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Celecoxib, a cyclooxygenase-2 inhibitor, prevents induction of liver preneoplastic lesions in rats☆

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***Background/Aims*: Several studies suggest that cyclooxigenase-2 (COX-2) inhibitors are chemopreventive agents against colon, breast and skin cancer. In this study, we evaluated the chemopreventive effect of celecoxib, a specific COX-2 inhibitor, on the development of liver preneoplastic lesions in rats.**

***Methods*: Male Sprague–Dawley rats were fed during 5 weeks either a control or an experimental diet containing 1500 ppm celecoxib on a medium-term hepatocarcinogenesis protocol. Livers were collected and evaluated by histological and biochemical assays.**

***Results*: A reduction by 80 and 90% both in the number and size of altered hepatic foci was observed in the group treated with celecoxib during hepatocarcinogenesis treatment, respectively. No evidence of apoptosis was observed in our present study, however, the expression of the proliferation markers such as PCNA and Ki-67 was drastically reduced. Interestingly, neither COX-2 expression nor prostaglandin-E_2 (PGE_2) production were altered by the hepatocarcinogenic treatment or celecoxib treatment. Finally, celecoxib inhibited the translocation of Rel A/p65 to the nucleus with significant effect on stability of the repressor IκB-α.**

***Conclusions*: This is the first demonstration that a specific COX-2 inhibitor, celecoxib, possesses striking chemopreventive activity, inhibiting preneoplastic lesions during hepatocarcinogenesis in vivo, suggesting that celecoxib effects are mediated by PGE_2-independent mechanisms.**

***Keywords*: Selective cyclooxygenase-2 inhibitor; Chemoprevention; Hepatocarcinogenesis**

1. Introduction

Primary liver cancer is the fifth most common cancer in the world and the fourth most common cause of cancer mortality. Therapeutic possibilities are limited and prognosis is usually poor. Chronic cytotoxic and inflammatory events favour the development of this disease, indicating that inflammation mediators, such as prostaglandins (PGs), are involved in its pathogenesis [1]. PGs are produced from arachidonic acid by either of two enzymes: COX-1 and COX-2. COX-1 is constitutively expressed in most tissues, and this isoenzyme is important for the maintenance of homeostatic functions. COX-2 is an inducible isoenzyme, recently associated with cell proliferation, angiogenesis,

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Abbreviations: 2-AAF, 2-acetylaminofluorene; COX, cyclooxygenase; DEN, diethylnitrosamine; GGT, (-glutamyltranspeptidase; GST-P, glutathione S-transferase placental form; HPT, hepatocarcinogenic treatment; NT, non-treated; NSAID, non-steroidal anti-inflammatory drugs; PARP, poly(ADP-ribose)polymerases; PCNA, proliferating cell nuclear antigen; PG_S, prostaglandins.

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invasiveness and resistance to apoptosis; all these processes are involved in tumorigenesis [2,3]. A number of preclinical studies have suggested that COX-2 inhibitors such as celecoxib have anticancer effects in animal models of colon, urinary bladder, skin, and breast [4–7]. Furthermore, in patients with familial adenomatous polyposis, the daily administration of 800 mg of celecoxib reduces the number of colorectal polyps [8]. Although the chemopreventive efficacy of this drug has been demonstrated in some types of cancer, its mechanisms of action need to be clarified. Available data have indicated that COX-2 inhibitors prevent cancer through inhibition of cell proliferation and angiogenesis, and induction of apoptosis [9,10]. Nevertheless, interpretation of the celecoxib-mediated chemopreventive effect is complicated by the presence of COX-2-independent mechanisms [11,12] or the involvement of COX-2 inhibitors with other molecular targets such as NF-κB, which is highly linked to the carcinogenesis process [13].

Non-specific inhibitors of COX-1 and COX-2 have been shown to produce side effects, such as gastrointestinal bleeding and ulceration, while the specificity of celecoxib (SC-58635) as COX-2 inhibitor makes it less toxic for gastrointestinal tract [14]. This feature has added to the celecoxib anticancer effects shown in several animal models, suggesting that it is a good chemopreventive candidate whose attributes should be explored in other tumours. In this context, our study was aimed to: (1) determine whether celecoxib exerts a chemopreventive effect on hepatic preneoplastic lesions induced by DEN, 2-AAF and partial hepatectomy; (2) investigate the role of celecoxib in cell proliferation and apoptosis in in vivo hepatocarcinogenesis; (3) examine the participation of COX-2 and NF-κB in this medium-term rat hepatocarcinogenesis assay and the effect of celecoxib on these molecular targets.

2. Materials and methods

2.1. Chemicals and reagents

DEN and 2-AAF were purchased from Sigma Chemical Co (St Louis, MO). Electrophoresis reagents were from Bio-Rad (Hercules, CA). Antibodies were from Santa Cruz Biotech (Santa Cruz, CA) and Dako Corporation (Carpinteria, CA).

