Expression of PCNA and Ki-67 in the rat submandibular gland after one year cadmium intoxication - a preliminary study

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Abstract

The aim of our study was to determine to what degree one-year exposure of female rats to cadmium at a dose of 5, 50 and 100 mg Cd/l affects cell proliferation in the submandibular gland, shown by PCNA and Ki-67 expression. In the present study, we found a positive nuclear reaction for PCNA in single cells in microscopic preparations of control rats. In Group I, an increase was observed in the number of PCNA-positive cells, compared to that in the control. In Group II, the number of PCNA-positive cells was markedly higher than that in Group I and in the control. In the submandibular glands of rats in Group III, the number of PCNA-positive cells was similar to that, found in Group II. However, Ki-67 expression was sporadically observed in control submandibular glands, but not in Groups I, II and III.

Key words: submandibular gland, rat, expression of PCNA and Ki-67.

Introduction

Cadmium (Cd) is a trace metal which accumulates in the body organs with age [1, 2, 3]. Until now, it has been demonstrated that a 24-week exposure to cadmium at a dose of 5 and 50 mg Cd/l not only leads to its accumulation in the submandibular gland, but it also increases the percentage of cells, showing a positive reaction for the proliferating cell nuclear antigen (PCNA) in both experimental groups, compared to the control, with lack of human Ki-67 antigen (Ki-67) expression [4]. PCNA is treated as a co-factor for DNA polymerase in both the S phase of the cell cycle and in DNA synthesis, associated with DNA repair [5]. The Ki-67 antigen occurs in proliferating cells in the G_1, G_2, S and M phases of the cell cycle, but is absent during DNA repair [6]. The aim of the present study was to determine to what a degree one year exposure of female rats to cadmium at a dose of 5, 50 and 100 mg Cd/l affects cell proliferation in the submandibular gland, manifested in PCNA and Ki-67 expression.

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Material and methods

The study involved twenty-two young female Wistar rats, allocated to 4 groups. Six control rats received only redistilled water to drink. Eighteen experimental rats were given an aqueous solution of cadmium chloride (CdCl2): six rats in Group I received a dose of 5mg CD/l, six animals in Group II received a dose of 50 mg Cd/l and the remaining four in Group III - 100 mg Cd/l. The animals were sacrificed in pentobarbital narcosis. Immunocytochemical reaction was performed, using specific monoclonal mouse antibodies against PCNA 1:50 Clone PC10, Code No. M0879 (Dako) and monoclonal human antibodies against Ki-67 1:50 Clone MIB-1, Code No. M 7240 (Dako). The sections were counterstained with Mayer's haematoxylin.

Results

After one-year of the experiment, we observed a positive nuclear reaction for PCNA in single cells in microscopic preparations of the control rats (Fig. 1). In the submandibular glands of Group I rats, we found an increased number of PCNA-positive cells, compared to that in the control (Figs. 1, 2). In Group II, the number of PCNA-positive cells was markedly higher than that in Group I and in the control (Figs. 1-3). In the submandibular glands of Group III rats, the number of PCNA-po-

Figure 1. The control. Strong immunoreactivity for PCNA in single serous cell nuclei. (X 400).

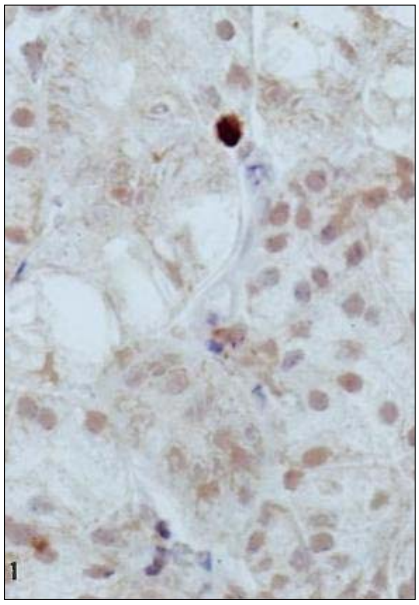


Figure 2. Group I. 5 mg Cd/l. Strong immunostaining of PCNA is present in a few serous cell nuclei. (X 400).

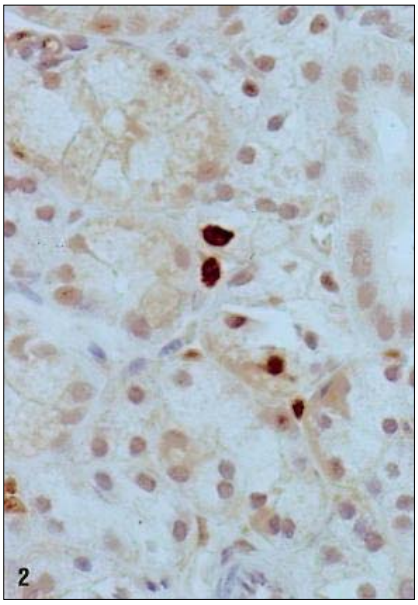


Figure 3. Group II. 50 mg Cd/l. Strong reaction for PCNA in many serous cell nuclei. (X 400).

