EFFECT OF CADMIUM FLUORIDE AND QUERCETIN ON IN VIVO ACTIVITY OF INDOLEAMINE 2,3-DIOXYGENASE IN MICE LIVER AND KIDNEY

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SUMMARY: The present study was undertaken to study the dose response of cadmium fluoride (CdF₂) on indoleamine 2,3-dioxygenase (IDO) activity in the liver and kidneys of mice. The possible protective effect of quercetin on CdF₂-induced alteration in enzyme activity was also studied. The following six groups, each consisting of six mice, were studied: (i) normal (control) group; (ii) mice treated with single i.p. injection of 1 mg/kg body weight (bw) CdF₂; (iii) mice treated with single i.p. injection of 2 mg/kg bw CdF₂; (iv) mice treated with single i.p. injection of 4 mg/kg bw CdF₂; (v) mice treated with single i.p. injection of quercetin (100mg/kg bw) alone; (vi) mice treated with i.p. injection of 100 mg/kg bw of quercetin followed by i.p. injection of CdF₂ (2 mg/kg bw). Mice receiving 4 mg/kg bw of CdF₂ died within 2 hours while those which received 2 mg/kg bw of CdF₂ demonstrated a significant decrease in IDO activity in kidneys (p<0.001) and liver (p<0.01) compared to that of the control group. On the other hand, the 100 mg quercetin/kg bw injection resulted in a significant increase (p<0.001) in IDO activity in both organs when compared to control group of mice. Administration of quercetin 2 hours before injection of CdF₂ resulted in resulted in the IDO activity in both liver and kidney remaining at near normal levels which were not significantly different from the control levels. In conclusion, CdF₂ at different doses led to inhibition of IDO activity which may be due to stimulation of the nitric oxide synthase system.

Keywords: Cadmium fluoride; Indoleamine 2,3-dioxygenase; Kidney; Liver; Quercetin.

INTRODUCTION

Indoleamine 2,3-dioxygenase (IDO) is an immuno-modulatory enzyme and its activity is elevated in several inflammatory conditions, such as infection, autoimmune disorders, and malignancies. IDO is a rate-limiting enzyme in the kynurenine pathway of tryptophan metabolism and is expressed in various cells and tissues including dendritic cells and macrophages in response to interferon gamma (IFN-γ), Tumor Necrosis Factor alpha (TNF-α), and lipopolysaccharide (LPS). Increase in IDO activity leads to immunosuppression as the enzyme has the ability to inhibit lymphocyte proliferation. The enzyme is also linked with neurotoxicity through the generation of quinolinic acid and other toxins. Signs of increased IDO activity correlate with tryptophan depletion, progression of systemic and brain HIV-1 infection, and HIV-1-associated dementia (HAD). Accumulating evidence suggests that IDO serves immunoregulatory, and tolerogenic functions. A number of studies indicate that IDO over expression by antigen processing cells (APCs) may result in immune suppression and reduced T-cell responses by enhancing Fas-mediated apoptosis in them. The capability of transferring a pro-oxidant (O₂⁻) into antioxidants makes IDO a powerful...

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Fluoride (F) at various doses has been shown to induce lipid peroxidation and changes in drug metabolizing enzymes. Cadmium fluoride (CdF₂) is the most hepatotoxic and lethal of all Cd-containing compounds. The toxic effects of CdF₂ appear to depend on its elimination and detoxification. The present in vivo study was therefore conducted to study the effect of different doses of cadmium fluoride (CdF₂) on IDO activity in mice liver and kidneys. In addition, the possible protective effect of quercetin on CdF₂-induced changes on IDO activity was examined in the liver and kidneys of mice.

**MATERIALS AND METHODS**

**Chemicals:** All reagents and chemicals, including ascorbic acid, bovine serum albumin, catalase, p-dimethaminobenzaldehyde (Ehrlich’s reagent), kynurenine, trichloroacetic acid, methylene blue, potassium dihydrogen phosphate, CdF₂, and L-tryptophan were purchased from Sigma Chemical Company, St Louis, MO, USA.