2.2. Experimental diet and treatments

Celecoxib was extracted from the commercial drug Celebrex to purity above 99% determined by Nuclear Magnetic Resonance (NMR) in the Chemistry Department at CINVESTAV (Mexico City). The experimental diet was prepared with Purina Test Diet, US and included 1500 mg of celecoxib/kg of food (1500 ppm).

Six-week-old male Sprague–Dawley rats were purchased from Harlan Industries (Mexico City) and were randomly divided into three treatment groups following the scheme depicted in Fig. 1.

The HPT group and the HPT plus celecoxib group received the hepatocarcinogenic treatment of the Semple and Roberts modified model [15]. Serum samples were taken from the caudal artery of each animal weekly and tested for celecoxib levels using high-performance liquid chromatography (HPLC). All animal experiments were performed according to the guidelines and with approval of the local institutional animal care committee.

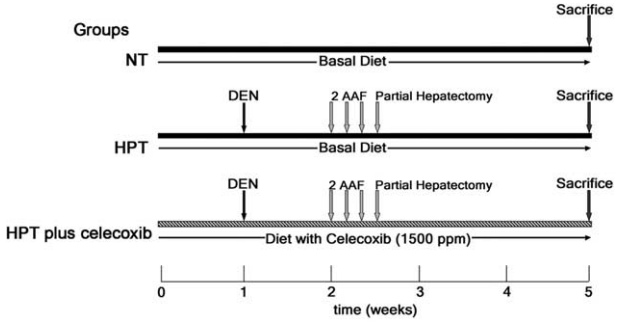

Fig. 1. Celecoxib administration in the hepatocarcinogenesis model. Group NT; rats maintained on basal diet ($n=5$). Group HPT; treatment was initiated with 150 mg/kg of DEN i.p.; at days 7, 8 and 9, 2-AAF was administered by gavage at doses of 20 mg/(kg/day), and partial hepatectomy was performed on day 10. Group HPT plus celecoxib; celecoxib was administered 7 days before DEN administration until sacrifice. All animals were sacrificed 5 weeks after the experiment was initiated.

2.3. Histology analysis

For histopathological evaluation, liver tissues were fixed in 10% buffered formalin and embedded in paraffin blocks. Then 5 μm thick sections were cut and processed by routine histological methods with H&E staining.

2.4. GGT histochemical staining

GGT activity was measured as described by Rutenburg [16]. Briefly, the histological liver sections of 15 μm were fixed in absolute ethanol during 5 min at 4 °C; next, γ-glutamyl-4-methoxy-2-naphthylamine, glycyl-glycine and 4-benzoylamino-2, 5-diethoxybenzene-diazonium chloride hemi(zinc chloride(salt were added during 30 min; then, cupric sulphate was added for 2 min. Finally, the GGT positive foci were quantified using an image analysis software (AnalySIS Soft Imaging System GmbH).

2.5. Immunoblotting

Microsomal, cytosolic and nuclear extracts were prepared as described previously [17–19]. The extracts were resolved on SDS-PAGE gels under denaturing and reducing conditions. After electrophoresis, the proteins were transferred to a nitrocellulose membrane and incubated with the indicated antibodies. The membranes were developed using a chemiluminescence system. Protein levels were quantified by densitometric analysis using a Gel Analysis Software (SIGMA GEL.LNK).

2.6. Tunel assay and immunohistochemical analysis

Apoptosis was determined by the Colorimetric TUNEL System (Promega Corporation) and the in situ Cell Death Detection Kit, Fluorescein (Roche Molecular Biochemicals) according to the manufacturer's protocols. Immunostaining was performed using the LSAB Plus-kit applied according to the manufacturer's instructions.

2.7. DNA gel electrophoresis

DNA was extracted using DNAzol® Reagent (Invitrogen Corporation, MI) following the manufacturer's protocol. The amount of DNA was

determined by spectrophotometric measurement. Each DNA sample was analyzed on a 1.5% agarose gel, stained with ethidium bromide and photographed under ultraviolet light.

2.8. PGE_2 measurement

To determine basal PGE_2 levels, 50 mg of frozen tissues were homogenized at ice temperature with a microtube pestel and vortexed thoroughly for 2 min in 0.25 ml of 0.1 M Tris–HCl buffer containing 5.6 μM indomethacin and 15% methanol. The mixture was centrifuged for 10 min at 400*g*. The quantity of PGE_2 in supernatants was immediately determined with the PGE_2 Monoclonal Enzyme Immunoassay Kit (Cayman Chemical), according to the manufacturer's instructions.

2.9. Statistical analysis

Data are expressed as mean ± SD. All experiments were carried out in at least with five animals per treatment group. Statistical significance between groups was determined by Student's *t*-test. *P* value of <0.05 was considered statistically significant.

