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**Original Paper**

# Celecoxib and Ibuprofen Restore the ATP Content and the Gluconeogenesis Activity in the Liver of Walker-256 Tumor-Bearing Rats

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**Key Words**
Cancer • Non-steroidal anti-inflammatory • Gluconeogenesis • ATP • Carnitine palmitoyl transferase 1 • Liver perfusion

**Abstract**
***Background/Aims:*** The main purpose of this study was to investigate the effects of celecoxib and ibuprofen, both non-steroidal anti-inflammatory drugs (NSAIDs), on the decreased gluconeogenesis observed in liver of Walker-256 tumor-bearing rats. ***Methods:*** Celecoxib and ibuprofen (both at 25 mg/Kg) were orally administered for 12 days, beginning on the same day when the rats were inoculated with Walker-256 tumor cells. ***Results:*** Celecoxib and ibuprofen treatment reversed the reduced production of glucose, pyruvate, lactate and urea from alanine as well as the reduced production of glucose from pyruvate and lactate in perfused liver from tumor-bearing rats. Besides, celecoxib and ibuprofen treatment restored the decreased ATP content, increased triacylglycerol levels and reduced mRNA expression of carnitine palmitoyl transferase 1 (CPT1), while ibuprofen treatment restored the reduced mRNA expression of peroxisome proliferator-activated receptor alpha (PPARα) in the liver of tumor-bearing rats. Both treatments tended to decrease TNFα, IL6 and IL10 in the liver of tumor-bearing rats. Finally, the treatment with celecoxib, but not with ibuprofen, reduced the growth of Walker-256 tumor. ***Conclusion:*** Celecoxib and ibuprofen restored the decreased gluconeogenesis in the liver of Walker-256 tumor-bearing rats. These effects did not involve changes in tumor growth and probably occurred by anti-inflammatory properties of these NSAIDs, which increased expression of genes associated with fatty acid oxidation (PPARα and CPT1) and consequently the ATP production, normalizing the energy status in the liver of tumor-bearing rats.



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## Introduction

Walker-256 tumor-bearing rats, an animal model widely used to study metabolic alterations induced by cancer, exhibit cachexia, anorexia and several disturbances in the metabolism of protein, carbohydrates and lipids [1]. Liver perfusion studies revealed several changes in hepatic metabolism of Walker-256 tumor-bearing rats. For example, glycolytic flux is diminished in the liver of Walker-256 tumor-bearing rats and this seems to be caused, at least in part, by an impaired glucokinase activity [2]. These animals also showed decreased hepatic capacity for oxidizing fatty acids [3, 4], a phenomenon which seems to be caused by reduction in the activity of the mitochondrial carnitine palmitoyl transferase-1 (CPT1) [3] and -2 (CPT2) [5]. In addition, liver gluconeogenesis from alanine, pyruvate and lactate was decreased in this cancer-cachexia experimental model in our [6] and others [4, 7, 8] studies. However, the mechanism involved in this inhibition is still unknown.

Tumor necrosis factor-α (TNFα) and interleukin-1 (IL1), which are pro-inflammatory cytokines increased by cancer [9-11], have been associated with the gluconeogenesis inhibition. It was observed that intravenous administration of TNFα [12, 13] or IL1β [12] reduced the gluconeogenesis from alanine, pyruvate and lactate in liver perfusion. Nevertheless, we observed that treatment with infliximab, an antibody that neutralizes TNFα, did not affect the gluconeogenesis inhibition from alanine in Walker-256 tumor-bearing rats [14].

Further the cytokines, the prostaglandins (PGs), especially prostaglandin E2 (PGE2), which is involved in inflammation caused by the Walker-256 tumor [15], seem to be associated to the inhibition of gluconeogenesis. However, few studies have assessed the effects of non-steroidal anti-inflammatory drugs (NSAIDs), which decrease PGs synthesis by inhibiting cyclooxygenase-1 (COX-1) and/or cyclooxygenase-2 (COX-2), on gluconeogenesis in pathological states.

The treatment with indomethacin (NSAID, COX-1 and COX-2 inhibitor) reduced the inhibition of gluconeogenesis from alanine in liver of arthritic rats [16]. In addition, treatment with celecoxib (NSAID, COX-2 inhibitor) abolished the inhibition of gluconeogenesis from alanine in liver perfusion of Walker-256 tumor-bearing rats [8], which shows high levels of PGE2 in plasma [15]. However, in this latter study the celecoxib treatment also reduced tumor growth [8] and so the production of factors with gluconeogenesis inhibitory properties, such as the cytokines TNFα and IL1. Therefore, the contribution of PGs to inhibition of gluconeogenesis in Walker-256 tumor-bearing rats still remains inconclusive.