**Animal Care:** Healthy 4-to-5-week-old mice weighing 25–30g (male/female) were obtained from the Animal Breeding Laboratory, King Saud University, Riyadh, Saudi Arabia. A week after acclimatization the mice were divided into different groups consisting of six mice each as described below. The experimental protocol was approved by the King Saud University Research Center, and the ethical animal care guidelines were followed.

**Dose-response of CdF₂ on indoleamine 2,3-dioxygenase activity in mice liver and kidney:** For these experiments, four groups of six mice each were studied: (i) normal (control) mice; (ii, iii, and iv) CdF₂-treated mice administered single i.p. injections of 1, 2, and 4 mg CdF₂/kg bw, respectively. After 24 hr the animals were sacrificed by asphyxiation with carbon dioxide.

**Protective effect of quercetin on CdF₂-induced alteration in indoleamine 2,3-dioxygenase activity in mice liver and kidney:** The following two groups of six mice each were studied: (v) mice injected i.p. with 100 mg quercetin/kg bw; and (vi) mice injected i.p. with 100 mg quercetin/kg bw followed 2 hours later with i.p. injection of 2 mg CdF₂/kg bw. After 24 hr all mice were sacrificed by asphyxiation with carbon dioxide.

**Preparation of liver and kidney samples:** After the animals were killed, the liver and kidneys were dissected out, cleared of adhering tissues, and weighed. The organs were then homogenized in normal saline (10% w/v). The homogenates were centrifuged at 13,000 rpm for 60 min and used for determination of IDO activity.

**Measurement of indoleamine 2,3-dioxygenase activity (IDO):** The activity of indoleamine 2,3-dioxygenase in the liver and kidney extracts was measured by the method of Kudo and Boyd with slight modifications. Briefly, 0.4 mL of enzyme extract was added to the buffer mixture. The buffer mixture consisted of 0.1 mL of 10 mM L-tryptophan, 0.2 mL of 200 µM of methylene blue, 0.2 mL of 400 mM ascorbic acid, 77 µL of suitably diluted catalase which had an enzyme activity of...
200 units/mL (from stock of 518,700 units/mL), and 0.5 mL of 0.4 M pH 6.5 potassium phosphate buffer. Before mixing, the enzyme supernatant and the incubation buffer mixture were pre-heated to 37°C. The final volume of the reaction mixture was made up to 2 mL with double distilled water. The mixture was incubated for 20 min at 37°C, and 0.2 mL of 30% (w/v) trichloroacetic acid was added to stop the reaction. The mixture was heated at 50°C for 30 min to hydrolyze the N-formyl kynurenine formed from L-kynurenine released by the action of indoleamine 2,3-dioxygenase on the L-tryptophan. The mixture was then centrifuged at 6,000 rpm at room temperature to remove sediment. To 1.0 mL of the supernatant was added 1.0 mL of 1% (w/v) p-dimethylaminobenzaldehyde in acetic acid. The absorbance was determined at 480 nm to measure the amount of L-kynurenine by a standard intensity calibration. The enzyme activity was expressed as mean±SD×10^{-3} micrograms of L-kynurenine formed/min/mg protein. Protein concentration was determined by the method of Markwell et al. using bovine serum albumin as standard.

**Statistical Analysis:** Samples were run in duplicate. IDO activity between groups was compared using one-way ANOVA analysis followed by Tukey’s test for multiple comparisons. Values were considered significant at p<0.05. Receiver operating characteristic analysis was done. Area under the curve, specificity and sensitivity were calculated.

**RESULTS**

Figure 1 shows the effect of CdF$_2$ on IDO activity in liver and kidney of mice.

**Figure 1.** Effect of different i.p. injection dosages of CdF$_2$ on indoleamine 2,3-dioxygenase (IDO) activity in liver and kidney tissues. Enzyme activity is expressed as mean±SD×10^{-3} (n=6) micrograms of L-kynurinine formed/min/mg protein.***p<0.001 when compared to control group (Independent Samples T-Test between the control and the treated groups in kidney). **p<0.01 when compared to control group (Independent Samples T-Test between the control and the treated groups in kidney).
Both 1 mg and 2 mg/kg bw caused a highly significant decrease of IDO activity in kidneys and liver. Figure 2 shows the protective effect of i.p. injection of quercetin (100 mg/kg bw) on changes induced by CdF$_2$ (2 mg/kg bw) in kidney and liver IDO activity of mice.