3. Results

3.1. General observations

Average body weight of animals treated with vehicle or carcinogenic treatment and fed the control diet or celecoxib remained similar throughout the experiment (data not shown).

3.2. Plasma celecoxib levels

To determine whether celecoxib reaches seric levels similar to the chemopreventive levels previously reported in other models of carcinogenesis [20,21], celecoxib was determined weekly during this medium-term rat hepatocarcinogenesis assay by HPLC. Drug levels were: after 1 week 1.32 ± 0.15 μg/mL, on the second week 3.33 ± 0.39 μg/mL, the highest level of 8.82 ± 0.78 μg/mL was found on the third week after celecoxib administration, which correlates with the post-hepatectomy stage; the fourth week, one day before sacrifice, the celecoxib serum level was 5.76 ± 0.68 μg/mL. In conclusion, at the end of the experiment our animals showed celecoxib serum levels similar to those required for chemoprevention of mammary (5.1 μg/mL) and colon carcinogenesis (4.29 μg/mL) [20,21].

3.3. Prevention of preneoplastic lesions by celecoxib

Celecoxib was tested for its chemopreventive effect in the hepatocarcinogenesis assay, induced by DEN as initiator and 2-AAF as promoter (Fig. 1). The microscopic findings in the liver of the HPT plus celecoxib group showed a decrease of 85% in the number of preneoplastic nodules as compared with the HPT group. Moreover, in this latter group of rats, sacrificed 5 weeks after the experiment was initiated, the nodules were larger and caused distortion of the hepatic architecture (Fig. 2B). No differences in liver

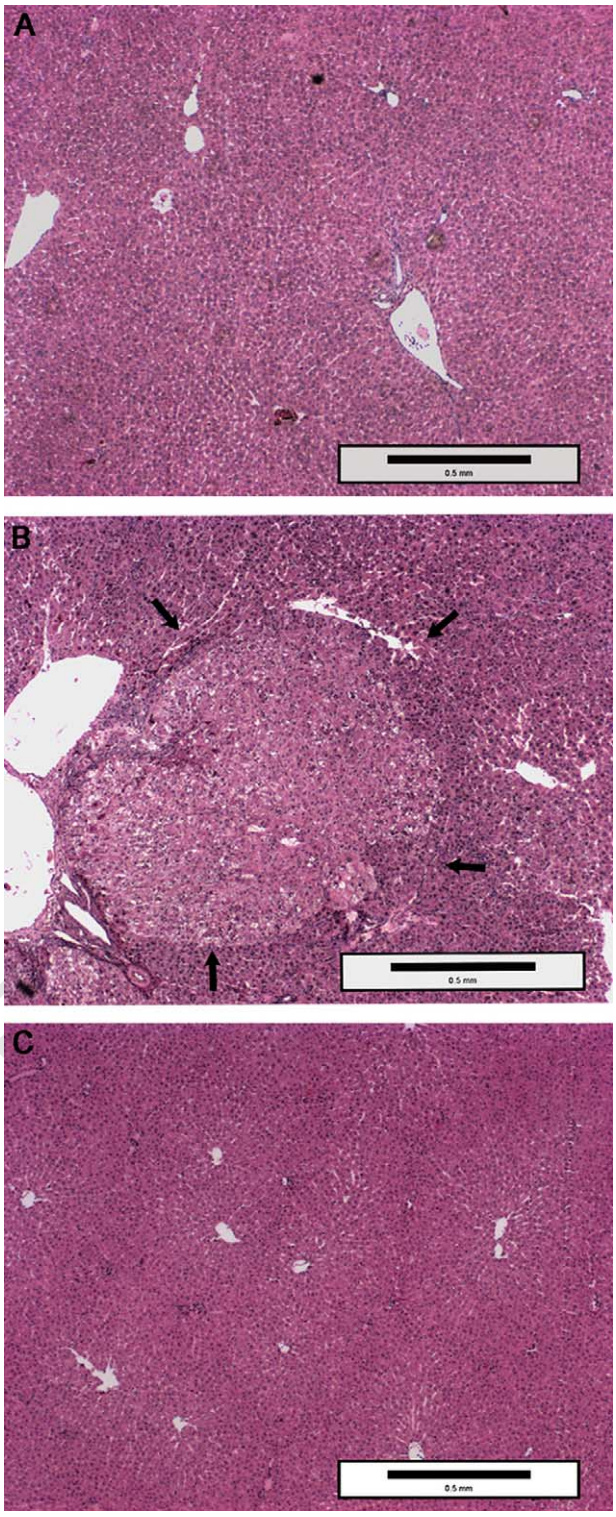

Fig. 2. Representative histological sections from livers of rats sacrificed 5 weeks after experiment initiation. NT (A), HPT (B) and HPT plus celecoxib (C). (A) Note the normal architecture of liver. (B) A distorted general appearance of tissue architecture. Nodules are distinguished, which show cells with larger cytoplasm and more eosinophils than cells in non-nodular tissue. Arrows show the periphery of the nodule compressing the non-nodular tissue. (C) Note that, in spite of HPT, the architectural framework is preserved. Photographs were taken at 40-fold magnification.

