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Effect of dexamethasone, 2-bromopalmitate and clofibrate on L-FABP mediated hepatoma proliferation

G. Rajaraman and F. J. Burczynski

Abstract

Cytosolic liver fatty acid binding protein (L-FABP) is involved in many intracellular functions including cellular mitogenesis. We investigated the role of L-FABP and the plasma membrane liver fatty acid binding proteins (L-FABP$_{pm}$) in the modulation of hepatoma growth and proliferation, hypothesizing that agents that affect either the content of, or ligand binding to, L-FABP would affect hepatocellular mitogenesis. L-FABP expressing 1548-rat hepatoma cells were treated with 0.5 μM dexamethasone or 500 μM clofibrate for 4 days to downregulate and upregulate L-FABP expression, respectively. The competitive inhibitor 2-bromopalmitate (BrPA, 600 μM) was used to inhibit ligand binding to L-FABP. The peripherally present plasma membrane fatty acid transporter was inactivated by treating cells with 1:50 rabbit antisera (FABP-Ab) raised against L-FABP. Western blot analysis was used to monitor L-FABP levels while [^{3}H]-thymidine incorporation and growth curves were used to monitor hepatocellular proliferation. [^{3}H]-Palmitate clearance studies were performed using monolayer cultures. Palmitate clearance in dexamethasone-, BrPA- and FABP-Ab-treated cells was significantly reduced when compared with control ($P < 0.05$), while clofibrate treatment moderately increased the rate. [^{3}H]-Thymidine incorporation by dexamethasone- and BrPA-treated cells was significantly lower than control ($P < 0.05$), suggesting that hepatocellular proliferation was inhibited. Clofibrate treatment did not statistically affect growth rate. Lowering L-FABP using dexamethasone or interfering with its activity using BrPA significantly affected hepatocellular proliferation. This may be due to the non-availability of long-chain fatty acids or other intracellular mediators that are transported by L-FABP to the nucleus.

Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada

G. Rajaraman, F. J. Burczynski

Department of Pharmacology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada

F. J. Burczynski

Correspondence: F. J. Burczynski, Faculty of Pharmacy, University of Manitoba, 50 Sifton Road, Winnipeg, Manitoba, R3T 2N2, Canada.
E-mail: burczyn@cc.umanitoba.ca

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Introduction

The primary biological function of hepatic cytosolic fatty acid binding protein (L-FABP) is thought to be the binding and intracellular transport of long-chain fatty acids (LCFA) (Bass 1988; Kaikaus et al 1990; Paulussen & Veerkamp 1990; Glatz & van der Vusse 1996). Cellular uptake of LCFA is known to parallel L-FABP levels – an increased L-FABP level is associated with increased uptake and conversely a decreased level is associated with decreased uptake (Luxon & Weisiger 1993; Burczynski et al 1997, 1999; Milliano & Luxon 2001; Hung et al 2003). This 14 kDa protein, however, is also responsible for the translocation of many other hydrophobic ligands, including bilirubin, prostaglandins, squalene, etc. (Kaikaus et al 1990; Thumser et al 1994; Thompson et al 1997). Other ligands that bind to L-FABP include the genotoxic metabolites of aminoazo dyes (Ketterer et al 1976), peroxisome proliferators (Brandes et al 1990) and hydroxy and hydroperoxy metabolites of arachidonic acid (Raza et al 1989). Some mitogenic peroxisome proliferators, such as the amphipathic carboxylates and tetrazole-substituted acetophenones, specifically require L-FABP for their action. These peroxisome proliferators have been reported to enhance the multiplication of L-FABP expressing hepatoma cells but failed to induce growth in L-FABP non-expressing cells (Khan & Sorof 1994).

