydevold and Bremer, 1989).

We now present a detailed study of the response of this range of liver cell lines to the peroxisome proliferator nafenopin. Cell growth kinetics, maintenance of differentiated function, maintenance of a response to peroxisome proliferators and mode of cell death are all compared, to assess these cell lines for use in studies of the mechanism of hepatocellular carcinogenesis caused by this important class of non-genotoxic carcinogens.

**MATERIALS AND METHODS**

**Materials**

Nafenopin (2-methyl-2-[p-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxyl]propionic acid) was a gift from Ciba-Geigy, Basel, Switzerland. Trypsin was from Worthington, USA, and foetal bovine serum was from Techgen, UK. Acridine orange (AO) and ethidium bromide were purchased from Molecular Probes Inc., Oregon, USA. Peroxidase-labelled secondary antibodies were from DAKO Ltd., Denmark and the anti-albumin antibody was from Serotec, UK. The enhanced chemiluminescence (ECL) kit was purchased from Amersham, UK. All other chemicals were of analytical grade and were purchased from Sigma, UK, or from Aldridge, UK. All other tissue culture reagents were purchased from Sigma, UK, Flow, UK, or Gibco, UK. All plastics were obtained from Nunc, UK. 7800C1 and MH1C1 cells were a gift from Professor Gautvik, Institute of Medical Biochemistry, Oslo. FaO cells were a gift from Dr Mary Weiss, Pasteur Institute, Paris. HTC cells were purchased from Flow, UK, and RH1 cells were from the tissue culture cell bank at ICI, Alderley Park, UK.

**Culture of hepatoma cell lines**

RH1 cells were cultured in DMEM supplemented with 10% foetal bovine serum and HTC cells were cultured in the same medium further supplemented with 1% non-essential amino acids. JB1 cells were cultured in Williams E medium supplemented with 5% foetal calf serum. 7800C1 and FaO cells were both cultured in Ham’s modification of F12 medium supplemented with 15% horse serum and 2.5% foetal bovine serum or with 10% foetal bovine serum, respectively. MH1C1 cells were cultured in Ham’s F10 medium supplemented with 15% horse serum, 5% bovine serum. In addition, all culture media contained 2 mM L-glutamine, 100 units/ml penicillin and 100 units/ml streptomycin. Cell lines were routinely passaged at ratios of 1:3 to 1:10 twice a week and their medium was changed every 48 hours. All cell lines were screened for the presence of mycoplasma on a monthly basis and were found to be negative.

**Treatment of cells with nafenopin**

Cell lines were changed into complete medium containing 50 µM nafenopin from a 20 mM stock dissolved in dimethyl formamide (DMF) and incubated for the times indicated. Control flasks were treated with DMF to the same final concentration.

In the experiments to investigate the effects of nafenopin on apoptosis, 50 µM nafenopin was added as the FaO and HTC cells approached confluence.

**Growth response to nafenopin**

Cells were seeded in 4 ml medium at the densities indicated in 25 cm² tissue culture flasks. At 24 hours after seeding, cells were treated with nafenopin as described. At 24- to 48-hour intervals, cells were removed by trypsinisation, resuspended in a defined volume of medium, and counted using a haemocytometer. Cell viability was established by trypan blue exclusion.

**Analysis of nuclear morphology by acridine orange (AO) staining**

The nuclear morphology of attached and detached cells from a confluent monolayer was analysed by AO staining. Detached cells were collected 1-2 hours after addition of fresh medium to the flasks. Cells were pelleted by centrifugation at 150 g and resuspended in 100 µl medium. A 6 µl sample of cell suspension was
mixed on a slide with an equal volume of AO solution (10 µg/ml in phosphate buffered saline). Monolayer cells were stained similarly following removal from the culture flask by trypsinisation. Green fluorescence was detected between 500-525 nm using an Olympus microscope with fluorescence attachment. Cells with brightly staining condensed chromatin were classed as apoptotic (Dive et al., 1992) and expressed as a percentage of total cell number. Weakly staining cell ghosts were not counted. Images were recorded using a Biorad MRC 600 confocal microscope.