tissue architecture were observed in the NT and HPT plus celecoxib groups (Fig. 2A and C). Additionally, the chemopreventive effect of celecoxib on preneoplastic lesion development was quantified by measuring preneoplastic lesions with two markers, GGT and GST-P; both markers are considered the most sensitive for detection of altered hepatic foci induced by most chemicals [22]. In the HPT plus celecoxib group, both number and area of GGT-positive liver foci were significantly reduced by 80% ($P \leq 0.0001$) and 90% ($P \leq 0.001$), respectively, compared with the HPT group (Fig. 3). There were no GGT-positive liver foci in the NT group, using a detection parameter for focus size larger than 0.01 mm^2. Rat GST-P expression is minor in the normal liver but high in hyperplastic nodules and in hepatocellular carcinomas during chemical hepatocarcinogenesis [23]. GST-P expression was analyzed by Western blot assay of cytosol liver proteins. The HPT group displayed GST-P expression increase GST-P 2.8 times higher than the NT group (Fig. 4). In contrast, GST-P expression in the HPT plus celecoxib group GST-P was almost the same as in the NT group, with a difference that was not statistically significant ($P > 0.05$). In summary, these results demonstrate that celecoxib inhibited the expression of GST-P by 90% in liver carcinogenesis ($P < 0.001$) (Fig. 4).

3.4. Celecoxib did not cause apoptosis during in vivo hepatocarcinogenesis

To determine if celecoxib induces apoptosis after DEN administration, after 2AAF administration, after hepatectomy or at the end of the experiment, we used four different procedures applied to the three groups (Fig. 5). These were applied to the groups: NT, HPT and HPT plus celecoxib. First, we analyzed the expression of mitochondrial proteins such as Bax, Bcl-xL, which were not modified after treatment with celecoxib. Second, according to this observation neither mitochondrial levels of cytochrome *c* nor the target proteins caspase 3 and PARP were affected. Third, no DNA ladders in DNA fragmentation analysis were found. Finally, in the TUNEL assay no significant positive cells were observed. All these data suggest the lack of a pro-apoptotic effect of celecoxib on hepatocarcinogenesis in vivo.

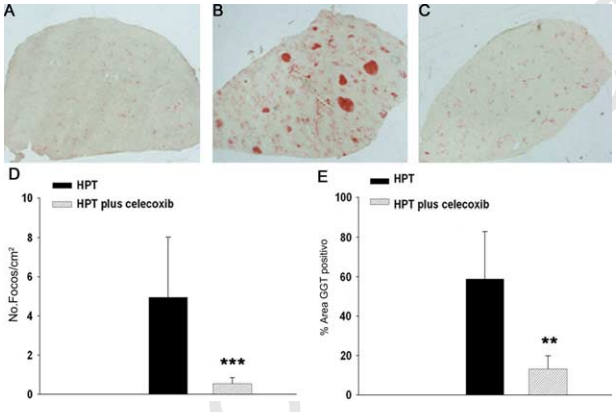

Fig. 3. Expression of GGT tumoral marker on liver slices. (A) Group NT ($n = 5$), (B) Group HPT ($n = 9$), (C) Group HPT plus celecoxib ($n = 9$). The bar graphs indicate the quantification of number (D) and area of GGT^+ foci (E) from an average of four liver histological sections for each rat chosen randomly for densitometric analysis. In the HPT plus celecoxib group both the number and area of γ-glutamyltranspeptidase (GGT) positive foci decreased by 80 and 90%, respectively, compared with HPT group. GGT^+ foci were not detected in the NT group using a detection parameter for focus size larger than 0.01 mm^2. Asterisks indicate significant difference in comparison with HPT group ($P \leq 0.001$, ***$P \leq 0.0001$).**