The notion that L-FABP is intricately involved in cellular mitotic activity is supported by studies showing markedly elevated levels in hepatocarcinogenesis or regeneration (Custer & Sorof 1984, 1985). In actively replicating cells mitotic activity typically occurs 6–8 h following DNA replication (Grisham 1962). This time frame parallels the major growth period of the liver. Results from our laboratory indicated that the

increased L-FABP level in regenerating cells following 70% partial hepatectomy coincided with DNA synthesis (Wang et al 2004). Thus, available evidence suggests that increased L-FABP level is associated with enhanced cell replication. L-FABP may, therefore, be one of the key factors responsible for the successful occurrence of hepatic regeneration and may indeed be a pivotal protein required for cell replication. Pharmacological agents that target L-FABP could potentially affect hepatocellular proliferation. Agents such as dexamethasone and 2-bromopalmitate that are known to respectively lower the content of, and ligand binding to, L-FABP might potentially suppress hepatoma proliferation and growth. Conversely, the peroxisome proliferator clofibrate would then be expected to enhance cellular replication as it increases L-FABP expression. However, whether very high L-FABP levels further enhance mitotic activity is not known. Although these agents possess other pharmacotoxicological effects independent of L-FABP, all evidence points to a common mechanism of their mitomodulatory action through L-FABP. Also investigated in this report was the role of the liver plasma membrane fatty acid transport proteins (L-$FABP_{pm}$) on mitotic activity.

Materials and Methods

[^{3}H]-Palmitic acid ([^{3}H]-PA, 56.5 Ci mmol^{-1}) and [^{3}H]-thymidine (~1 mCi) were purchased from New England Nuclear (Boston, MA). Electrophoresis reagents were purchased from FisherBiotech (Pittsburgh, MA) and trypsin-EDTA and 2-bromopalmitate (BrPA) were purchased from Gibco BRL (Burlington, ON) and Acros Organics (Ottawa, ON), respectively. All other chemicals, including bovine serum albumin (essentially fatty acid-free BSA), fetal bovine serum (FBS), fetal calf serum (FCS), penicillin–streptomycin (50 U mL^{-1}), dexamethasone, clofibrate and Dulbecco's modified essential medium (DMEM) were purchased from Sigma Chemical Co. (St Louis, MO). The aqueous buffer used throughout all experiments was phosphate-buffered saline (PBS), which had a composition of (in mM) 137 NaCl, 2.68 KCl, 1.65 KH_2PO_4 and 8.92 Na_2HPO_4, pH adjusted to 7.4 using 0.1 M NaOH.

Preparation of L-FABP antisera ($FABP_c$-Ab)

Studies were performed in accordance with the University of Manitoba Animal Care Committee guidelines. Details of the isolation and purification of rat liver L-FABP used in the preparation of L-FABP antisera were described previously (She et al 2002). Antisera was raised against rat liver L-FABP by intradermal injection of the purified antigen in Freund's complete adjuvant into female New Zealand White rabbits, followed by biweekly intramuscular booster injections of the antigen (Hung et al 2003). Rabbit plasma was stored frozen (−20°C) until required.

Cell culture and drug treatment

L-FABP expressing CRL-1548 hepatoma cells (derived from epithelial liver hepatoma of *Rattus norvegicus*, obtained from ATCC (Manassas, VA), were maintained in 25-cm^2 culture flasks supplemented with DMEM containing 10% FBS (DF-10), penicillin and streptomycin (50 U mL^{-1}) at 37°C in a humidified atmosphere of 5% CO_2. Dexamethasone (0.5 μM), clofibrate (500 μM), and BrPA (600 μM) were dissolved in dimethyl sulfoxide (DMSO) and later added to DF-10 media containing 300 μM BSA (DMSO < 0.1% v/v in final solution). Concentrations of the pharmacological interventions selected were based on the maximum effective dose above which these drugs affected cell adherence and viability. Control cells were incubated with DMSO (same final volume). Cells were supplemented with DF-10 media containing dexamethasone or clofibrate at the time of cell seeding and were exposed for 4 days to drug treatment. BrPA treatment (600 μM) and FABP-Ab treatment (1:50) of culture plates was performed 30 min before [^{3}H]-PA and [^{3}H]-thymidine uptake studies.

Measurement of cell growth

Hepatoma cells were seeded at 2.0×10^4 cells/well on 24-well plates as triplicate cultures. Cells were supplemented with DF-10 media containing 300 μM BSA with and without the pharmacological agent employed (dexamethasone, clofibrate or FABP-Ab). Cells were allowed to grow for 7 days in the presence of the pharmacological agents. At predetermined time intervals, cells were trypsinized, harvested and viable cells counted (as determined by trypan blue exclusion) in an inverted Nikon microscope using a haemocytometer. Control and treated cell viability were assessed to be more than 95%. A growth curve was generated by plotting cell number against time.

