EFFECTS OF FLUORIDE ON C-REACTIVE PROTEIN, ADENOSINE DEAMINASE, AND CERULOPLASMIN IN RABBIT SERA

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SUMMARY: Twenty healthy 6-month-old male and female New Zealand rabbits with a mean body weight of 3.5±0.5 kg were fed a standard commercial rabbit diet and provided with water *ad libitum* containing 40 mg F/L for 70 days. Blood samples were obtained from each rabbit, and sera adenosine deaminase (ADA) activity and C-reactive protein (CRP) and ceruloplasmin (CP) levels were determined with the following results at the start, at day 35, and at day 70, respectively: ADA activity 9.55±0.66, 14.78±1.11, and 19.56±1.85 U/L; CRP level 18.1±2.6, 85.5±20.3, and 123±23.26 ng/mL; CP level 22.19±2.66, 19.49±1.18, and 14.75± 0.96 mg/dL. By days 35 and 70 significant increases in ADA activity and CRP levels had occurred, along with a significant decrease in the CP level. These results demonstrated that fluoride intoxication caused significant alterations in ADA, CRP, and CP in rabbit sera.

Keywords: Adenosine deaminase, C-reactive protein; Ceruloplasmin; Fluoride in rabbits.

INTRODUCTION

Fluorosis is an important public health problem throughout many parts of the world. After chronic administration of fluoride to animals, various changes occur in the blood, brain, liver, muscle, heart, kidney, and spinal cord. These changes include abnormal behaviour patterns, altered neuronal and cerebrovascular integrity, and metabolic lesions. Generation of lipid peroxidation, free radicals, and altered antioxidant defence systems are considered to play an important role in the toxic effects of fluoride.[1-7] Exposure to fluorides can induce inflammatory reactions and cell cycle arrest[8] and an increase in the levels of markers of inflammatory reactions.[9] C-reactive protein (CRP) is synthesized in appreciable amounts following tissue injury, and is used as a marker of an inflammatory reaction.[10] Ceruloplasmin (CP) is produced by hepatocytes, and its synthesis is accelerated in response to inflammation.[11] Adenosine deaminase (ADA) also plays an important role in acute and protracted inflammatory responses.[12]

The aim of the present study was to investigate alterations in sera ADA activity and CRP and CP levels caused by elevated levels of fluoride ingestion from drinking water in rabbits.

MATERIALS AND METHODS

Animals and experimental procedure: Twenty healthy 6-month-old male and female New Zealand rabbits with a mean body weight of 3.5±0.5 kg were used. The rabbits were obtained from the Ondokuz Mayis University Animal Laboratory (Samsun, Turkey), and the study was approved by the University Ethics Committee.

After their clinical health was verified, the animals were housed in a well-ventilated, temperature-controlled (23±2ºC) hygienic room at 60% relative humidity under a 12-hr light/dark cycle. Throughout the study, the rabbits were

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allowed free access to standard rabbit chow (Samsun, Turkey) and drinking water containing 40 mg F/L. On day zero 7 mL of blood was collected from each rabbit via puncture of *v. auricularis magna* to determine the ADA activity and CRP and CP levels in the sera. Subsequently, on days 35 and 70, blood samples were again collected from each animal. All blood samples were placed in tubes and centrifuged (1550 g, 10 min, +4ºC). The separated serum samples were then stored at –80ºC until analysed.

Biochemical analyses: Serum ADA activities were measured using the Giusti method.[13] CRP was analysed with diagnostic commercial Elisa kits (Life Diagnostics, Inc. 2210-5). Serum CP analysis was conducted by a spectrophotometric method, which included use of *p*-phenyldiamine dichloride (PPD).[14]

Statistical analysis: The significance of differences between pre- and post-testing results was determined using the paired *t*-test.

RESULTS

Results of the analyses are given in the figure.