Given that ibuprofen (NSAID, COX-1 and COX-2 inhibitor), as opposed to celecoxib, did not reduce Walker-256 tumor growth in our previous investigation, the aim of this study was to compare the effects of these two NSAIDs (celecoxib or ibuprofen) on the inhibition of gluconeogenesis from alanine, pyruvate and lactate caused by Walker-256 tumor in rats. The effects of celecoxib or ibuprofen on the ATP content, triacylglycerol level, mRNA expression of carnitine palmitoyl transferase 1 (CPT1) and peroxisome proliferator-activated receptor alpha (PPARα), as well as on the levels of TNFα, IL6 and IL10 in the liver were also assessed. Besides, the effects of the NSAIDs on the tumor growth were examined.

## Materials and Methods

*Drugs and chemicals*

Celecoxib (Celebra®) was purchased from Pfizer (Guarulhos, Brazil) and ibuprofen from Medley (Campinas, Brazil). The perfusion fluid salts and other chemicals (98-99.8% purity) were obtained from Sigma Chemical Co. (St Louis, USA), Merck (Darmstad, Germany) and Reagen (Rio de Janeiro, Brazil). The high-capacity cDNA Reverse Transcription Kit was acquired from Applied Biosystems (USA). Protease inhibitor cocktail was purchased from Sigma-Aldrich (St. Louis, USA) and Kits for determination of TNFα, IL6 and IL10 (DuoSet ELISA®) from R&D Systems (Minneapolis, USA).

*Animals and Walker-256 tumor inoculation*

Male Wistar rats (220-230 g) fed with a standard rodent chow (Nuvilab CR-1 Nuvital®, Colombo, Brazil) were used in all experiments. All protocols were approved by the Ethics Committee for Animal Experimentation of the State University of Londrina.

Walker-256 carcinosarcoma cells were injected in tumor-bearing rats as previously described [1, 17]. The Walker-256 cells were suspended in phosphate buffered saline (PBS: 16.5 mM phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4) and each rat was subcutaneously inoculated with $8.0X10^7$ viable cells on the right flank. Control (healthy) rats were inoculated with PBS in the same place.

*Treatment protocol*

Walker-256 tumor-bearing rats were treated with celecoxib (25 mg/Kg) or ibuprofen (25 mg/Kg), by gavage, once a day (9:00 a.m.), for 12 days, starting on the day the rats were inoculated with Walker-256 tumor cells. Celecoxib and ibuprofen were daily diluted in water and doses and treatment protocols were based on previous studies [8, 18, 19]. Drug-free control rats (Walker-256 tumor bearing or healthy) received water, by gavage, instead of celecoxib or ibuprofen.

The rats were fasted for 24 hours before all tests to prevent the glucose arising from glycogenolysis to influence the measurement of gluconeogenesis activity and also to prevent the influence of anorexia of tumor-bearing rats on the other parameters evaluated. Since the Walker-256 tumor-bearing rats survived for an average of 14 days after tumor inoculation, all assessments were carried out 12 days after of the treatments with celecoxib, ibuprofen or water (control rats).

*Assessment of liver gluconeogenesis*

To assess the gluconeogenesis, Walker-256 tumor-bearing rats were weighed, anesthetized with sodium pentobarbital (40 mg/kg) and subjected to *in situ* liver perfusion as previously described [6, 20, 21]. The perfusion fluid, Krebs-Henseleit buffer (KHB: 115 mM NaCl, 26 mM $NaHCO_3$, 5.8 mM KCl, 1.2 mM $Na_2SO_4$, 1.18 mM $MgCl_2$, 1.2 mM $NaH_2PO_4$ and 2.5 mM $CaCl_2$, pH 7.4), at 37ºC, and saturated with a 95%:5% $O_2$:$CO_2$ mixture, was introduced into the liver (4 mL/min per gram of liver) through a cannula inserted into the portal vein, while a second cannula in the inferior vena cava was used to collect the effluent perfusate at 2 min intervals, to assess the production of glucose [22], lactate [23], pyruvate [24] and urea [25]. The livers were perfused with KHB for 10 min and then with KHB plus alanine (2.5 mM), or pyruvate (5.0 mM), or lactate (2.0 mM) for 30 min. To calculate the areas under the curve (AUC), the differences in production of glucose, lactate, pyruvate and urea, before and during the infusion of gluconeogenesis precursors, were used. At the end of the experiments, the tumors were carefully dissected and weighed to measure tumor growth.