DNA agarose gel electrophoresis

The DNA from attached and detached cells was analysed by agarose gel electrophoresis. In summary, 10^6 cells were pelleted by centrifugation at 150 g. Pellets were resuspended in 20 µl of lysis buffer (10 mM ethylenediaminetetraamine (EDTA), 50 mM Tris-HCl, pH 8, containing 0.5% sodium lauryl sarcosinate and 0.5 mg/ml proteinase K). A 10 µl sample of 10 mM EDTA, pH 8, containing 1% low-gelling-temperature agarose melted at 70 °C was added before loading the samples onto a 2% agarose gel containing ethidium bromide (0.5 mg/ml). A phage φX174 DNA/HaeIII marker (fragment sizes 1353, 1078, 872, 603, 310, 276, 234, 194, 118 and 72 bp) was run adjacent to the samples. Samples were allowed to solidify for 5 minutes before the gel was flooded with Tris-phosphate-EDTA (TPE) running buffer. DNA was subjected to electrophoresis at 40 volts for 3 hours. The gel was then incubated for 3 hours with TPE running buffer containing 0.1 mg/ml RNase. DNA was visualised under UV illumination and gels were photographed on Kodak polaroid 667 film.

Western blotting

SDS-polyacrylamide gels were prepared according to the method of Laemmli and Favre (1973). A 50 µg sample of protein was loaded per lane on to 12.5% acrylamide gels with 3.5% stacking gels. Proteins were blotted onto nitrocellulose following the method of Towbin et al. (1979). Antibodies were localised using horseradish peroxidase-linked secondary antibodies and enhanced chemiluminescence.

Immunocytochemistry

Cells were fixed and permeabilised in ice-cold methanol prior to incubation with anti-albumin and anti-cytochrome p450IVA1 (Bains et al., 1985) antibodies. Primary antibodies were visualised using a peroxidase-linked secondary antibody and an amino ethyl carbazole (AEC) substrate. Counterstaining was achieved using Mayer’s haematoxylin.

RESULTS

Growth kinetics of cell lines

The growth curves in full (10%) serum conditions (Fig. 1) illustrate a progressive decrease in growth rate from RH1 (Fig. 1A) to MH1C1 (Fig. 1E), measured over a 12-day period. RH1 and HTC cells grew rapidly to confluence with doubling times of 16 and 24 hours, respectively. At confluence, the cells began to ‘pile up’ and then detached from the monolayer. FaO cells also had a doubling time of about 24 hours but, in contrast, detached from the monolayer before reaching confluence. 7800C1 and MH1C1 cells behaved quite differently with slower doubling times (36 hours). These cells became contact inhibited at confluence with little cell detachment at 12 days.

A reduction in the level of serum in the medium of FaO cells to 2% significantly depressed both the growth rate and the confluent density of the cells (P=0.002), where P=the probability of such a decrease happening by chance. (Fig. 2).

Effect of nafenopin on growth kinetics in full serum (10%)

The presence of 50 µM nafenopin in FaO, 7800C1 and MH1C1 culture medium had no apparent effect on the growth rate or the confluent density of the cells over a 12-day period in 10% serum (Fig. 1). In addition, the growth rate of solvent-treated control cultures remained unchanged. However, a slight decrease in the growth rate of HTC and RH1 cells treated with nafenopin was observed, although the difference was not found to be significant using the Student’s t-test.

Effect of nafenopin on the growth rate of FaO cells in reduced serum (2%)

The addition of 50 µM nafenopin to the culture medium of FaO cells growing in reduced (2%) serum levels had no
statistically significant effect on the growth rate or the confluent density of the cells over a 12-day period (Fig. 2).

**Maintenance of liver-specific markers**

Western blots showed that FaO, MH1C1 and 7800C1 express both albumin and PPAR (Fig. 3A and B, Table 1). Expression of both markers was highest in the FaO cells. Albumin expression was not detected in HTC cells, which also expressed only low levels of PPAR. RH1 cells did not express albumin (Table 1) or PPAR (data not shown). JB1 cells demonstrated the lowest expression of albumin (Fig. 3A) and were therefore excluded from further experiments. There was little or no increase in PPAR expression in any of the cell lines in response to nafenopin (Table 1). Further investigations using immunocytochemistry (Fig. 4) confirmed the presence of albumin in FaO cells, but again HTC cells gave a negative response.