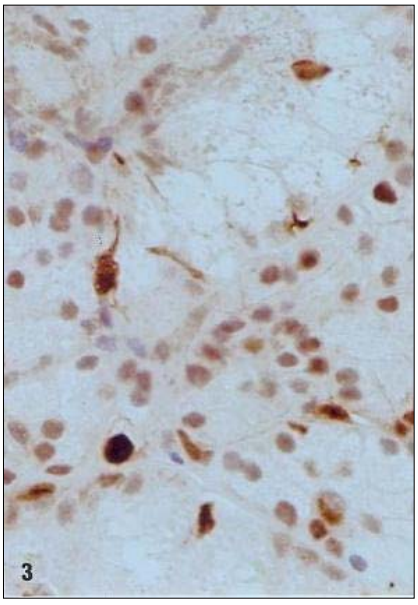


Figure 4. Group III. 100 mg Cd/l. Strong immunoreactivity for PCNA in many serous cell nuclei. (X 400).

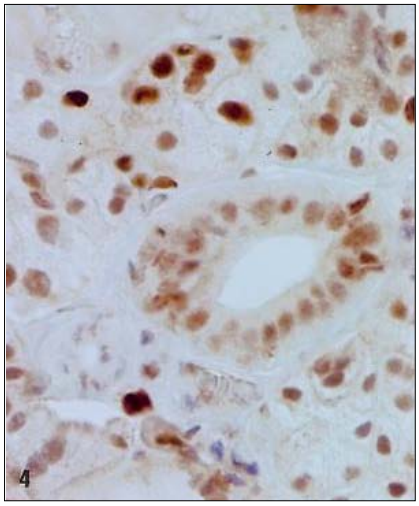


Figure 5. Control. Weaker immunostaining of Ki-67 is present in single serous cell nuclei. (X 400).

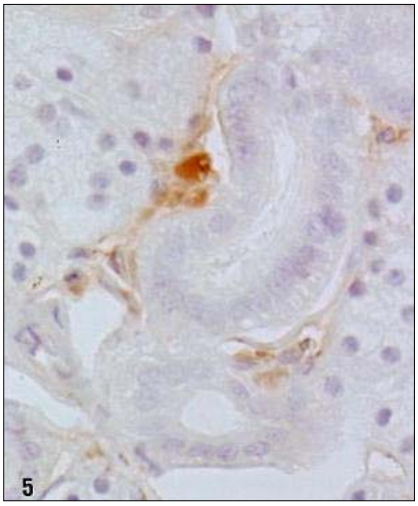
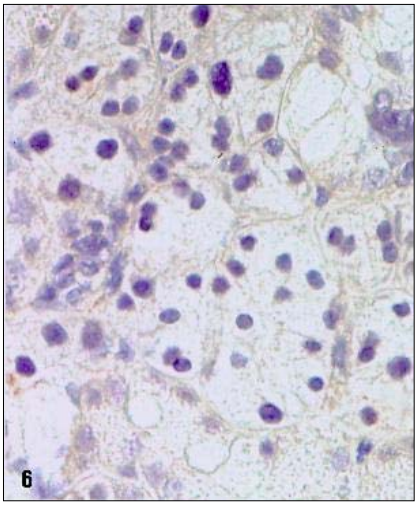


Figure 6. Group III. 100 mg Cd/l. Negative reaction for Ki-67 in the cell nucleus. (X 400).



sitive cells was similar to that, found in Group II (Figs. 3, 4). Ki-67-reaction was sporadically found to be positive in the submandibular glands of the control rats, and was negative in Groups I, II and III (Figs. 5, 6).

Discussion

In the present study, after one-year exposure of female rats to cadmium at a dose of 5, 50 and 100 mg Cd/l, an increase was found in the number of PCNA-positive cells in the submandibular glands in all the three experimental groups, compared to that in the control. Considerably more PCNA-positive cells were observed in the submandibular glands of the rats in Group II and Group III, receiving 50 and 100 mg Cd/l, respectively. We obtained similar results in our previous experimental model, during a 24-week exposure of rats to cadmium at a dose of 5 and 50 mg/Cd/l, when the increase in the number of PCNA-positive cells in the rat submandibular glands was dose-dependent [4]. In the present study, the reaction for Ki-67 was negative in all the three experimental groups, compared to respective values in the control. That was similar to our previous model of a 24-week exposure to cadmium, when the reaction for Ki-67 was negative in both experimental groups, compared to that in the control [4]. The increased number of PCNA-positive cells in the submandibular glands of the rats in Groups I, II and III could be associated either with an enhanced proliferation of those cells or with DNA repair processes. In our experiment, the increased number of PCNA-positive cells seems to indicate a predominance of DNA repair processes, rather than cell proliferation, hence the lack of Ki-67 expression, which is a proliferation exponent [6]. Cadmium destroys DNA both in cell culture with

cadmium and in acute or subacute exposure of rat to this metal [7]. We believe that also in the case of one-year exposure of female rats to cadmium, cell DNA is damaged, which is evident in disturbed cell proliferation.

The present experiment indicates that one-year administration of cadmium to rats at a dose of 5, 50 and 100 mg Cd/l, leads to disorders in cell proliferation in the submandibular glands, which is manifested by an increased PCNA expression in cell nuclei and by the lack of Ki-67 expression.

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