*Assessment of ATP, triacylglycerol, CPT1, PPARα and cytokines in the liver*

In order to quantify these parameters, livers of rats anesthetized with sodium pentobarbital (40 mg/kg) were removed by the freeze-clamp technique, immediately frozen by immersion in liquid nitrogen and stored at -80ºC for later evaluation of the parameters. Thereafter, the tumors were dissected and weighed to measure tumor growth.

To assess the ATP content, frozen samples were crushed in liquid nitrogen, deproteinized in cold percloric acid and subjected to differential centrifugation. The collected supernatant was precipitated with potassium carbonate (pH 7.4) and used for the enzymatic determination of the ATP in the liver [26].

To evaluate the triacylglycerol level, the lipids were extracted from liver samples with chloroform-methanol, as described by Folch [27]. The triacylglycerol in the lipid extract were determined by enzymatic assay based on the Trinder reaction [28].

To assess the expression of CPT1 and PPARα, which was evaluated by real-time PCR (qRT-PCR), total mRNA was extracted from liver samples with Trizol® reagent and used for cDNA synthesis, with the high-capacity cDNA reverse transcription Kit. CPT1 and PPARα gene expression was normalized by the expression of ribosomal protein L19 (RPL-19) using the CT (threshold cycle) comparative method [29].

To measure the cytokines, liver samples were homogenized in RIPA buffer (0.625 % Nonidet P-40, 0.625 % sodium deoxycholate, 6.25 mM sodium phosphate and 1 mM EDTA, at pH 7.4), containing 10 µg/mL protease inhibitor cocktail. The homogenates were centrifuged and the supernatant was utilized to determinate the total protein concentration by Bradford assays and the levels of TNFα, IL10 and IL6 by ELISA.

*Statistical analysis*

Normal distribution and variance homogeneity were tested and the one-way ANOVA test was employed to analyze the results. Statistical analysis was carried out using the Statistica 6.0 or GraphPad Prism 5.0 programs, being accepted as different for $p<0.05$. Results are expressed as mean ± *s*tandard error of the mean (SEM).

## Results

Compared to healthy rats, Walker-256 tumor-bearing rats showed a reduction of approximately 50% in the glucose production from alanine (Fig. 1A), which was almost completely abolished ($p<0.05$) by celecoxib or ibuprofen treatment, as demonstrated by the AUCs (Fig. 1B). Tumor-bearing rats also showed a reduction of almost 50% in the pyruvate production (Fig. 2A), 45% in the lactate production (Fig. 2B) and 75% in the urea production

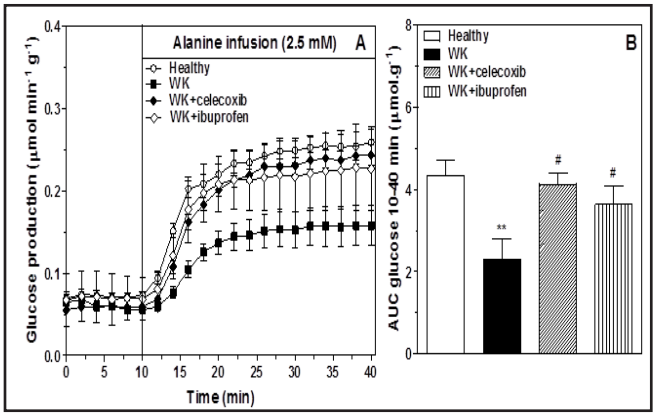


**Fig. 1.** (A) Production of glucose and (B) the respective areas under the curves (AUCs) in livers of healthy rats and Walker-256 tumor-bearing rats treated with celecoxib (WK+celecoxib) or ibuprofen (WK+ibuprofen) for 12 days or untreated (WK). Livers of 24h-fasted rats were perfused as described in Materials and Methods. L-Alanine (2.5 mM) was infused between 10 and 40 min. Data are presented as mean ± SEM of 7-9 experiments. **p<0.01 *vs.* healthy; #p<0.05 *vs.* WK (one-way ANOVA followed by Newman-Keuls).

