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Possible role of cell redox state on collagen metabolism in carbon tetrachloride-induced cirrhosis as evidenced by adenosine administration to rats

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Abstract

Marked changes in the redox state of liver cells in carbon tetrachloride (CCl_4)-induced cirrhosis after chronic treatment with the hepatotoxin (4–8 weeks) were observed. A shift of the redox state towards the reduced side is noticed in both compartments, cytosol and mitochondria. At 8 weeks of treatment an imbalance between these two compartments was evident. The alteration produced by the CCl_4 treatment in the cell redox state might be related to the mitochondrial damage elicited by the hepatotoxin. Adenosine treatment to CCl_4-poisoned rats was able to counteract the effect of the hepatotoxin on the redox equilibrium; hence, it could be linked to the beneficial action of the nucleoside in the maintenance of mitochondrial function. The changes in the hepatocyte redox state, induced by CCl_4 and/or adenosine, seem to modify collagen and nitrogen metabolism, indicating a linear correlation between the redox state and the collagen synthesis rate, whereas an inverse relationship was observed with collagenase activity. The possible role of the changes in cell redox state as signals for communication between parenchymal and mesenchymal liver cells is discussed. The results suggest an important correlation among mitochondrial function, cellular redox state, and regulation of collagen metabolism that could be relevant for the physio-pathology of this model of experimental cirrhosis.

Key words: Hepatotoxicity; NAD/NADH ratio; Mitochondrial damage; Fibrosis; (Liver)

1. Introduction

A direct carbon tetrachloride (CCl_4) toxicity is exerted on all the tissues capable of metabolizing it to free radicals with the participation of cytochrome *P*-450 [1]; but other tissues might also be affected by its metabolic products. However, since the liver accounts for most of CCl_4 metabolism, its chronic administration to rats results in a degenerative liver damage that resembles, in many aspects, human cirrhosis [2]. The role of mitochondrial injury, as a possible underlying event associated to CCl_4-induced liver fibrosis, has become an issue of study in the mechanisms of experimentally induced cirrhosis [3–5]. The present study attempts to evaluate mitochondrial function in vivo through the assessment of the mitochondrial and cytoplasmic redox states during generation of CCl_4-induced liver damage.

The cell redox state is an important regulatory factor of several metabolic fluxes [6], and it could be especially relevant in collagen metabolism, since the increase in hepatic reduced power, namely NAD(P)H, favours conversion of glutamate to proline [7], decreases proline degradation [8], slows proline transport to mitochondria [9] and its oxidation [10], and promotes interactions between proline and metabolic pathways, such as the hexose monophosphate shunt [11]. As a whole, these actions could elicit an increase in liver proline, which is an important component of collagen: an elevation in collagen synthesis could lead to liver fibrosis.

Adenosine administration to chronic CCl_4-treated rats improves liver function, reduces collagen accumulation, and maintains cell energy availability [12]. The nucleoside has been shown to protect completely the liver mitochon-

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drial function in rats made cirrhotic by CCl_4 poisoning [5]. In addition, acute adenosine administration elevates cytoplasmic and mitochondrial NAD/NADH ratios in vivo and in vitro [13,14].

The aim of the present study was to evaluate whether CCl_4-induced cirrhosis promotes disturbances in cell redox states, as calculated from the cytoplasmic [lactate]/[pyruvate] ratio and from the β-hydroxybutyrate/acetoacetate and [glutamate]/[oxoglutarate][ammonia] ratios in mitochondria, and whether the possible alterations are counteracted by simultaneous administration of adenosine. Also, we tried to correlate the changes in cellular redox state with collagen and nitrogen metabolisms, which are markedly affected during CCl_4-induced liver damage [2,15].

2. Material and methods

2.1. Materials

CCl_4 was purchased from Merck (Mexico). All other reagents were from Sigma (St. Louis, MO).

2.2. Animal procedures

Male Wistar rats (90–100 g, initial body weight) were used throughout this study. The animals were fed with lab chow and had water ad libitum; injected intraperitoneally with CCl_4 (0.025 ml, diluted in peanut's oil) and adenosine (200 mg/kg body weight, pH 7.4), three times weekly. The experimental groups and the treatment schedule have been described elsewhere [12]. Two days after treatment cessation, animals were fasted overnight and, under general anesthesia with sodium pentobarbital (40 mg/kg body weight), liver samples were taken and acid extracts ($HClO_4$, 6% w/v) were rapidly obtained to quantify metabolites. From the remaining liver, samples were used to determine the rate of collagen synthesis and breakdown. Histological and biochemical assessments of the CCl_4-induced necrosis and fibrosis were performed as described elsewhere [12].