[^{3}H]-Thymidine incorporation assay

Sub-confluent (< 40%) 24-well plate cultures of 1548 cells in DF-10 (control and treated cells) were used for studying [^{3}H]-thymidine uptake. Briefly, 1 μCi of [^{3}H]-thymidine was added to each well and incubated for the final 3 h of the 48-h culture period. At the end of incubation, cells were rinsed twice with ice-cold PBS and 5% trichloroacetic acid was added to the plates and allowed to stand for 30 min. Cells were lysed using 500 μL of 4 M NaOH of which 400 μL was used in measuring [^{3}H]-thymidine incorporation with a Beckman LS6500TA liquid scintillation counter and 10 μL was used to measure protein content by the Bradford method (Bradford 1976). [^{3}H]-Thymidine incorporation by dexamethasone-, clofibrate- and FABP-Ab-treated cells was expressed as Treatment Index (TI), calculated as a percent ratio of total radioactivity obtained from treated cells (DPM_t) to control cells (DPM_c), adjusted for cellular protein content.

$$TI = (DPM_t/DPM_c) \times 100 \quad (1)$$

Western blot analysis

Cellular proteins were extracted and resolved on 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel and later transferred to nitrocellulose paper. Immunoblot analysis was carried out using polyclonal antisera raised

against L-FABP as described previously (Hung et al 2003). Western blot bands were quantitated by scanning densitometry and using Scion Image Software (Frederick, USA).

[^{3}H]-PA Clearance by 1548 cells

Manufacturer-supplied [^{3}H]-PA was further purified before use as described previously (Borgstrom 1952; Burczynski & Cai 1994). [^{3}H]-PA uptake was performed as described previously (Burczynski et al 1993). Cells grown in culture flasks were subcultured and seeded onto 24-well plates (3.0×10^5 cells/well). Dexamethasone- and clofibrate-treated cells (4-day treatment in presence of serum) were further incubated for 4 h in the presence of serum-free DMEM. Control experiments for FABP-Ab studies were performed by incubating cells with 1:50 rabbit serum. Before uptake, media was quickly aspirated, cells washed once with 37°C PBS and incubated with 100 μM BSA solution containing purified [^{3}H]-PA. At 30-s intervals, the incubation solution was quickly aspirated and uptake stopped by adding 0.5 mL ice-cold stop solution (PBS pH 7.4) to each well. After 10 s, the stop solution was quickly aspirated, washed once with ice-cold PBS and finally cells were incubated with lysis buffer (1% Triton buffer) for a further 30 min. The cell lysate was counted for radioactivity using a Beckman LS6500TA liquid scintillation counter. [^{3}H]-PA clearance was calculated as μL/10^6 cells/s and finally expressed as a percentage of control.

Statistical analyses

Data are presented as mean ± s.e.m. The n value refers to the number of culture plates or number of replicates performed for each study. Data were analysed using one-way analysis of variance with Dunnett's two-sided multiple comparison post-hoc test taking $P < 0.05$ as the level for significance. In growth curve plots, individual differences in cell numbers between various drug treatments were analysed using one-way analysis of variance with Dunnett's test (statistical significance set at $P < 0.05$).

Results

L-FABP levels

Western immunoblot was employed to assay the L-FABP levels in control and treated cells. Figure 1 shows that the L-FABP content between control and BrPA-treated cells were similar. The 4-day dexamethasone-treated cells, however, expressed a much lower amount of L-FABP compared with control (57 ± 3%; n = 4). Dexamethasone treatment has been shown to significantly reduce L-FABP levels both in-vivo and in primary hepatocyte culture in rats, which is likely secondary to changes in cellular lipid metabolism (Foucaud et al 1998). Conversely, clofibrate significantly increased the L-FABP content (187 ± 14%; n = 4) following the 4-day treatment period. As clofibrate is one of the potent peroxisome proliferators of its class (Goll et al 1999), the magnitude of increase in L-FABP on clofibrate treatment of cells is not surprising and similar to that reported by others (Bass et al 1989; Khan & Sorof 1994; Milliano & Luxon 2001). Clofibrate increases L-FABP levels by increasing the transcription rate of the L-FABP gene through activation of peroxisome proliferator activated receptor (PPARα) (Lee et al 1995).