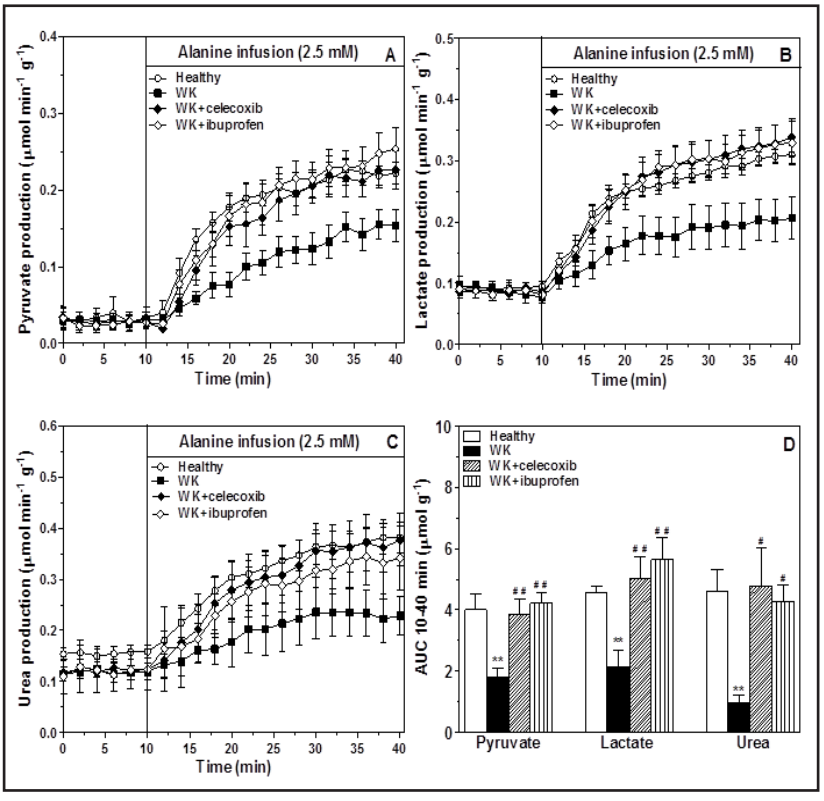


**Fig. 2.** Production of (A) pyruvate, (B) lactate, (C) urea and (D) the respective areas under the curves (AUCs) in livers of healthy rats and Walker-256 tumor-bearing rats treated with celecoxib (WK+celecoxib) or ibuprofen (WK+ibuprofen) for 12 days or untreated (WK). Livers of 24h-fasted rats were perfused as described in Materials and Methods. L-Alanine (2.5 mM) was infused between 10 and 40 min. Data are presented as mean ± SEM of 7-9 experiments. **p<0.01 *vs.* healthy, #p<0.05 and ##p<0.01 *vs.* WK (One-way ANOVA followed by Newman-Keuls).

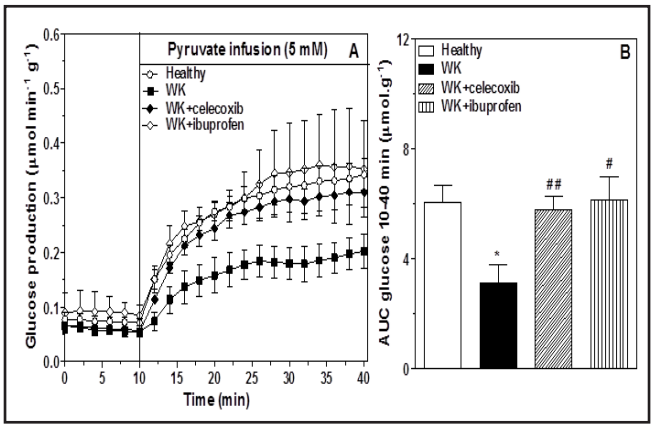


**Fig. 3.** (A) Production of glucose and (B) the respective areas under the curves (AUCs) in livers of healthy rats and Walker-256 tumor-bearing rats treated with celecoxib (WK+celecoxib) or ibuprofen (WK+ibuprofen) for 12 days or untreated (WK). Livers of 24h-fasted rats were perfused as described in Materials and Methods. Pyruvate (5 mM) was infused between 10 and 40 min. Data are presented as mean ± SEM of 4-6 experiments. *p<0.05 *vs.* healthy; #p<0.05 and ##p<0.01 *vs.* WK (One-way ANOVA followed by Newman-Keuls).

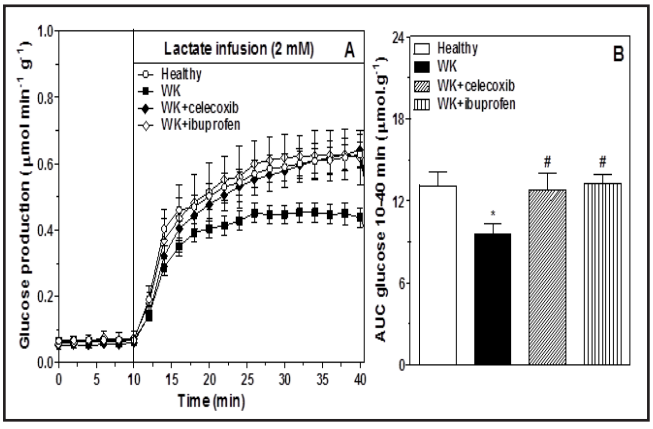


**Fig. 4.** (A) Production of glucose and (B) the respective areas under the curves (AUCs) in livers of healthy rats and Walker-256 tumor-bearing rats treated with celecoxib (WK+celecoxib) or ibuprofen (WK+ibuprofen) for 12 days or untreated (WK). Livers of 24h-fasted rats were perfused as described in Materials and Methods. Lactate (2 mM) was infused between 10 and 40 min. Data are presented as mean ± SEM of 7-21 experiments. *p<0.05 *vs.* healthy; #p<0.05 *vs.* WK (One-way ANOVA followed by Newman-Keuls).