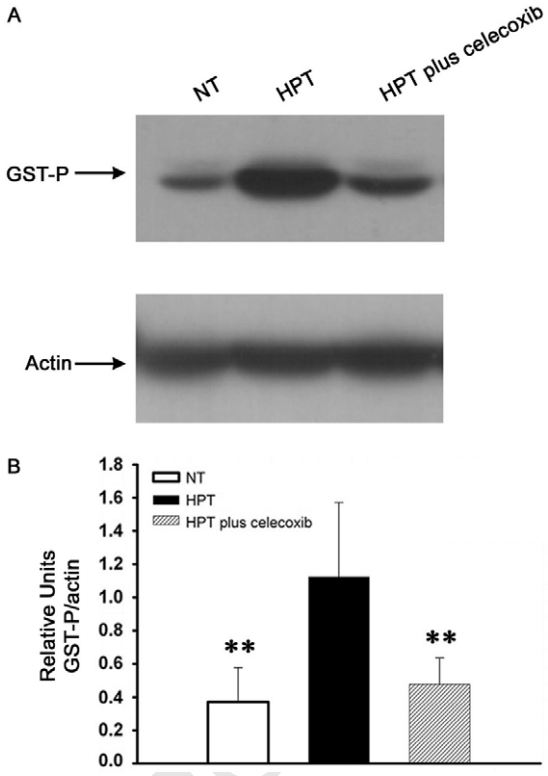

Fig. 4. Effect of celecoxib treatment on GST-P expression. (A) Representative Western blot results of the GST-P of nine animals per group. Actin expression was determined as a control for loading. (B) The bar graphs indicate the relative amounts of GST-P after normalization with respect to the amount of actin loaded. The HPT plus celecoxib group showed a decrease of 90% in GST-P expression compared with the HPT group. Asterisks indicate statistical significant from HPT group with a *P* value ≤ 0.001.

3.5. Celecoxib reduced proliferation during in vivo hepatocarcinogenesis

To explore whether the chemopreventive effect of celecoxib is associated to proliferation, we analyzed the nuclear expression of PCNA, a protein required for DNA synthesis, and a common technique to study the proliferating activity of cancer cells [24]. In the HPT

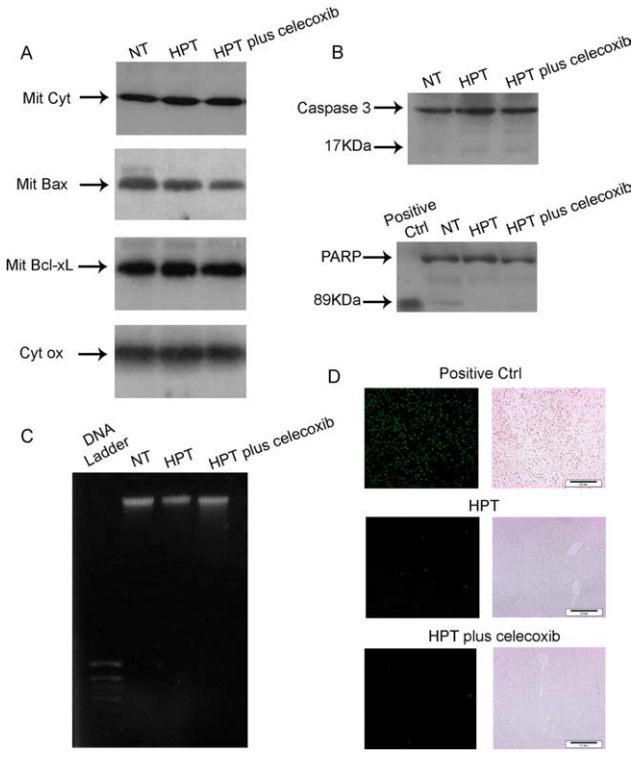

Fig. 5. Apoptosis analysis in the hepatocarcinogenesis model. The results are representative of five animals per group: NT, HPT and HPT plus celecoxib. (A) Cytochrome *c*, Bax and Bcl-xL levels in mitochondria were analyzed by Western blot. Cytochrome oxidase expression was determined as loading control. (B) Cleavage of Caspase 3 and PARP proteins analyzed by Western blot. HeLa cells treated with Fas ligand were used as positive control for PARP cleavage. (C) DNA gel electrophoresis. (D) Tunel assay was analyzed both colorimetrically (left panel) and fluorometrically (right panel). Sections pretreated with DNAse were used for positive control in the Tunel assays. All these assays show that celecoxib was unable to induce an apoptotic effect in the hepatocarcinogenesis model.

plus celecoxib group, the nuclear concentration of PCNA was drastically reduced with respect to the HPT group, as is clearly shown by the immunohistochemical assay (Fig. 6B, B′ and C). It is noteworthy that positive stained nuclei were scarce in the NT group (Fig. 6A), very numerous in HPT animals (in nodular and as well in non-nodular tissue, Fig. 6B and B′) and less than half of the latter in the HPT plus celecoxib group (Fig. 6C). To quantify these phenomena, PCNA and another proliferation marker, Ki-67, were analyzed by Western blot from nuclear extracts. PCNA expression showed a decrease of 60% in HPT plus celecoxib animals ($P<0.05$ versus HPT group) (Fig. 6D and E). Furthermore, the nuclear expression of Ki-67, which is expressed in proliferating cells and necessary during all active phases of the cell cycle except in the resting cell [25], the treatment with celecoxib caused a marked downregulation in approximately 90% of the nuclear expression of this protein ($P<0.05$ versus HPT group) (Fig. 6D and E).