Quercetin injection alone caused significant increases (p<0.001) in IDO activity and CdF$_2$ injections alone caused significant decreases in IDO activity of kidneys (p<0.001) and liver (p<0.01), but administration of quercetin 2 hr before injection of CdF$_2$ resulted in the IDO activity in both liver and kidney remaining at near normal levels which were not significantly different from the control levels. Figures 3–6 show the ROC analysis of the IDO activity of all treated groups in kidney and liver. It was found that treatments with quercetin, CdF$_2$ and quercetin + CdF$_2$ in kidneys showed 100, 83.3 and 83.3% sensitivity and 100, 100 and 72.2% specificity respectively and 100,100 and 66.7% sensitivity and 61.1, 72.2 and 50% specificity in liver. The Table shows effect of CdF$_2$ and quercetin on protein content in the kidneys and liver of mice. The results showed non-significant increase in protein contents of groups treated with 2 mg/kg bw of CdF$_2$ when compared with control group. A relatively significant increase in protein content was found in mice treated with 1 mg/kg bw of CdF$_2$ (p<0.05). Quercetin alone also caused significant increase in protein contents and when administered before 2 hr protein values tend to return to normal levels although not significantly.
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Figure 3. Receiver operating characteristic (ROC) curve of kidney and liver IDO activity in group treated with CdF₂ (2 mg/kg bw) alone.

Figure 4. ROC curve of kidney and liver IDO activity in group treated with quercetin (100 mg/kg bw).
Figure 5. ROC curve of kidney in group treated with quercetin + CdF₂.

Figure 6. ROC curve of liver in group treated with quercetin + CdF₂.
Table. Effects of cadmium fluoride and/or quercetin on protein content in the kidneys and liver of mice

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Kidneys</th>
<th>Liver</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>4.14 ± 1.07</td>
<td>4.10 ± 1.29</td>
</tr>
<tr>
<td>1 mg CdF₂/kg bw</td>
<td>5.75 ± 1.17*</td>
<td>7.14 ± 1.52</td>
</tr>
<tr>
<td>2 mg CdF₂/kg bw</td>
<td>5.55 ± 1.36NS</td>
<td>7.46 ± 1.96</td>
</tr>
<tr>
<td>100 mg quercetin/kg bw</td>
<td>6.20 ± 2.20*</td>
<td>4.33 ± 0.50</td>
</tr>
<tr>
<td>100 mg quercetin + 2 mg CdF₂/kg bw</td>
<td>5.90 ± 1.64*</td>
<td>3.80 ± 0.47</td>
</tr>
</tbody>
</table>

Values are expressed as mg/mL protein. *p<0.05 when compared to control group (Independent Samples T-Test between the control and the treated groups). NS as compared to control group (Independent Samples T-Test between the control and the treated groups).

Figures 7–10 show the correlations between protein content and the enzyme activity in kidneys of all treated groups of mice.

**Figure 7.** Correlation between protein content and the enzyme activity with 1 mg CdF₂/kg bw in kidney with best fit line curve (non significant positive correlation).

**Figure 8.** Correlation between protein content and the enzyme activity with 2 mg CdF₂/kg bw in kidney with best fit line curve (non significant positive correlation).
The groups receiving CdF$_2$ were found to have non significant positive correlations between protein content and enzyme activity in kidney while the groups treated with quercetin, alone and followed by CdF$_2$, showed non significant negative correlations. Figures 11–14 show the correlations between protein content and the enzyme activity in liver of all treated groups of mice.

**Figure 9.** Correlation between protein content and the enzyme activity with 100 mg quercetin /kg bw in kidney with best fit line curve (non significant negative correlation).

**Figure 10.** Correlation between protein content and the enzyme activity with 100 mg quercetin/kg bw followed by 2mg CdF$_2$/kg bw in kidney with best fit line curve (non significant negative correlation).

**Figure 11.** Correlation between protein content and the enzyme activity with 1 mg CdF$_2$/kg bw in liver with best fit line curve (non significant positive correlation).
**Figure 12.** Correlation between protein content and the enzyme activity with 2 mg CdF₂/kg bw in liver with best fit line curve (non significant positive correlation).