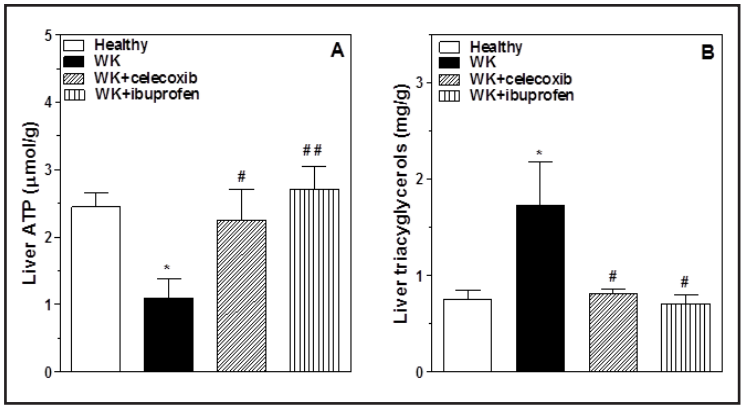


**Fig. 5.** Contents of (A) adenosine triphosphate (ATP) and (B) triacylglycerol in livers of healthy rats and Walker-256 tumor-bearing rats treated with celecoxib (WK+celecoxib) or ibuprofen (WK+ibuprofen) for 12 days or untreated (WK). Rats fasted for 24 hours. Data are presented as mean ± SEM of 6-8 experiments. *p<0.05 *vs.* healthy; #p<0.05 and ##p<0.01 *vs.* WK (One-way ANOVA followed by Newman-Keuls).

(Fig. 2C) from alanine, compared to healthy rats, and the treatments with celecoxib or ibuprofen completely reversed the decrease in pyruvate (p<0.01), lactate (p<0.01) and urea (p<0.05) production, as indicated by the AUCs (Fig. 2D).

Hepatic glucose production was about 50% lower from pyruvate (Fig. 3A) and 30% lower from lactate (Fig. 4A) in tumor-bearing rats as compared with healthy rats, and the treatments with celecoxib or ibuprofen reversed the decreased gluconeogenesis from pyruvate (p<0.05) and lactate (p<0.01) , as demonstrated by the AUCs (Figs. 3B and 4B).

The ATP content in tumor-bearing rats livers was almost 60% lower than in healthy rats livers and the treatment with celecoxib (p<0.05) or ibuprofen (p<0.01) restored the hepatic ATP content (Fig. 5A). Tumor-bearing rats also showed an increase of 130% (p<0.05) in the triacylglycerol level in the liver, as compared to healthy rats. The celecoxib or ibuprofen

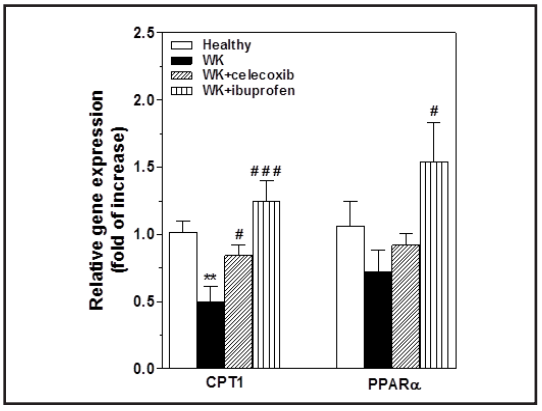


**Fig. 6.** Relative gene expression of CPT1 and PPARα in livers of healthy rats and Walker-256 tumor-bearing rats treated with celecoxib (WK+celecoxib) or ibuprofen (WK+ibuprofen) for 12 days or untreated (WK). Rats fasted for 24 hours. Data are presented as mean ± SEM of 5-8 experiments. *p<0.05 *vs.* healthy; #p<0.05 and ###p<0.001 *vs.* WK (One-way ANOVA followed by Newman-Keuls).