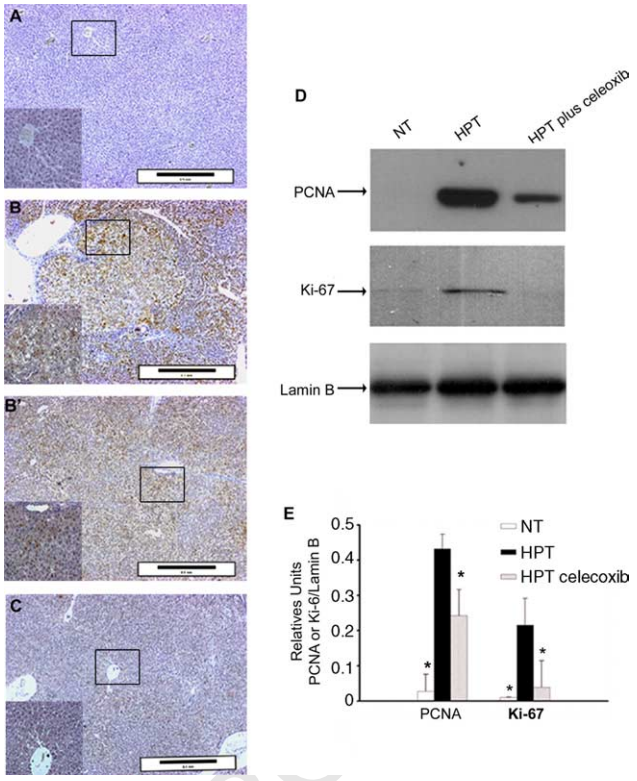

Fig. 6. Effect of celecoxib on the proliferation markers PCNA and Ki-67. Representative pictures of liver samples stained for PCNA obtained 5 weeks after experiment initiation from five animals per group. (A) Group NT, absence of immunostaining. Group HPT, prominent immunostaining of PCNA, both intra-nodular (B) and extra-nodular (B′). (C) Group HPT plus celecoxib, showing appreciable decrease of PCNA staining. The photographs show a 40-fold magnification, and a detail (×200) is shown in the inset. (D) Western blot of nuclear extracts for analysis both PCNA and Ki-67 corresponding to the NT, HPT and HPT plus celecoxib groups. The HPT plus celecoxib group showed a decrease of 60 and 90% in the expression of PCNA and Ki-67, respectively. Lamin B expression was determined as a control for loading. (E) The bar graphs indicate the relative amounts of PCNA and Ki-67 after normalization with respect to the amount of lamin B loaded. *P value is <0.05 in comparison with HPT group.

3.6. COX-2 expression and PGE_2 levels were not induced by carcinogenic treatment in the medium-term rat hepatocarcinogenesis assay

Up-regulation of COX-2 has been observed in a number of tumors including gastrointestinal tumors, which suggests a role for COX-2 in gastrointestinal tumorigenesis. In order to investigate the participation of COXs in the development of preneoplastic lesions in this hepatocarcinogenesis model, 5 weeks after experiment initiation, we analyzed by Western blot the expression of COX-1 and COX-2 proteins from liver microsomal extracts and total homogenates of five animals per group. The treatment of this hepatocarcinogenesis model did not induce the over-expression of COX-1 or of COX-2 in the HPT group (Fig. 7A and B). No statistically significant changes were observed with respect to basal levels in HPT and in HPT plus celecoxib ($P>0.05$)

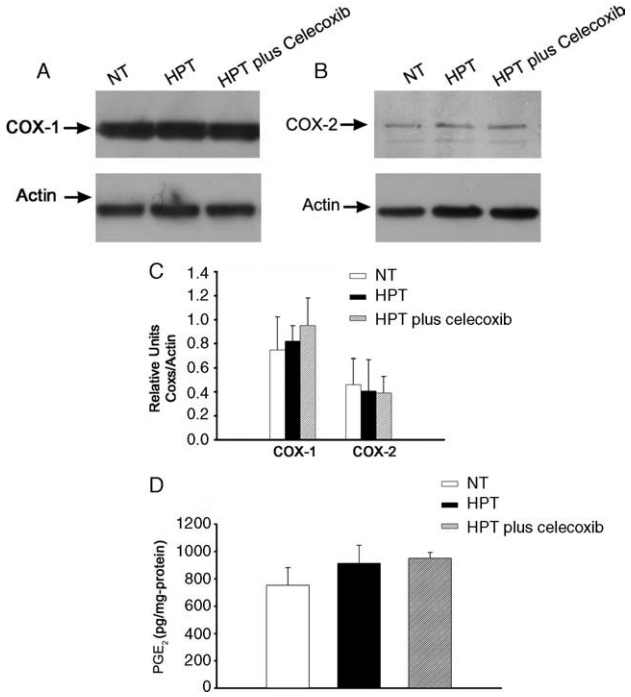

Fig. 7. Analysis of COXs and PGE_2 in the medium-term rat hepatocarcinogenesis assay (A). Representative Western blot assay of both COX-1 and COX-2 of six animals per group. Actin expression was determined as a control for loading. (B) PGE2 levels in groups NT, HPT and HPT plus celecoxib. These parameters were analyzed 5 weeks after experiment initiation. Data were not significantly different from the HPT group (P value > 0.05).