**Maintenance of cytochrome p450IVA1 induction by nafenopin**

The presence of cytochrome p450IVA1, a marker associated with peroxisome proliferation, was monitored in each of the cell lines before and after treatment with nafenopin. RH1 and HTC cells showed no p450IVA1 expression (Fig. 3C, Table 1) whereas MH1C1, 7800C1 and FaO cells contained some endogenous p450IVA1. On treatment with nafenopin, expression of p450IVA1 increased markedly in FaO cells (2.2-fold), a phenomenon not observed in any of the other cell lines (Table 1). Immunocytochemistry supported these results (Fig. 4) and, in addition, demonstrated a heterogeneity of expression of p450IVA1 between neighbouring FaO cells.

**Cell death kinetics**

In all five cell lines the percentage of trypan blue-excluding cells present in both semi-confluent and confluent monolayers was always greater than 91%. However, cells were seen to detach from the monolayer as the culture approached confluence. In a sample of the cells that

![Fig. 2. Growth kinetics of FaO cells in 2% serum, treated with and without nafenopin.](image)

![Fig. 3. Expression of constitutive and inducible markers in hepatoma cell lines. (A) Constitutive albumin expression; (B) and (C) constitutive and nafenopin-induced PPAR and cytochrome p450IVA1 expression, respectively.](image)

<table>
<thead>
<tr>
<th>Table 1. Quantification of albumin (A), PPAR (B) and cytochrome p450IVA1 (C) expression in nafenopin-treated and control cells by laser densitometry of western blots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area under peak (AU2)</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>RH1</td>
</tr>
<tr>
<td>HTC</td>
</tr>
<tr>
<td>7800C1</td>
</tr>
<tr>
<td>MH1C1</td>
</tr>
<tr>
<td>FaO</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>HTC</td>
</tr>
<tr>
<td>7800C1</td>
</tr>
<tr>
<td>MH1C1</td>
</tr>
<tr>
<td>FaO</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>RH1</td>
</tr>
<tr>
<td>HTC</td>
</tr>
<tr>
<td>7800C1</td>
</tr>
<tr>
<td>MH1C1</td>
</tr>
<tr>
<td>FaO</td>
</tr>
</tbody>
</table>

The albumin standard (0.5 µg), the PPAR standard (absolute amount unknown) and the cytochrome p450IVA1 standard (1.5 pM) gave AU2 values of 1.36, 0.16 and 0.70, respectively.
detached over a 2-hour period, 54% of cells exhibited a pattern of chromatin condensation characteristic of apoptotic cells when stained with AO (Fig. 5) and 17% of cells included trypan blue (Table 2). The remainder of detached cells exhibited mitotic figures or were cell ghosts where the mode of cell death could not be established (data not shown). In HTC cultures, cell detachment and death occurred more rapidly than in FaO cultures. After 45 min-

Fig. 4. Immunocytochemical localisation of albumin and cytochrome p450IVA1 in FaO (A-E) and HTC (F-H) hepatoma cell lines. (A) and (F) control; (B) control, stained for albumin; (C) and (G) nafenopin-treated, stained for albumin; (D) control, stained for p450IVA1; (E) and (H) nafenopin treated, stained for p450IVA1. Bars, 40 µm.
utes, 53% of detached cells appeared apoptotic and 10% of cells included trypan blue (Table 2). The DNA isolated from the detached FaO and HTC cells exhibited the banding pattern or DNA ‘ladder’ characteristic of apoptosis when run on an electrophoretic agarose gel (Fig. 6). The DNA fragments consisted of multiples of approximately 180 base-pairs, indicative of the endonucleocytic cleavage at internucleosomal sites (Arends et al., 1991). The detached cells collected from FaO and HTC cultures after longer incubation times contained a higher proportion of cell ghosts and the DNA from these cell samples exhibited a smear typical of non-specific degradation.