2.3. Analytical procedures

The acid extracts, obtained as described above, were neutralized with 4 M K_2CO_3 and the metabolites were enzymatically quantified as follows: lactate according to Noll [16], pyruvate by the method of Lamprecht and Heinz [17], and acetoacetate (AA) and β-hydroxybutyrate (β-HB) using the assay described by Kientsch-Engel and Siess [18]. Glutamate (GLU) an glutamine (GLN) were assayed by the method of Hughey et al. [19], and α-ketoglutarate (α-KG) by that of Bergmeyer and Bernt [20]. Ammonia was measured by the GDH reaction, according to the method of Schmidt [21], and urea was estimated by the colorimetric method of Gutman and Bergmeyer [22]. The hepatic free proline pool, rate of collagen synthesis in vivo (evaluated by labelled proline incorporation to collagen-bound hydroxyproline), and collagenase activity in vitro were measured as previously described in detail [12].

2.4. Calculations

Cytoplasmic and mitochondrial NAD/NADH ratios were calculated using the following equation: NAD/NADH = [oxidized substrate]/[reduced substrate] $\times 1/K_{eq}$, taking into account the K_{eq} of lactate dehydrogenase for the cytosolic fraction, and the K_{eq} of β-hydroxybutyrate (β-HBDH) and glutamic (GDH) dehydrogenases for the mitochondrial fraction [23]. Redox potentials (E_h), defined as the ability to dissect an overall electron transfer into two half-reactions [24], were calculated by means of the Nernst equation, as follows: $E_h = E'_0 + 0.03 \log NAD/NADH$, where $E'_0 = 0.314$ V.

Table 1
Hepatic levels of lactate, pyruvate, β-hydroxybutyrate, and acetoacetate after chronic CCl_4 and adenosine administration

Parameter (μmol/g of liver)	Treatment			
	control	adenosine	CCl_4	CCl_4 + adenosine
4 weeks				
Lactate	1.65 ± 0.16	1.57 ± 0.10	2.72 ± 0.42	1.54 ± 0.12 **
Pyruvate	0.17 ± 0.02	0.19 ± 0.02	0.11 ± 0.01 *	0.14 ± 0.01
β-Hydroxybutyrate	0.24 ± 0.02	0.23 ± 0.03	0.24 ± 0.03	0.14 ± 0.01 *,**
Acetoacetate	0.20 ± 0.03	0.22 ± 0.03	0.10 ± 0.0 *	0.13 ± 0.01
β-HB + AA	0.44 ± 0.06	0.45 ± 0.06	0.34 ± 0.03	0.27 ± 0.02 *
8 weeks				
Lactate	1.74 ± 0.18	1.64 ± 0.12	2.42 ± 0.34	2.15 ± 0.27
Pyruvate	0.16 ± 0.02	0.18 ± 0.02	0.13 ± 0.01	0.19 ± 0.02 **
β-Hydroxybutyrate	0.27 ± 0.03	0.24 ± 0.03	0.48 ± 0.07 *	0.36 ± 0.05
Acetoacetate	0.22 ± 0.03	0.23 ± 0.03	0.15 ± 0.01	0.30 ± 0.05 **
β-HB + AA	0.49 ± 0.07	0.47 ± 0.06	0.63 ± 0.07	0.66 ± 0.10

The redox-pair metabolites were enzymatically quantified in liver extracts after 4 and 8 weeks of treatment. The results are expressed as mean ± S.E. of at least seven individual observations. Statistical significance: $P < 0.01$ as compared to the control group (*) or CCl_4 + saline group (**).

2.5. Statistics

All results are expressed as mean ± S.E. The significance of the differences among groups was assessed by two-way ANOVA test.