(Fig. 7C). Results of COX-1, COX-2 were similar when analyzed after DEN administration, after 2-AAF administration, and after 24 and 48 h of partial hepatectomy (data not shown). In order to determine whether COX-1 and COX-2 were induced by the hepatocarcinogenic treatment in the neoplastic nodules, COX-1 and COX-2 immunostaining was performed. Using a conventional labeled streptavidin–biotin system (Dako LSAB kit), we found that neither COX-1 nor COX-2 were detectable (data not shown). To detect this low basal COX-1 and COX-2 expression, it was necessary to use a Catalyzed Signal Amplification System (DakoCSA System), which is highly sensitive and allows for the detection of extremely small quantities of target proteins. Using this system we detected equal non-preferential COX-1 and COX-2 expression in all the treatment groups (NT, HPT and HPT plus celecoxib groups), with prominent staining in central vein and portal area. Furthermore, no differences in the COXs expression were observed between nodular and non-nodular tissue in the HPT groups (data not shown). In conclusion, these data support our WB results, in which COX-1 and COX-2 expression was not considerably modified by this hepatocacinogenic treatment or celecoxib treatment. To analyze the activity of the COXs, we measured PGE_2 levels in the liver tissues. Results of PGE_2 production in the HPT group are in accordance with the protein expression of the COXs, in which the levels were similar to animals of the NT group. Likewise, no changes were found between HPT and HPT plus celecoxib groups, analyzed five weeks after experiment initiation ($P > 0.05$) (Fig. 7D), 24 and 48 h after partial hepatectomy (data not shown).

3.7. NF-kappa B nuclear translocation blockage by celecoxib

The activation of NF-κB induces transcription of proteins that participate in cell proliferation; therefore, it is a key component in the initiation and progression of carcinogenesis and is considered a good target for chemoprevention [26]. Activation of NF-κB requires nuclear translocation mainly of the Rel A/p65 subunit. To verify if HPT or HPT plus celecoxib had any effect at this level we measured the p65 protein both in nuclear and cytosolic extracts 48 h after the partial hepatectomy proliferative stimulus. The results indicate that celecoxib inhibited the activation of NF-κB, given that p65 decreased

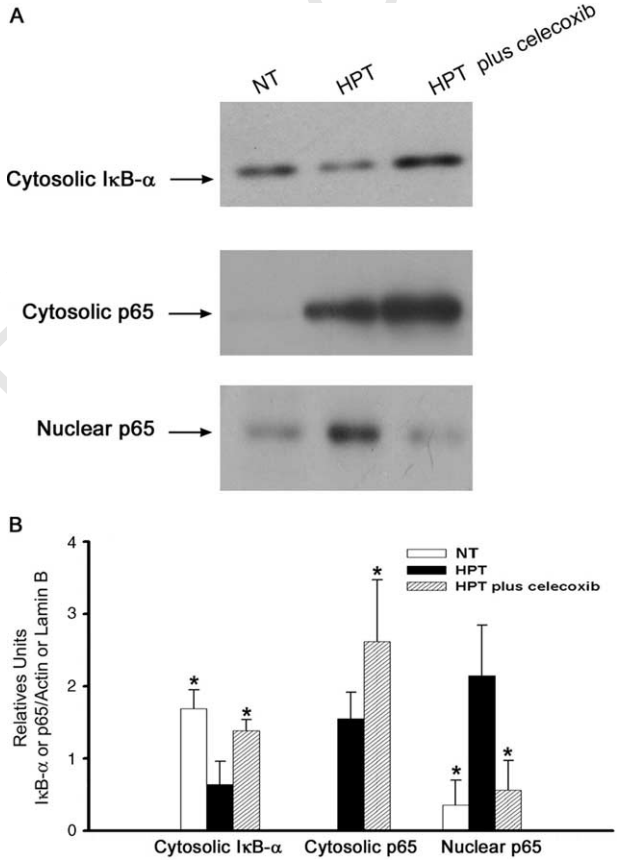

Fig. 8. Effect of celecoxib on NF-κB activation. (A) Western blot of IκB-α and p65 48 h after partial hepatectomy in the hepatocarcinogenesis model. (B) The bar graphs indicate the relative amounts of p65 and IκB-α after normalization with respect to the amount of lamin B and actin loaded. In the HPT plus celecoxib group, both IκB-α and p65, at cytosolic levels, were significantly increased by 55 and 65%, respectively. Also, an inhibition of 60% in the p65 nuclear levels was found. *P value is < 0.05 ($n = 6$) in comparison with HPT group.

in nuclear fractions by 60% and increased by 65% at cytosolic level with respect to the HPT group ($P<0.05$) (Fig. 8). It is known that the translocation of NF-κB is preceded by proteolytic degradation of IκB-α; therefore, we measured the IκB-α protein at cytosolic levels. IκB-α degradation, visualized by western blotting, correlates with NF-κB translocation to the nucleus in the HPT group. Furthermore, the level of IκB-α was significantly increased by 55% following treatment with celecoxib with respect to HPT group ($P<0.05$) (Fig. 8).