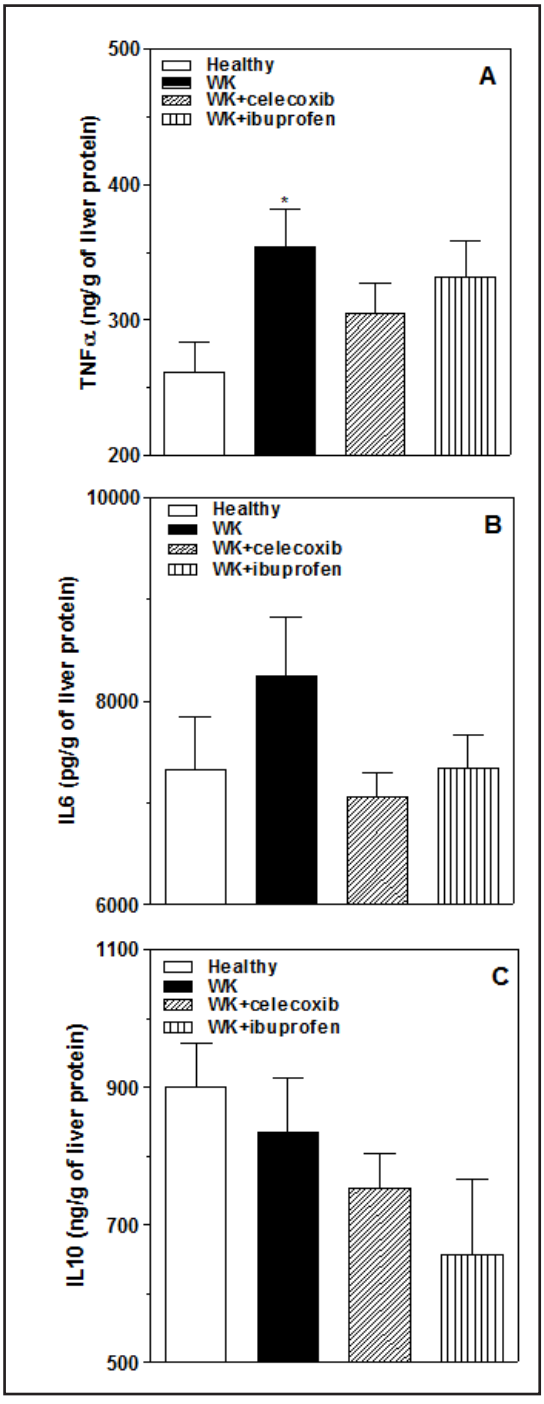


**Fig. 7.** Contents of (A) tumor necrosis factor alpha (TNFα), (B) interleukin 6 (IL6) and (C) interleukin 10 (IL10) in livers of healthy rats and Walker-256 tumor-bearing rats treated with celecoxib (WK+celecoxib) or ibuprofen (WK+ibuprofen) for 12 days or untreated (WK). Rats fasted for 24 hours. Data are presented as mean ± SEM of 5-8 experiments. *p<0.05 *vs.* healthy (One-way ANOVA followed by Dunnet's).

**Table 1.** Tumor mass from Walker-256 tumor-bearing rats treated with celecoxib (WK+celecoxib) or ibuprofen (WK+ibuprofen) during 12 days or untreated (WK). Data are as mean ± SEM. ###p<0.01 *vs.* WK (ANOVA One-Way followed by Newman-Keuls)

| | WK | WK+celecoxib | WK+ibuprofen |
|---|---|---|---|
| Tumor mass (g) | 22.17±1.41 | 10.06±0.91### | 19.64±1.45 |
| | (n=23) | (n=28) | (n=22) |

treatment reduced (p<0.05) the triacylglycerol level in the liver of tumor-bearing rats to near the level found in healthy animals (Fig. 5B).

CPT1 expression was decreased (p<0.05) and PPARα expression tended to be lower in the livers of tumor-bearing rats as compared to healthy rats. The celecoxib treatment increased CPT1 expression (p<0.05) and tended to increase PPARα expression in the liver, whereas the ibuprofen treatment increased both CPT1 (p<0.001) and PPARα (p<0.05) expression (Fig. 6).

When compared to healthy rats livers the levels of TNFα was higher (p<0.05) (Fig. 7A), of IL6 tended to be higher (Fig. 7B) and of IL10 tended to be lower (Fig. 7C) in tumor-bearing

rat livers, and treatments with celecoxib or ibuprofen tended to decrease the levels of TNFα, IL6 and IL10 in tumor-bearing rats livers.

Finally, celecoxib treatment reduced ($p<0.01$) the Walker-256 tumor mass by approximately 40%, while the ibuprofen treatment did not affect tumor growth (Table 1).