3. Results

3.1. Levels of redox coupled metabolites

The effects of chronic administration of CCl_4 and/or adenosine on marker metabolites for hepatic cytoplasmic and mitochondrial redox states are depicted in Table 1. After 4 weeks, CCl_4 induced a significant diminution of oxidized metabolites, pyruvate and AA, without significant changes in their reduced couple. At 8 weeks, the decrease in pyruvate and AA was not significant, whereas a striking increase in β-HB was observed (Table 1). The simultaneous administration of adenosine to CCl_4-poisoned rats reduced the drop of oxidized metabolites and diminished the mitochondrial reduced substrate (β-HB) after 4 weeks of treatment (Table 1). After 8 weeks, the simultaneous treatment with the nucleoside resulted in a significant elevation of pyruvate and AA levels, as compared to the CCl_4 plus saline group (Table 1). Other marker metabolites of the mitochondrial redox state are those participating in the GDH reaction, namely GLU, α-KG, and ammonia (Table 2). CCl_4 administration showed a similar effect to that observed in the other mitochondrial redox-pair metabolites, that is, a decrease of the oxidized substrate (α-KG), at 4 and 8 weeks of treatment, and a significant increase of liver GLU level after 8 weeks (Table 2). Ammonia content showed minor variations in the presence of the hepatotoxin. Adenosine administration to the CCl_4-treated group, partially prevented the changes in GLU and α-KG levels induced by CCl_4; moreover, a significant decrease of liver ammonia was recorded after 8 weeks of treatment (Table 2).

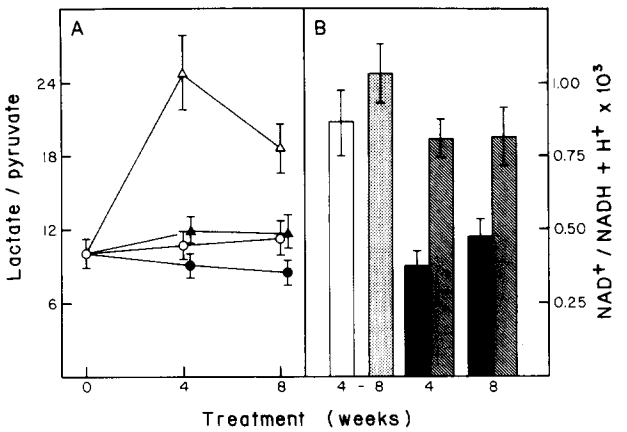

Fig. 1. Effects of chronic administration of CCl_4 and adenosine on cytoplasmic NAD/NADH ratio. For the lactate/pyruvate ratio (A), data were taken from Table 1. The cytoplasmic NAD/NADH ratios (B) were calculated using the equilibrium constant of the LDH reaction (see Materials and methods). Symbols are as follows: control group (empty circles and bars), adenosine alone (filled circles and dotted bars), CCl_4 plus saline (empty triangles and solid bars), and CCl_4 plus adenosine group (filled triangles and shaded bars).

3.2. The NAD / NADH ratios and redox potential

From the values of the redox couples, we calculated the reduced/oxidized substrate ratios, the NAD/NADH ratio, and the redox potential. CCl_4 administration produced a significant increase in the lactate/pyruvate ratio at 4 and 8 weeks of treatment, resulting in a marked diminution of the cytosolic NAD/NADH ratio (Fig. 1A and B). Adenosine treatment of the CCl_4-intoxicated rats elicited a protection against the hepatotoxic effects (Fig. 1A and B).

Similar results were obtained for the mitochondrial NAD/NADH ratio, calculated from the β-HB/AA ratio (Fig. 2A). A significant decrease of the NAD/NADH ratio was noticed after 4 and 8 weeks of CCl_4 administration, simultaneous administration of the nucleoside also prevented these changes (Fig. 2A and B). CCl_4 also induced changes in the metabolites involved in the GDH reaction,

Table 2
Effects of CCl_4 and adenosine on liver levels of metabolites involved in the GDH reaction and nitrogen metabolism