Figure 1 Western blot analyses of L-FABP levels in CRL-1548 hepatoma cells. Equal amounts of protein obtained from control, 600 μM BrPA-treated, 0.5 μM dexamethasone (DEX)-treated and 500 μM clofibrate (CLO)-treated cells were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose paper. The obtained blot underwent treatment with primary and secondary L-FABP antibody. The antibody-bound L-FABP band was visualized using an enhanced chemiluminescence detection kit. Quantitative measurements of these bands were determined using Scion Image Software (Frederick, USA). BrPA treatment did not affect L-FABP levels, whereas dexamethasone and clofibrate treatment decreased (43%) and increased (187%) the levels, respectively.

Cell growth

In the presence of 0.5 μM dexamethasone, cell proliferation was significantly reduced by 37 ± 2% (n = 6; $P < 0.05$) at day 1 and 67 ± 1% at day 5 compared with control. The diminished growth rate was consistent with significantly reduced [^{3}H]-thymidine incorporation (Figure 2) (TI = 74 ± 1%; $P < 0.05$, n = 6). Figure 3 shows that 500 μM clofibrate did not alter hepatocellular growth rate. Growth curves revealed no statistical difference between clofibrate-treated cells and control cells. The [^{3}H]-thymidine incorporation assay complemented the observed

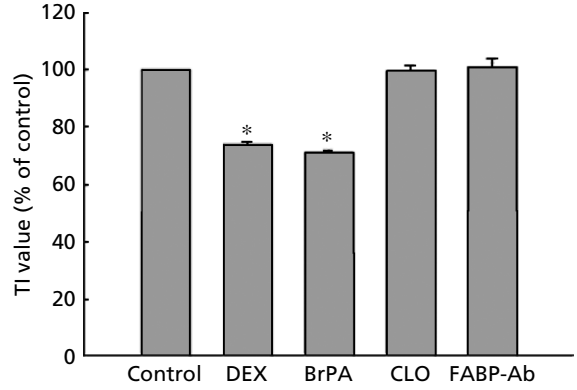

Figure 2 [^{3}H]-Thymidine incorporation values of dexamethasone (DEX)-, BrPA-, clofibrate (CLO)-, and FABP-Ab-treated CRL-1548 hepatoma cells expressed as a Treatment Index (TI, % of control). While BrPA and dexamethasone treatment of cells resulted in statistically lower TI values (*$P < 0.05$), clofibrate- and FABP-Ab-treated cells were not different from control. Data are mean ± s.e.m., n = 6.

growth curves and the TI value of clofibrate treatment ($100 \pm 2\%$, $n = 6$) was not significantly different from control (Figure 2). The [^{3}H]-thymidine incorporation of BrPA-treated cells ($TI = 71 \pm 1\%$, $n = 6$) was significantly reduced ($P < 0.05$) from control (Figure 2), suggesting that hepatocellular proliferation was affected when binding of LCFA to L-FABP was inhibited. Thus, the role of L-FABP in hepatocellular mitogenesis appears to be mediated through its ability to bind and transport LCFA to target sites.

It is known that polyclonal antibody raised against L-FABP binds to $FABP_{pm}$ (Stremmel et al 1985a, b; Schwieterman et al 1988; Paulussen & Veerkamp 1990; Hung et al 2003). Therefore, we employed a rabbit antisera (FABP-Ab) raised against L-FABP (1:50) to irreversibly bind and inactivate $FABP_{pm}$. Both the growth plot ($\leq$4 days and day 7) and the [^{3}H]-thymidine incorporation assay ($TI = 101 \pm 2\%$, $n = 6$) showed that FABP-Ab treatment did not affect hepatoma proliferation (Figures 2 and 3). Although some data points showed a statistically lower cell number in FABP-Ab-treated cells (4–6 days) compared with control, this difference was not thought to be of physiological importance. Also, the effect of dexamethasone on hepatoma proliferation appears to be mediated through L-FABP as the results from FABP-Ab treatment rule out the possibility that the reduced cell proliferation might have been mediated through plasma membrane fatty acid binding proteins.