**Figure 13.** Correlation between protein content and the enzyme activity with 100 mg quercetin /kg bw in liver with best fit line curve (non significant positive correlation).

**Figure 14.** Correlation between protein content and the enzyme activity with 100 mg quercetin/kg bw followed by 2mg CdF₂/kg bw in liver with best fit line curve (non significant negative correlation).
All groups were found to have non significant positive correlations between protein content and enzyme activity except the group treated with quercetin followed by CdF₂ which had a non significant negative correlation.

**DISCUSSION**

Indoleamine 2,3-dioxygenase (indoleamine-pyrrole 2,3-dioxygenase), was detected as a tryptophan cleaving enzyme widely distributed in the body and showing a wider substrate specificity than the classical liver tryptophan 2,3-dioxygenase.¹⁶,¹⁷ We tested the dose dependent effect of cadmium fluoride (CdF₂ 1, 2 and 4 mg/kg body weight) on IDO activity in liver and kidney of mice as it is already reported that CdF₂ at various concentrations induces toxicity and changes in drug metabolizing enzymes.¹² Further the protective effect of quercetin on CdF₂-induced changes on IDO activity was examined in both liver and kidney. Earlier, it was reported that rats exposed to NaF had an increased production of superoxide anions by the neutrophils.¹⁸ Since IDO uses superoxide anion radical (O₂⁻) as a cofactor in its catalytic process,¹⁹,²⁰ its activity should increase in all toxicity reactions. In the present study, CdF₂ caused a decrease in IDO activity which may indicate an impaired tryptophan metabolism. Kidneys showed a more significant decrease when compared with liver. Chronic renal diseases lead to the induction of IDO activity and INF-γ.²¹ Besides its ability to induce IDO activity INF-γ also induces nitric oxide synthase (NOS). NOS in turn leads to augmented nitric oxide (NO) synthesis, and NO is reported to have high affinity for haeme iron present in IDO enzyme. Also NO may interact with superoxide ion which is an essential cofactor for the IDO. Hence the stimulation of NOS activity leads to inhibition of IDO activity. Thus in our study decrease in IDO activity may be due to stimulation of NOS activity by CdF₂. Impaired IDO mediated tryptophan catabolism has also been reported in patients with autoimmune disease such as multiple sclerosis, Grave’s disease, autoimmune diabetes and rheumatoid arthritis.²²-²⁵ Polyphenols as antioxidants are shown to exhibit anticancer activities. However, the underlying mechanism has not been entirely characterized. Certain flavone molecules profoundly inhibit the enzymatic activity of IDO-1 but quercetin, a flavonol has been interestingly shown to stimulate IDO activity.²⁶ Our results are consistent with these studies. We also found that quercetin stimulates the enzymatic activity significantly when mice were treated alone with it at the dosages of 100 mg/kg bw. Also mice treated 2 hr before the exposure to CdF₂ showed the reversal of IDO activity to almost the same levels as in control mice hence proving its role as a protective agent. We chose a quercetin treatment period of 2 hours before of exposure to CdF₂ based on the previous data published on quercetin which state that the time-course of the plasma concentration of quercetin in humans is biphasic, with half-lives of 2–2.4 hr for the distribution phase and 16.8 hr for the elimination phase.²⁷ Interestingly we noticed that there was a marked decrease in the body weights of mice treated with quercetin alone after 24 hours which may be due to an increase in the expenditure of energy used to eliminate the quercetin (unpublished data). Receiver operating characteristic (ROC) curves analysis showed more sensitivity and specificity in kidneys than liver indicating the former to be more sensitive than liver. This may
be due to metabolism by the liver which has active xenobiotic metabolizing machinery. In this study there is a non significant positive correlation between protein content and IDO activity in kidneys in the groups receiving 1 mg/kg bw CdF$_2$ and 2 mg/kg bw CdF$_2$ while the groups receiving quercetin alone and quercetin followed by CdF$_2$ showed non significant negative correlation. All the treated groups showed non significant positive correlations in the liver except that of the group which received quercetin followed by CdF$_2$ which showed a non significant negative correlation. A positive correlation between the enzyme activity and protein content may indicate that the enzyme activity parallels the protein content.

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