4. Discussion

One important feature of the resistant hepatocyte model is the induction of persistent nodules that allows early studies of chemopreventive agents in the sequence, starting with nodule appearance and ending with cancer [27]. It is outstanding in this experimental model that rat livers present foci and nodules fairly early and they are quite easily detected by their histological characteristics and staining-pattern for GGT and GST-P markers. In previous work, this model allowed us to evaluate the chemoprotective effect of CAPE on the promotion stage of hepatocarcinogenesis [28]. On the other hand, the data presented here demonstrate for the first time that administration of celecoxib is highly effective in inhibiting the multiplicity and size of liver preneoplastic lesions induced by DEN, 2-AAF and partial hepatectomy. The exact mechanism of action by which celecoxib decreases the number and size of liver preneoplastic lesions remains to be clarified. Many other reports have shown that celecoxib has apoptosis and antiproliferative effects in neoplastic cell lines [9,29]. These *data* prompted us to analyze the effects of celecoxib in a whole animal carcinogenesis model. The present study with celecoxib did not provide evidence of apoptosis in the in vivo hepatocarcinogenesis model. This result is supported by a previous in vivo colorectal cancer study [30], and in vitro studies using hepatic and mammary tumor cell lines [31,32], in which COX-2 inhibitors including celecoxib were able to block tumor growth without apoptosis induction. Furthermore, previous in vivo studies have shown that the simultaneous inhibition of COX-2 and NOS-2 activity is required to induce apoptosis in partially hepatectomized mice, indicating that PGE_2 and nitric oxide (NO) play an important role in protecting from apoptotic death in vivo [33]. In this context, we consider that the absence of apoptosis in our system is possibly because the PGE_2 synthesis in liver was not suppressed by celecoxib treatment.

Our proposal is that celecoxib did exert antineoplastic effects during early stages of hepatocarcinogenesis through its antiproliferative activity. This proposal is supported by the down-regulation of the proliferation markers PCNA and Ki-67 in animals treated with celecoxib during hepatocarcinogenesis, which correlated with the decrease in preneoplastic lesions.

On the other hand, our results showed that neither COX-2 expression nor PGE_2 production were altered by HPT or celecoxib treatment. This finding is in concordance with previous reports in which treatment of celecoxib did not alter COX-2 expression or prostaglandin production in a UVB-induced skin cancer model [34]. Therefore, it can be considered that the chemopreventive activity of celecoxib is mediated by PGE_2-independent mechanisms in the present experimental model. The main effect of celecoxib was a clear blockage of the nuclear translocation of NF-κB induced 48 h post-hepatectomy in this hepatocarcinogenesis model. It is known that NF-κB participates in the regulation of cyclins and modulates the cell cycle [35] thus lending support to our hypothesis that celecoxib modulates critical steps, closely bound to the promotion stage, after the proliferation stimulus of hepatectomy of liver carcinogenesis, and consequently to carcinogenesis progression by other molecular targets besides COX-2. On the other hand, the COX-2 inhibitors could also inhibit growth through peroxisome proliferator-activated receptor gamma (PPARγ) activation. PPARγ agonists induce cell-cycle arrest by downregulation of cyclin D1 in several tumor cell lines, including those derived from hepatocellular carcinoma [36] and recent in vivo data suggest that COX-2 inhibitors increase the activity of PPARγ by acting as PPARγ-ligands [37].

Although neither COXs nor PGE_2 were induced by this hepatocarcinogenic treatment, which suggests a potential limitation of this animal model for evaluating the role of COX-2 inhibitors in preventing hepatocarcinogenesis, our results clearly show that the administration of celecoxib produces a striking chemopreventive effect on the development of preneoplastic lesions that correlates with the decrease in cell proliferation markers and reduction of nuclear traslocation of NF-κB. Additional studies will be necessary to determine the mechanisms involved in the chemopreventive efficacy of celecoxib against hepatocarcinogenesis. Finally, although several COX-2 inhibitors have demonstrated efficacy in chemoprevention, it is important to mention that not all selective COX-2 inhibitors are good candidates for chemoprevention because they increase the risk of cardiovascular events.

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