Metabolite (μmols/g)	Treatment				
	Control	4 weeks		8 weeks	
		CCl_4	CCl_4 + adenosine	CCl_4	CCl_4 + adenosine
GLU	4.30 ± 0.42	4.20 ± 0.37	4.00 ± 0.36	5.72 ± 0.2 *	4.74 ± 0.40
αKG	0.36 ± 0.06	0.24 ± 0.02	0.28 ± 0.02	0.22 ± 0.02	0.44 ± 0.04 **
Ammonia	1.03 ± 0.05	1.24 ± 0.19	1.16 ± 0.15	1.15 ± 0.10	0.80 ± 0.09 **
NAD/NADH [GDH]	22.1 ± 2.3	18.2 ± 1.9	20.8 ± 2.0	11.3 ± 0.9 *	19.0 ± 1.8 **
GLN	3.66 ± 0.24	3.09 ± 0.15	4.33 ± 0.20	3.29 ± 0.15	5.07 ± 0.31 ***
Urea	5.27 ± 0.47	6.19 ± 0.47	5.80 ± 0.90	7.00 ± 0.43	6.76 ± 0.71
Urea/GLN	1.56 ± 0.11	2.00 ± 0.1 *	1.34 ± 0.13 **	2.13 ± 0.1 *	1.33 ± 0.1 **
Proline	0.32 ± 0.03	0.52 ± 0.0 *	0.39 ± 0.02 **	0.54 ± 0.0 *	0.35 ± 0.03 **

Metabolites were determined colorimetrically or enzymatically after 4 and 8 weeks of treatment. Mitochondrial NAD/NADH was calculated using the K_{eq} of GDH as described under Materials and methods. Results are expressed as mean ± S.E. of at least seven individual experiments. Statistical significance is as described in Table 1.

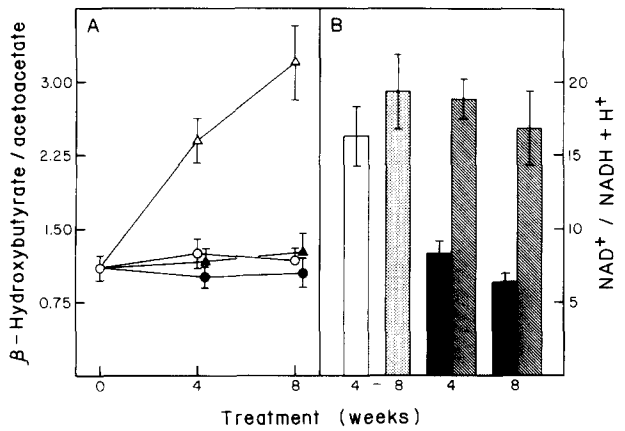

Fig. 2. Changes of the mitochondrial NAD/NADH ratio after chronic administration of CCl_4 and adenosine. The β-hydroxybutyrate/acetoacetate ratio (A) was calculated from the data shown in Table 1. The mitochondria NAD/NADH ratios (B) were assessed using the equilibrium constant of β-HBDH (see Materials and methods). Symbols are as described in Fig. 1.

reflecting a lower mitochondrial NAD/NADH ratio (Table 2). On the other hand, the redox potentials of the cytosol ($E_{h,cyt}$) and mitochondria ($E_{h,mit}$), calculated from the values mentioned above, were $E_{h,cyt} = -223.6 \pm 8.8$ mV, and $E_{h,mit} = -273.1 \pm 10.1$ mV, giving an $E_{h,mit} - E_{h,cyt}$ difference of 49.5 ± 1.6 mV in the control groups. Adenosine alone did not change these values, keeping the difference between both compartments in 51.3 ± 1.7 mV. After 4 weeks of CCl_4 treatment, the redox potentials became more negative ($E_{h,cyt} = -236.8 \pm 8.8$ mV, and $E_{h,mit} = -286.3 \pm 11.3$), but the potential difference was maintained (49.5 ± 1.9 mV). After 8 weeks of treatment, the changes promoted by the hepatotoxin ($E_{h,cyt} = -232.7 \pm 8.3$ mV, and $E_{h,mit} = -289.4 \pm 10.7$ mV) resulted in a difference between the potentials of 56.7 ± 1.8 mV, indicating a small but significant shift of around 7 mV ($P < 0.02$, against the control group). Concomitant administration of adenosine blocked completely the effect of the hepatotoxin, maintaining the mentioned difference of 50.5 ± 2.0 mV ($E_{h,cyt} = 228.3 \pm 8.0$ mV, and $E_{h,mit} = 278.8 \pm 9.2$ mV) after 8 weeks.