## Discussion

Treatment with either celecoxib or ibuprofen, drugs that inhibit PGs synthesis, completely reversed the decreased activity of gluconeogenesis from alanine, pyruvate or lactate in perfused livers from Walker-256 tumor-bearing rats.

The doses of celecoxib and ibuprofen used were based on previous studies [8, 18-19], being almost the double of the maximum recommended daily dose for humans. Although the PGs levels were not assessed in our study, it is well established that the anti-inflammatory properties of celecoxib and ibuprofen are achieved by blocking PGs production, since celecoxib and ibuprofen reduced PGE2 production by about 90% [30].

The restoration of the decreased gluconeogenesis from alanine, by treatments with celecoxib or ibuprofen (Fig. 1), involved an improvement in conversion of alanine to pyruvate, as indicated by the full reversion of the inhibition of hepatic production of pyruvate, lactate and urea in tumor-bearing rats (Fig. 2). The reduced hepatic production of pyruvate, lactate and urea from alanine observed in tumor-bearing rats (Fig. 2) confirms our previous findings [6] and can be due to an inhibition of alanine aminotransferase, as suggested by others [31, 32].

The observation that the treatment with celecoxib or ibuprofen reversed the reduced glucose production from pyruvate (Fig. 3) and lactate (Fig. 4) in the liver of tumor-bearing rats shows that these NSAIDs improve other steps of the gluconeogenesis pathway besides that catalyzed by alanine aminotransferase. The inhibition of glucose production from pyruvate and lactate, but not from glutamine [7] and glycerol [6], in tumor-bearing rats shows that this inhibition occurs at steps preceding the entry of glutamine and glycerol into the gluconeogenic pathway, such as the conversion of pyruvate to oxalacetate, a mitochondrial step catalyzed by pyruvate carboxylase, which is ATP dependent. In fact, a reduction in ATP content decreases the rate of gluconeogenesis by inhibiting pyruvate carboxylase [33].

In parallel with the inhibition of gluconeogenesis, Walker-256 tumor-bearing rats livers showed lower ATP content than in healthy rats livers (Fig. 5A), as also shown by others [7], and the treatments with celecoxib or ibuprofen restored the lowered ATP levels in the livers (Fig. 5A). These results indicate that the reversion of gluconeogenesis inhibition by these NSAIDs involved a normalization of the hepatic energy state of tumor-bearing rats.

Given that the rats were fasted for 24 hours, a condition that leads to depletion of the hepatic glycogen stores, reducing the endogenous glucose source for ATP production, and that the exogenous glucose source for ATP production is reduced in Walker-256 tumor-bearing rats due the decreased activity of glucokinase [2], it is likely that most of ATP in the liver of tumor-bearing rats originated from mitochondrial β-oxidation of fatty acids and not from glycolysis. Thereby, the lower ATP level in the liver of tumor-bearing rats could be due to reduction in mitochondrial β-oxidation of fatty acids. Really, it was shown that the hepatic capacity of fatty acids oxidation is decreased in Walker-256 tumor-bearing rats [3], a phenomena that seem to be caused by reduction of CPT1 and CPT2 activity, enzymes involved in the transport of fatty acids from cytosol to mitochondria [3, 5, 34].

A reduction of CPT1 and CPT2 activities could divert the fatty acids to esterification and so triacylglycerol synthesis, instead of oxidation. In fact, liver of tumor-bearing rats showed high levels of triacylglycerol (Fig. 5B) and lower mRNA expression of CPT1 (Fig. 6). These results are consistent with the reduction of hepatic fatty acid oxidation [3] and to the increase of free fatty acids and triacylglycerol levels in the liver [15] and blood [1] of Walker-256 tumor-bearing rats.

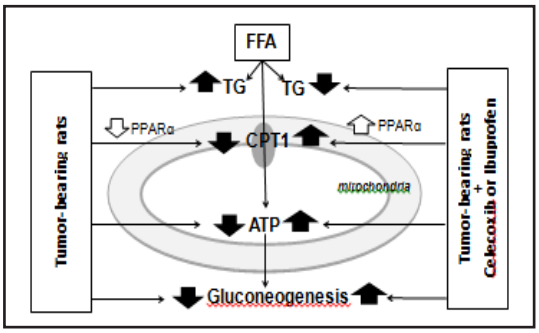


**Fig. 8.** Schematic representation of ibuprofen and celecoxib effects in the liver of Walker-256 tumor-bearing rats. Celecoxib and ibuprofen (both at 25 mg/Kg) were orally administered for 12 days, beginning on the same day when the rats were inoculated with tumor cells. FFA- free fatty acids; CPT1- carnitine palmitoyl transferase 1; PPARα- peroxisome proliferator-activated receptor alpha; TG- triacylglycerol; ⬆ - increased; ⬇ - decreased.