Preliminary analysis showed that the supernatant collected from dexamethasone-treated cells did not contain any non-viable cells, as visualised by trypan blue exclusion or viable non-adhered cells. This observation eliminated the possibility that the observed decrease in cell proliferation was due to cell death caused by dexamethasone treatment or that dexamethasone reduced the adherence of cells to the culture plate. As treatment (>4 h) with the competitive inhibitor BrPA interfered with the attachment of cells to the culture plate, we could not generate growth curves for BrPA treatment. Lowering the dose of BrPA or adding BrPA to the already adhered cells only resulted in a delayed detachment of cells on longer exposure times. Analysis of the supernatant (from culture plates) with trypan blue showed that the detached cells were viable ($>95\%$). Hence, the observed effect of BrPA appeared to be independent of dose and was consistent with other investigators who reported reduced adherence to culture plates of cells with BrPA treatment (Luxon 1996). Therefore, it became necessary to perform a [^{3}H]-thymidine incorporation assay as the procedure required shorter exposure times (<4 h) (Figure 2).

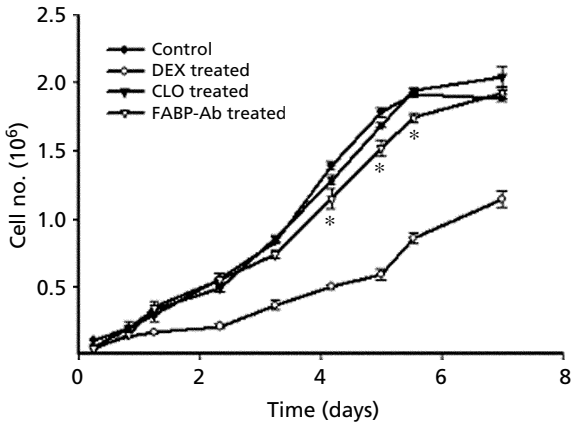

Figure 3 Growth curve plots were generated by plotting cell number (millions) vs time (days). Cells (CRL-1548 hepatoma cell line) were seeded at 20 000 cells/well on 24-well plates with or without dexamethasone (DEX), clofibrate (CLO), or FABP-Ab. Shown are changes in cell number from days 1–7. At frequent time intervals, cells were trypsinized and counted using a dye-exclusion haemocytometer procedure. Data show that hepatoma proliferation was significantly inhibited at all time points with dexamethasone treatment compared with control ($P < 0.05$) with the exception of the 6-h time point. Overall, FABP-Ab treatment did not affect cell proliferation except at the data points indicated (*$P < 0.05$). Clofibrate treatment did not differ from control. Each data point represents mean $\pm$ s.e.m., $n = 6$.

[^{3}H]-PA clearance

Figure 4 shows the 1548 hepatoma [^{3}H]-PA clearance (nL/10^6 cells/s) expressed as a percent ratio of treated cells to control cells. The reduced L-FABP level in dexamethasone-treated cells was associated with a significant reduction in [^{3}H]-PA clearance ($43 \pm 16\%$, $n = 6$) compared with control ($P < 0.05$). There was only a moderate increase in [^{3}H]-PA clearance by clofibrate-treated cells ($140 \pm 20\%$; $P > 0.05$, $n = 9$) over control cells. The considerable increase in L-FABP level did not result in significantly higher [^{3}H]-PA clearance compared with control, consistent with the indifference in cellular proliferation between clofibrate-treated and control cells. There was also a significant reduction in [^{3}H]-PA clearance ($29 \pm 3\%$, $n = 7$) by BrPA-treated cells compared with control ($P < 0.05$), consistent with previous observations (Luxon 1996). A 30-min FABP-Ab treatment significantly reduced ($54 \pm 2\%$, $n = 6$) [^{3}H]-PA

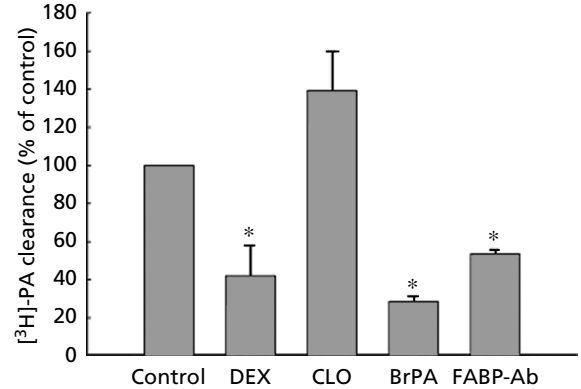

Figure 4 Purified [^{3}H]-PA clearance rate at 100 μM BSA by dexamethasone (DEX)-, BrPA-, clofibrate (CLO)-, and FABP-Ab-treated CRL-1548 hepatoma cells compared with control. While dexamethasone, BrPA and FABP-Ab reduced [^{3}H]-PA clearance significantly (*$P < 0.05$), clofibrate moderately increased the clearance rate. Data are mean $\pm$ s.e.m., $n = 6$.

clearance by hepatoma cells compared with control, consistent with observations made by other researchers (Stremmel et al 1985b; Berk et al 1990).