3.3. Metabolites involved in nitrogen and proline metabolism

The metabolites involved in the GDH reaction, besides being useful to calculate the mitochondrial NAD/NADH ratio, participate in the proline and nitrogen metabolisms. Table 2 shows that, after 4 weeks of treatment, CCl_4 promoted a significant diminution of α-KG, which was accompanied by a significant increase in the free proline pool. After 8 weeks, increased levels of liver GLU and a persistent decrease in α-KG were found in the CCl_4-treated rats; furthermore, liver proline remained high at this time (Table 2). Simultaneous adenosine treatment prevented the CCl_4-induced changes, mainly at 8 weeks, when a significant increase in α-KG level was noticed. Ammonia levels showed minor modifications in the presence of CCl_4, however, an unexpected reduction of the level of this compound was obtained in rats treated with CCl_4 plus adenosine. This result led us to investigate the metabolic fate of GLU's α-amino group and to determine the liver contents of GLN and urea (Table 2). The animals receiving adenosine, in the presence or absence of CCl_4, had higher levels of GLN, whereas urea did not change significantly in any of the experimental groups. As a consequence, the decrease of the urea/GLN ratio, induced by the nucleoside, suggests the participation of adenosine in nitrogen metabolism, which could be important for the decrease in the liver proline pool (Table 2).

3.4. Collagen metabolism and cell redox state

The rate of collagen synthesis, assessed by proline incorporation to collagen-bound hydroxyproline, was gradually enhanced by CCl_4 treatment, and this effect was accompanied by a progressive loss of collagenolytic activity in the liver samples from the CCl_4-treated animals.

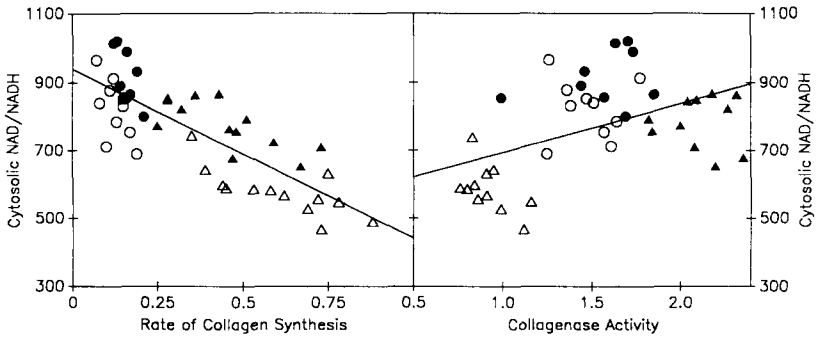

Fig. 3. Correlation between cytoplasmic redox state and parameters of collagen metabolism. The correlation between cytoplasmic NADH/NADH ratio with the rate of collagen synthesis (left panel) and against collagenase activity (right panel) was analyzed by plotting the values for controls ($n = 9$), adenosine alone ($n = 8$), CCl_4 alone ($n = 14$, 4 and 8 weeks), and CCl_4 plus adenosine treated animals ($n = 14$, 4 and 8 weeks). Left panel (correlation coefficient (r) = 0.81, $P < 0.005$), and right panel ($r = 0.51$, $P < 0.1$). Symbols for each experimental group as represented in Fig. 1.

Simultaneous administration of adenosine partially blocked the stimulated collagen synthesis, induced by the hepatotoxin, maintaining high levels of hepatic collagenase activity (Figs. 3 and 4). We plotted collagen synthesis and collagenase activity against either mitochondrial or cytoplasmic NAD/NADH ratio, searching for a possible correlation among these parameters. Fig. 3 shows the correlation among collagen synthesis, collagenase activity, and cytoplasmic NAD/NADH ratio. A highly significant inverse correlation between collagen synthesis and cytoplasmic NAD/NADH ratio ($r = 0.81$, $P < 0.005$) was observed, whereas the latter parameter seemed to have less influence on collagenase activity ($r = 0.51$, $P < 0.1$). On the other hand, mitochondrial NAD/NADH ratio showed a striking inverse correlation with the rate of collagen synthesis ($r = 0.84$, $P < 0.005$) and a linear relationship with collagenase activity ($r = 0.93$, $P < 0.001$, Fig. 4). Thus, these data suggest a putative relationship between cellular redox state and collagen metabolism.

4. Discussion

The present study demonstrates that chronic administration of CCl_4 induced drastic changes in cytoplasmic and mitochondrial redox states, promoting a more reduced state in the liver cell. Simultaneous administration of adenosine counteracted the CCl_4 effects on cellular redox state, maintaining the parameters within normal values.