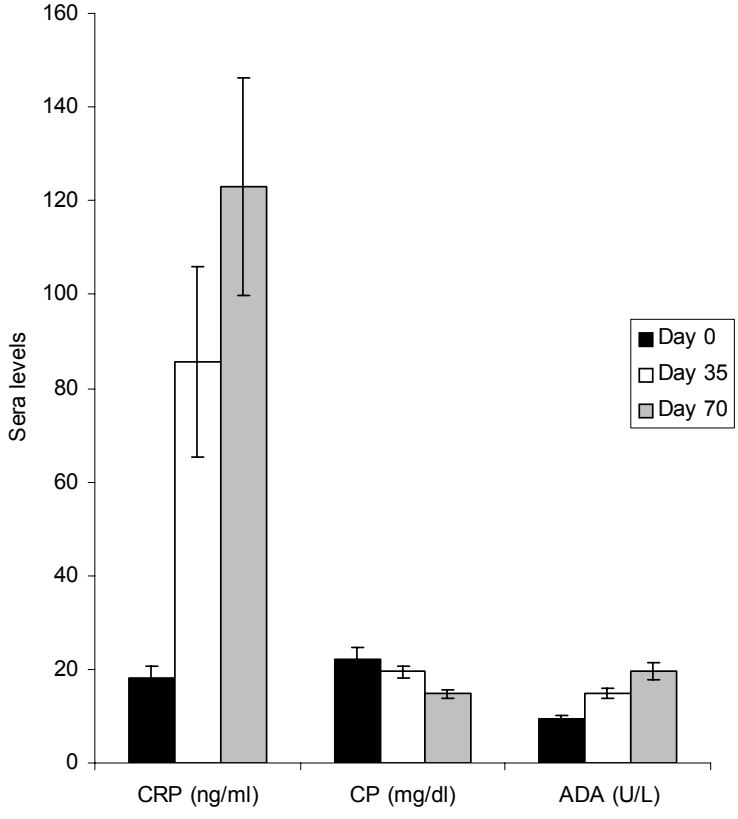

Figure. Rabbit sera CRP (ng/mL) activity, CP (mg/dL) levels and ADA (U/L) activity on days 0, 35, and 70(n=20).

As indicated by the data, a significant increase was observed in serum ADA activity ($p<0.001$) on days 35 and 70 and in the serum CRP level on day 35 ($p<0.01$) and 70 ($p<0.001$). For the CP level, however, a significant decrease was observed only on day 70 ($p<0.01$) compared to day 0.

DISCUSSION

The role of fluoride as a possible activator in the monocyte differentiation process also seems to be confirmed by the results of *in vivo* studies.[15] In addition, fluoride acts as an activator of alveolar macrophages enhancing the production of chemokines and pro-inflammatory cytokines (pro-inflammatory activity).[16] Furthermore, epithelial lung cell exposure to fluoride had been shown to release increased amounts of inflammatory cytokines, and fluoride has been reported to increase the production of cytokine.[17]

C-reactive protein (CRP) is a sensitive marker of systemic low-grade inflammation and is currently recommended as the principal inflammatory marker in research and clinical practice.[18] In our work, significantly higher CRP levels were observed in rabbit sera on days 35 ($p<0.01$) and 70 ($p<0.001$, compared to the level on day 0. Although few studies on CRP levels in fluorosis are on record, our findings are in accord with previous results reported by Susheela and Jethanandani.[9]

Adenosine deaminase (ADA) acts in differentiating lymphoid cells and is secreted in biological fluids during the cellular immune response against intracellular pathogens, but it can also be increased in other pathological processes.[19-23] Macrophages have been suggested as the cellular source of extracellular ADA activity. ADA is important in acute and protracted inflammatory responses. Recent work in our laboratory has demonstrated elevated ADA activity during inflammatory responses in macrophage-rich tissues, such as liver and spleen.[12,24,25] Similarly, the present study revealed significantly elevated sera ADA activity ($p<0.001$).

Ceruloplasmin (CP) is produced by hepatocytes, and its synthesis is accelerated in response to inflammation.[11] In our work, significant decreases ($p<0.01$) in CP levels were observed in rabbit sera on day 70, compared to that on day 0. Although not many reports were encountered about CP values in fluoride intoxication, results of Sharma[26] are concordant with those of the present study. Previous studies have also indicated that copper levels in serum decreased after chronic fluorosis.[27-29] Since CP is a copper-containing serum protein, this decrease in CP level may be related to the reduction in the levels of serum copper.

In conclusion, statistically significant increases in CRP levels and in ADA activities, along with the decrease in CP levels by day 35 and 70 observed in our study suggest that the ingestion of fluoride by rabbits via drinking water at 40 mg F/L caused acute phase response for both study periods. The data also indicate that acute phase protein CRP and CP levels and ADA activities, along with other parameters, may be determinative criteria for diagnosing fluoride poisoning.

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