**Cell death in the presence of nafenopin**

Preliminary results have shown that, under full serum conditions, the proportion of apoptotic cells detaching from confluent cultures is not affected by the presence of nafenopin (data not shown).

### DISCUSSION

The molecular mechanisms of non-genotoxic hepatocarcinogenesis are unclear and progress in their elucidation is severely hindered by a lack of appropriate in vitro models that can be readily manipulated. An ideal cell line for these purposes would exhibit markers of differentiated liver function, be responsive to peroxisome proliferators, retain the basic pathway for apoptosis and be amenable to experimentation. We have carried out a thorough characterisation of a range of hepatoma cell lines with a view to establishing a model system for studying the molecular mechanisms of the peroxisome proliferator class of non-genotoxic car-

---

**Table 2. Percentage of trypan blue positive and AO positive cells present in a sample of detached FaO and HTC cells**

<table>
<thead>
<tr>
<th></th>
<th>% Trypan blue positive (±s.d.)</th>
<th>% AO positive (±s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FaO</td>
<td>17 (9)</td>
<td>54 (8)</td>
</tr>
<tr>
<td>HTC</td>
<td>10 (2)</td>
<td>53 (5)</td>
</tr>
</tbody>
</table>

Values are means (±s.d.) of 3 counts made in each of 3 independent experiments. Detached cells were collected from confluent cultures between day 5 and day 10 after plating.

---

**Fig. 5.** FaO (A and B) and HTC (C and D) cells stained with acridine orange. Viable monolayer cells (A and C) and apoptotic detached cells (B and D) are shown. The diffuse staining patterns of the nuclei in the viable monolayer cells can be contrasted with the brightly staining, compact chromatin masses in the detached cells. Bars, 7.5 µm.

**Fig. 6.** Electrophoretic agarose gel containing DNA isolated from viable and apoptotic FaO (lanes 2 and 3) and HTC (lanes 4 and 5) cells. The high molecular weight DNA in monolayer cells (lanes 2 and 4) illustrates the viable nature of the cells. In contrast, DNA fragmentation patterns typical of apoptosis can be seen in DNA from detached cells (lanes 3 and 5). Lane 1 contains the DNA marker.
cinogens. Particular emphasis has been placed on modeling the putative role that peroxisome proliferators may play in suppressing apoptotic cell death in the liver. The five hepatoma cell lines studied varied widely in both growth rate and behaviour at confluence. The disparate growth rates appeared to be accompanied by variation in the differentiation status of the cell lines. The more rapidly growing cell lines RH1 and HTC did not express albumin and expressed only low levels of PPAR, whereas the slow-growing 7800C1 and MH1C1 cells expressed both of these markers of differentiated liver function. FaO cells displayed the most desirable characteristics, since they showed the strongest expression of the markers of differentiated function, albumin and PPAR, and had an intermediate growth rate.

The data demonstrate that the FaO and MH1C1 cell lines are both able to respond to the peroxisome proliferator nafenopin by induction of cytochrome p450IVA1, although the induction is strongest in the FaO line. Interestingly, these cells demonstrated a heterogeneity of response with variations in expression between neighbouring cells. This intercellular heterogeneity of cytochrome p450IVA1 induction is also seen in primary hepatocytes in culture (James et al., 1992) and in the liver in vivo, where levels are higher in centrilobular hepatocytes than in the perportal hepatocytes (Bars et al., 1989). Our data therefore show that FaO cells and to an extent MH1C1 cells have retained some ability to respond to peroxisome proliferators.

It has been established in vivo and in vitro that peroxisome proliferators stimulate cell division in hepatocytes (Bieri et al., 1984; Marsmann et al., 1988). However, no increase in growth rate of the cell lines studied here was seen in response to nafenopin, despite the ability of the some of these cell lines to respond to nafenopin as measured by the upregulation of inducible markers. This finding was confirmed even in the growth-retarded cells maintained in low serum. One explanation for this behaviour could be that the many different responses of a cell to peroxisome proliferators such as peroxisome proliferation, enzyme induction and mitosis do not share a common pathway and therefore can be segregated. If this is the case, these cell lines will enable us to dissect out those cellular responses to peroxisome proliferators that are relevant for mitosis, apoptosis and ultimately hepatocarcinogenesis.