Celecoxib and ibuprofen treatments completely abolished the rise in the levels of triacylglycerol (Fig. 5B) and the decreased CPT1 expression in the liver of tumor-bearing rats (Fig. 6), suggesting a role of PGE2, which is increased in the liver [34] and blood [15, 34] of Walker-256 tumor-bearing rats, in the inhibition of CPT1. Corroborating our suggestion, PGE2 decreased CPT1 expression in isolated hepatocytes and the PGE2-dependent repression of CPT1 resulted in inhibition of mitochondrial $\beta$-oxidation and triacylglycerol accumulation in hepatocytes [35]. In addition, the exercise training normalized the increased level of PGE2 in the liver of Walker-256 tumor-bearing rats, restored the decreased activity of CPT1 and CPT2 and prevented hepatic steatosis [34]. Moreover, treatment with PGE2 or PGE2 receptor agonist increased the triacylglycerol in the liver [36]. It was also shown that treatment with indomethacin, which reduces PGE2 synthesis, increased CPT2 activity in the liver of Walker-256 tumor-bearing rats [5].

The CPT1 and CPT2 expression [37] and the β-oxidative degradation of fatty acids [38] in liver is stimulated by PPARα. Interestingly, PPARα expression tended to be lower in the liver of Walker-256 tumor-bearing rats and celecoxib treatment tended to increase the PPARα expression, while ibuprofen treatment significantly increased the PPARα expression in liver. These results indicate that celecoxib and ibuprofen have a promoting effect on expression of genes associated with fatty acid oxidation. In agreement with our results, it was shown that several NSAIDs, including ibuprofen, are efficacious activators of PPARα [39]. Therefore, a higher expression of PPARα and consequently of CPT1 and/or CPT2 caused by celecoxib and ibuprofen may have increased mitochondrial fatty acid oxidation and, thereby, the production of factors that stimulate gluconeogenesis such as acetyl-CoA, NADH and ATP, reversing the inhibition of gluconeogenesis of tumor-bearing rats.

In addition, PPARα was shown to suppress the expression of pro-inflammatory genes, by inactivating the transcription factor NFκB, and thus reducing the production of pro-inflammatory cytokines [40, 41, 42]. Walker-256 tumor-bearing rats showed elevated TNFα levels in liver (Fig. 7) and we showed that TNFα inhibits hepatic gluconeogenesis from alanine and lactate probably by affecting the conversion of pyruvate to oxaloacetate, a step ATP-dependent [13]. Furthermore, TNFα induced the uncoupling of respiration in isolated mitochondria [43], an effect that reduces the ATP synthesis. Thus, a reduction of TNFα by treatment with celecoxib or ibuprofen, although not significant (Fig. 7), may have been mediated by increased expression of PPARα and may have contributed to the improvement of hepatic gluconeogenesis in Walker-256 tumor-bearing rats.

The effects of celecoxib and ibuprofen in normalizing gluconeogenesis in the liver of tumor-bearing rats were independent of their influence on tumor growth, since ibuprofen, unlike celecoxib, did not reduce the Walker-256 tumor growth (Table 1). The celecoxib antitumor effect is well established [44-49]. It was shown that treatment with celecoxib reduces Walker-256 tumor growth by mechanisms independent of inhibition in the COX-2/PGE2 pathway, which involve reduction in the expression of Bcl-xl anti-apoptotic protein, that is, by specific pro-apoptotic actions [19].

In conclusion, celecoxib and ibuprofen restored the gluconeogenesis activity from alanine, pyruvate and lactate that is decreased in the liver of Walker-256 tumor-bearing rats. These effects did not involve changes in tumor growth and were probably mediated

by anti-inflammatory properties of these NSAIDs, which increased the expression of genes associated with fatty acid oxidation (PPARα and CPT1) and consequently the ATP production in the liver of tumor-bearing rats (Fig. 8).

In contrast to the beneficial effects of celecoxib and ibuprofen in gluconeogenesis, we showed recently that treatment with celecoxib, but not with ibuprofen, prevented the loss of body, adipose and muscle mass, hypertriacyglycerolemia and inhibition of peripheral response to insulin in Walker 256 tumor-bearing rats, effects that were attributed to anti-tumor action and not to anti-inflammatory property of celecoxib [50]. So, it is possible that celecoxib by presenting both anti-tumor and anti-inflammatory properties may provide better clinical improvement that ibuprofen in the treatment of metabolic disorders induced by cancer.

## Disclosure Statement

The authors declare that there are no conflicts of interest.

## Acknowledgments

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