4.1. Effects of CCl_4 on liver redox state

The alterations in the cytoplasmic and mitochondrial redox state, towards a reduced condition after 4 and 8 weeks of treatment, could be related to a progressive and severe mitochondrial dysfunction induced by the hepatotoxin [3–5]. Indeed, we recently reported, in cirrhotic rats [5], a decrease in oxygen consumption, respiratory control and ADP/O values mainly with site I substrates, an important decrease in ATP synthesis, a 30-mV diminution of the membrane's electrical potential, as well as a deficient activity of the malate-aspartate shuttle, which constitutes the main liver pathway for the mobilization of cytoplasmic reducing equivalents into the mitochondria. It is possible that all CCl_4-induced mitochondrial alterations, are also reflected in the cytoplasmic redox state. The latter is suggestive of the important role played by the transport of the reducing equivalent pathway, as represented by the malate-aspartate shuttle, to maintain close to 50 mV the difference between the mitochondrial and the cytoplasmic redox potentials. Thus, we believe that, after 8 weeks of CCl_4 treatment, the mitochondrial damage in vivo is of such a magnitude that the difference between the redox potentials underwent a significant imbalance of 7 mV, as compared to the other groups. To support this statement are the reports that the perfusate of livers with secondary biliary cirrhosis also show an increment in lactate/pyruvate and β-HB/AA ratios, and these changes are accompanied by a reduced activity of the mitochondrial transport chain [25].

The nature of the mitochondrial injury induced by chronic CCl_4 exposure, has not been clearly established; however, it is known that CCl_4 induces profound alterations in the lipid composition of membranes [5,26], affecting the activities of enzymes attached to the inner mitochondrial membrane but not those of the matrix [27]. Aside from the mechanism involved in the CCl_4-induced mitochondrial injury, adenosine treatment completely protected against the alterations generated by the hepatotoxin.

4.2. Redox state linked to nitrogen and proline metabolisms

The difference in the NAD/NADH ratio, calculated by the GDH redox pair metabolites, in comparison with those involved in the β-HBDH reaction deserve a special comment. The latter is more representative of the mitochondrial NAD/NADH ratio, since its metabolites are not used by the liver, whereas the substrates and products of the GDH reaction participate in nitrogen and proline metabolism [28,29]. In this sense, the results obtained

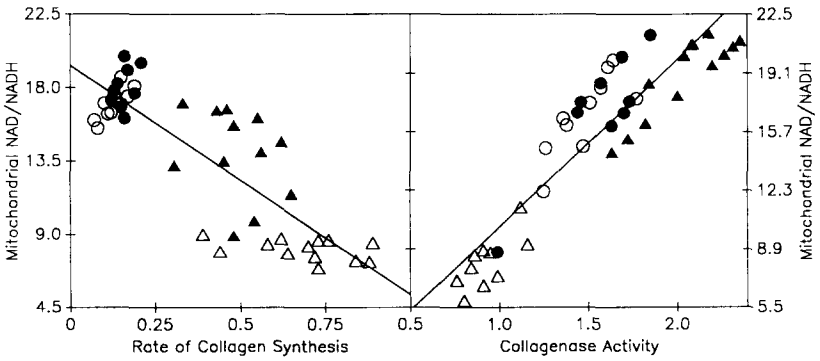

Fig. 4. Correlationship between mitochondrial NAD/NADH ratio and collagen synthesis and removal. We used the same number of data and symbols as described in Fig. 3. Left panel ($r = 0.84$, $P < 0.005$) correlation between mitochondrial NAD/NADH ratio and rate of collagen synthesis, and right panel ($r = 0.94$, $P < 0.001$) against collagenase activity. Same symbols as in Fig. 3.

suggest that the GLU and α-KG fluxes to proline and hydroxyproline are favored by the increase in the reduced state of the cell (Table 2); moreover, the adenosine effect, per se or in the presence of CCl_4, of increasing GLN (4–8 weeks) and decreasing liver ammonia (8 weeks) suggests that nitrogen metabolism is affected by the nucleoside (e.g., Glu conversion to GLN). At present, we do not know whether this action is directly associated to adenosine administration or is promoted by indirect means acting on mitochondrial metabolism and redox state. As a final result, an increased GLN formation will cause a decreased GLU level, which could facilitate normalization of the redox state.