Discussion

The role of L-FABP in hepatocellular growth and differentiation has been investigated using transfected L-FABP expressing and L-FABP non-expressing hepatoma cells (Keler et al 1992; Keler & Sorof 1993). Antisense oligodeoxynucelotides directed towards L-FABP reduced cellular proliferation rates and increased apoptosis (Das et al 2001). Such studies implicate L-FABP as a mediator of cellular mitogenesis but also raise questions on the effect of decreasing or increasing L-FABP levels on cellular proliferation.

In this study, we showed that dexamethasone treatment (0.5 μM) of 1548 cells resulted in a 43% decrease in L-FABP level, consistent with Foucaud et al (1998) who also reported a two-fold decrease in L-FABP levels following dexamethasone treatment of rats. The dexamethasone-mediated L-FABP down-regulation was likely due to secondary changes in cellular lipid metabolism rather than a direct endocrine effect. Presence of dexamethasone in the culture media also was shown to inhibit the proliferation rate of 1548 cells (Figure 3) by inhibiting S-phase entry of cells, as was evident from [^{3}H]-thymidine incorporation data (Figure 2). The mitoinhibitory action of dexamethasone is consonant with previous reports of dexamethasone-mediated inhibition of DNA synthesis in rat hepatocyte cultures and fetal rat livers (Klepac 1983; Vintermyr & Doskeland 1989). Such an action of dexamethasone is primarily thought to be mediated through its inhibitory effect on pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), that are necessary for normal hepatocyte proliferation (Fausto et al 1995; Debonera et al 2003). Studies reported by Lawrence and colleagues show that dexamethasone administration inhibited the peroxisome proliferator WY-14,643-induced hepatocyte proliferation (Lawrence et al 2001b) and that TNF-α was not required for WY-14,643-induced cell proliferation (Lawrence et al 2001a). Those results suggested that peroxisome proliferators such as amphipathic carboxylates and dexamethasone affect hepatic mitogenic response through a common molecular target. L-FABP may be that target as it is differentially regulated by both drugs. In our study, cellular [^{3}H]-PA clearance of dexamethasone-treated cells showed a 57% reduction compared with control (Figure 4). The reduced clearance corresponded to decreased L-FABP expression. As L-FABP plays a crucial role in the intracellular partitioning of LCFA between oxidation and esterfication thereby aiding in fatty acid metabolism (Mahadevan & Sauer 1971; Ockner & Manning 1976; Lands 1979; Hagve & Christophersen 1986), decreasing L-FABP levels would ultimately result in the reduction of LCFA intake and metabolism. Thus, dexamethasone appeared to slow down hepatoma proliferation by decreasing the L-FABP content, leading to a reduced cellular ability to target LCFA and other intracellular ligands to target sites. This may explain how dexamethasone co-administration selectively inhibited WY 16,463-induced cell proliferation in mice (Lawrence et al 2001b).

The 40 kDa plasma membrane FABP ($FABP_{pm}$) present on the extracellular side of the hepatocyte cell surface is believed to play an important role in the transmembrane flux of LCFA (Glatz & van der Vusse 1996; Glatz et al 2001). $FABP_{pm}$ is thought to act as a translocase or flippase, transporting the fatty acid to the cytoplasmic leaflet of the membrane. We do not know if dexamethasone also down-regulates $FABP_{pm}$ or affects its action. Thus, we used rabbit antisera that was raised against L-FABP to investigate whether $FABP_{pm}$ has any role in modulating cellular mitogenesis. Although FABP-Ab treatment reduced [^{3}H]-PA clearance by about 46% (Figure 4), it did not affect hepatoma proliferation as was evident from the unchanged growth curves ($\leq$4 days and day 7) and [^{3}H]-thymidine incorporation TI values (Figures 2 and 3). These findings indicated that a decrease in the intracellular LCFA transport to different target sites (mitochondria or peroxisome) is likely responsible for the inhibition of hepatocellular proliferation. Results from our study, therefore, lead us to believe that $FABP_{pm}$ does not play a significant role in hepatoma proliferation.