The mechanisms by which peroxisome proliferators cause hepatocarcinogenesis have yet to be established. One proposal is that peroxisome proliferators suppress or delay the onset of apoptosis, which would otherwise result in the removal of damaged or potentially tumorigenic cells from the liver (reviewed by Bursch et al., 1992). This proposal arises because cells displaying morphology consistent with apoptosis are found in the liver during the involution of hyperplasia which follows the removal of liver mitogens such as phenobarbital (Bursch et al., 1984). However, corroborative evidence has yet to be found, since the detection of an internucleosomal cleavage DNA ‘ladder’ from hepatocytes in vivo and in vitro remains elusive. This has led some authors to propose that this ‘laddering’ does not occur in hepatocytes undergoing apoptotic death (Oberhammer et al., 1992). In contrast, DNA ladders have been obtained on treatment of hepatocytes in vitro with TNFα (Shinagawa et al., 1991) and menadione (McConkey et al., 1988).

A well differentiated liver cell line that could model hepatocyte cell death would be useful in assessing the hypothesis that peroxisome proliferators act to inhibit the normal processes of apoptosis in the liver. Our data demonstrate that the liver cell lines studied here retain the ability to undergo the active process of apoptosis as they reach confluence. In both the poorly differentiated HTC cell line and the well differentiated FaO line, we have confirmed the mode of cell death as apoptosis: condensed chromatin and internucleosomal DNA fragmentation were detected, both processes associated with apoptotic death (Wyllie, 1980). It is interesting to note that apoptosis was detected only in detached cells, an observation that may reflect the cell shrinkage and detachment documented during in vivo studies of apoptosis. In vivo, such apoptotic cells are phagocytosed by macrophages or neighbouring cells (Wyllie et al., 1980). However, in vitro, in the absence of such phagocytic cells, we have observed that detached apoptotic cells rapidly undergo a secondary degradation process, resulting in the loss of both chromatin condensation patterns and internucleosomal DNA fragments. Thus, detached cells collected over a time period exceeding 2 hours no longer display these characteristic features of apoptosis.

As yet, we have been unable to demonstrate a suppression of apoptosis by nafenopin in any of the liver cell lines examined. It is possible that the mechanism by which nafenopin removal is coupled to the engagement of a programmed cell death may be absent in these cells. However, these initial observations were made following brief exposure of cultures approaching confluence in full-serum conditions to nafenopin. Further experiments are required to evaluate fully the potential of nafenopin to suppress liver cell apoptosis in vitro. Specifically, we are currently investigating whether co-culture with nafenopin inhibits chemically induced liver cell death. Additionally, removal of nafenopin from cells that have been adapted to growth in this agent will permit assessment of whether a period of adaptation is required for dependence on nafenopin for cell survival. The latter would mirror the observation made by us and others (Muakkassah-Kelly et al., 1987, R. G. Bars and C. R. Elcombe, personal communication) that prolonged exposure of hepatocytes to nafenopin in culture enables their adaptation and survival but compound withdrawal results in rapid death.

A large number of hepatoma cell lines are currently employed for in vitro studies of liver function. Whereas these cell lines do not obviate the need for in vivo studies and the use of primary hepatocyte cultures, they provide useful complementary mechanistic information. Current investigations of the growth, kinetics and cell death response to non-genotoxic carcinogens would benefit from the availability of such a cell line model. Little work has been carried out to evaluate the usefulness of cell lines for this application. The 7800C1 cell line has been found, in previous studies, to show a response to fatty acid peroxisome proliferators (Spydevold and Bremer, 1989). It is also interesting that the Reuber hepatoma cell line H4IEC3,
REFERENCES


Staubli, W., Schweizer, W., Suter, J. and Weibel, E.R. (1977). The