4.3. Correlation between redox state and collagen metabolism

A striking correlation of collagen synthesis and breakdown with the cytosolic and mitochondrial redox potentials was observed (Figs. 3 and 4). Considering that proline synthesis from glutamate is a pathway that requires NADH [7,30], the increase in the free-proline pool (Table 2) might be due to the presence of a more reduced state of the liver cell. Furthermore, the decrease of α-KG (Table 2), which is reflecting the drop of mitochondrial redox state, could be linked to a greater utilization for proline hydroxylation [31,32]. In addition, proline degradation depends on two NAD-dependent mitochondrial enzymes: proline oxidase [33] and pyrroline-5-carboxylate dehydrogenase [34], which can limit proline availability. Thus, the reduced cell state could induce a lower activity in these enzymes and, consequently, promote the free-proline pool. Besides, the increase of hepatic lactate per se, or by modifying the redox state, could provide a competitive inhibition of proline oxidase [35] and enhance proline incorporation into collagen hydroxyproline. These effects have been demonstrated in liver slices of CCl_4-induced cirrhotic rats [36] and in myofibroblasts obtained from livers of alcohol-treated baboons [37]. It has also been shown that lactate favours collagen synthesis by isolated fat-storing cells (Ito cells) [38].

All these considerations give further support to the inverse correlation shown in Figs. 3 and 4, where a diminution of the NAD/NADH ratio in cytosol and mitochondria is related with an increase in collagen synthesis. Simultaneous administration of adenosine prevented mitochondrial damage and the shift of cell redox state, and also avoided the enhancement of the free-proline pool (Table 2) and the rate of collagen synthesis [12]. The aforementioned agrees with the finding that the size of the proline liver pool is a controlling factor for collagen synthesis [39].

On the other hand, little is known about the factors that modulate collagenase activity, although it is accepted that up to 15% of all the synthesized collagen may be degraded intracellularly [40], that the activation of a latent pro-enzyme can occur in vivo by a disruption of a zinc-cysteine complex [41], and that factors, such as the transforming growth factor β (TGF-β), inhibit collagenase activity [42]. In this context, the possibility that the CCl_4-induced shift of the redox potential to the reduced side could influence the collagenase activity remains to be elucidated.

4.4. Cell redox state as signal for communication between liver parenchymal and mesenchymal cells

Ito cells and endothelial cells play a major role in collagen synthesis during CCl_4-induced liver fibrosis, whereas hepatocytes do not modify their rate of collagen formation during the generation of this type of liver damage [43–45]. We assume that the changes reported here in redox state are mainly reflecting the events occurring in the parenchymal cells (hepatocytes), where CCl_4 exerts its toxic action. Therefore, the question arises on what kind of communication exists between parenchymal and non-parenchymal liver cells. To this regard, a correlation between TGF-β and procollagen-type transcripts in mesenchymal cells during CCl_4-induced cirrhosis has been reported [45]. Thus, changes in the metabolic pathways of hepatocytes, controlled by the redox state, might produce metabolites involved in collagen metabolism that can trigger overproduction of collagenous proteins and modulate their deposition in liver tissue. It is possible that the adenosine treatment, by preventing the mitochondrial damage induced by the hepatotoxin [5], could maintain the redox state in both cellular compartments within normal range and, in this way, exert its beneficial action by conserving a normal or an increased collagen turnover [12].

4.5. Possible mechanisms involved in adenosine protection against CCl_4-induced damage

The nucleoside is a potent regulator of hepatic arterial blood flow [46]. This property of adenosine could counteract liver hypoxia, which has been claimed to occur as an underlying mechanism of CCl_4 hepatotoxicity [47–49]. Moreover, the effect of adenosine in preserving the function and membrane composition of liver mitochondria, and on increasing the activity of the malate-aspartate shuttle after chronic CCl_4 treatment [5], as well as in isolated hepatocytes [14], might play an important role in the effects of the nucleoside in maintaining the cellular redox state. The actions of adenosine led us to evaluate the changes in the liver redox potentials, as an important factor involved in the generation of liver fibrosis elicited by chronic CCl_4 treatment.

In summary, the data presented suggest a possible role of mitochondrial function, direct or indirectly, on collagen metabolism. Therefore, the therapeutic strategies for hepatic fibrosis might be focussed on preserving mitochondrial function and, in this way, possibly arrest the sequence of events leading to cirrhosis.

Acknowledgments

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