The brominated palmitic acid analogue BrPA was chosen as an inhibitor of LCFA binding to L-FABP. BrPA cannot be esterified to triglycerides and does not undergo oxidation by mitochondria (Goresky et al 1978; Luxon 1996). This ligand competitively binds to cytoplasmic L-FABP at the same ligand binding site as that of other LCFA (Oakes et al 1999). This effectively decreases the protein's ability to bind ligands and subsequently inhibits their transport to target sites. Previous work showed the rate of fluorescent 12-*N*-methyl-(7-nitrobenz-2-oxa-1,3-diazol) aminostearate (NBDS) cytoplasmic diffusion was reduced when binding to L-FABP was inhibited using BrPA (Luxon 1996). In this study, we showed a 71% decrease in cellular [^{3}H]-PA clearance (Figure 4) with BrPA treatment. This was consistent with the view that BrPA occupies palmitate's ligand binding site on L-FABP, decreasing the net cytoplasmic transport rate of palmitate. Consequently less palmitate is taken up by cells leading to a decrease in fatty acid metabolism with correspondingly lower acyl-CoA production. This may become extremely important if cellular uptake and metabolism of an essential fatty acid is altered. Those ligands are very much a requirement for normal cellular growth and proliferation. BrPA has been reported to reduce the rate of linoleic acid oxidation by 60–70% and increase the amount of free fatty acid recovered (Hagve & Christophersen 1987). In this study, the DMEM culture media contained 0.15 μM linoleic acid. The presence of 600 μM BrPA would be expected to inhibit the binding of linoleic acid to, and displacement of, the essential fatty acid from the L-FABP binding site. Thus, BrPA treatment would effectively reduce both linoleic acid intake and metabolism, affecting normal hepatocellular growth and proliferation. These views parallel our finding that [^{3}H]-thymidine incorporation of BrPA-treated cells was statistically lower than control cells (Figure 2), suggesting

that hepatoma proliferation was affected by BrPA treatment. Thus, BrPA interfered with LCFA uptake and transport by binding to L-FABP, leading to hepatoma mitoinhibition. Moreover, the argument that dexamethasone may affect hepatoma proliferation by altering cellular lipid metabolism independent of L-FABP levels is not supported by the action of BrPA, as this ligand specifically targets L-FABP.

The considerable increase in L-FABP level (Figure 1) caused by clofibrate treatment did not parallel the moderate increase (40%) in [^{3}H]-PA clearance (Figure 4). This finding may be explained by understanding the influx processes – one being a saturable component through a plasma membrane transporter (such as $FABP_{pm}$), the other being a nonsaturable diffusive component. The L-FABP antisera used in this study inactivates the saturable component without affecting the diffusive component. Interestingly, the [^{3}H]-thymidine incorporation assay (Figure 2) and growth curve plots (Figure 3) showed that hepatoma proliferation was unaffected by clofibrate treatment. In the presence of 10% FBS, hepatoma cells might already be multiplying at a maximal rate and hence clofibrate-mediated L-FABP up-regulation beyond basal expression levels in a hepatoma cell line did not further increase cell proliferation.

In summary, reducing the L-FABP level or function through pharmacological intervention was associated with reduced cellular replication. We speculate that L-FABP channels LCFA (and other intracellular ligands) to various target sites. Channelling of ligands appears to be crucial for normal cell growth and proliferation. Interfering with the activity of such a transporter may be expected to affect proliferation as is the case with dexamethasone and BrPA. Though clofibrate increases the intracellular level of L-FABP, saturation of the target site (e.g. efficiency of mitochondria or peroxisomes in processing the LCFA) may then explain the indifference in mitotic activity.

Conclusion

The results lead us to accept the hypothesis that decreasing L-FABP content or decreasing its ability to bind and transport LCFA reduces hepatocyte mitotic activity. Pharmacological intervention using dexamethasone or BrPA resulted in a significant reduction in hepatoma proliferation. Intervention with clofibrate did not alter the hepatoma mitogenic activity though it significantly increased L-FABP levels from basal expressive levels. Pharmacological treatment aimed at reducing L-FABP expression or function reduces cell proliferation. It appears that L-FABP warrants further investigation as a potential pharmacological target in understanding hepatocellular carcinogenesis.

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