

Effect of patulin on the interdigitating dendritic cells (IDCs) of rat thymus

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Patulin is a common fungal contaminant of ripe apples used for the production of apple juice concentrates and it is also present in other fruits, vegetables and food products. Patulin is a secondary metabolite produced by species of the genera *Penicillium*, *Aspergillus* and *Byssoschlamys*. Patulin has been reported to be mutagenic, carcinogenic and teratogenic. Antigen-presenting cells (APCs) are of prime importance in the innate immune response; they capture antigen in tissues and then migrate to the lymphoid organs to present the antigen to T lymphocytes. Thus, they are crucial for the initiation of immunity. Interdigitating dendritic cells (IDCs) are a subset of APCs that are present at the lymphatic organs. In the thymus, they act in positive and negative selection during T cell development. In the present study, patulin was administered orally to growing male rats aged 5–6 weeks. A dose of $0.1 \text{ mg kg}^{-1} \text{ bw day}^{-1}$ was given to rats for a period of 60 or 90 days daily. The effect of patulin on the IDCs of thymus was investigated by transmission electron microscopy (TEM), and the results were evaluated in terms of cell destruction. In the rats of the control group, it was observed that the IDCs had an indented nucleus, a clear cytoplasm and numerous membrane extensions. In the cytoplasm, a well-developed golgi complex, mitochondria, granular endoplasmic reticulum and a small number of lysosomal structures were observed. At day 60 of patulin-treated rat groups (P-60), loss of cristae in mitochondria and chromatin margination and lysis in the nucleus were found. It was observed that the IDCs had a perinuclear area of cytoplasm surrounded by a peripheral electron-lucent zone. In the cytoplasm of the 90-day patulin-treated rat group (P-90), a peripheral electron-lucent zone was also found, similar to the P-60 group. Additionally increase in vesicular and lysosomal structures, increase in apoptotic bodies and condensation of chromatin in the nucleus were noted. It was observed that patulin leads to apoptotic body formation and cell apoptosis in the IDCs of rat thymus especially in the P-90-treated groups. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS — thymus; patulin; Interdigitating dendritic cell (IDC)

INTRODUCTION

Patulin is a mycotoxin produced by certain species of *Penicillium*, *Aspergillus* and *Byssoschlamys* moulds that contaminate many common fruits and some vegetables. However, the major sources of contamination are apples and apple products. Although patulin had originally been considered as an antibiotic, it has adverse effects on health. It has been suggested that

patulin consumption may stimulate neurotoxic, immunotoxic, immunosuppressive, genotoxic, teratogenic and carcinogenic effects.¹ The reason of such toxic effects has not been completely understood. Patulin is believed to cause cell damage. Dendritic cells (DCs) are an essential component of the immune system. In the initiation of an immune response, some of the most important cells are the key antigen presenting DCs that possess the ability to stimulate naive T cells. DCs originate from haematopoietic progenitors in the bone marrow² and circulate in the blood as immature precursors prior to migration into peripheral tissues. Mature DCs migrate to lymphoid organs where they

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present antigen to T cells and can initiate immune responses.^{3–5} DCs that are immunologically active are classified into five groups.⁶

1. Langerhans cells: in the skin.
2. Interstitial DCs: in connective tissue of most organs.
3. Interdigitating dendritic cells (IDCs): in lymphoid tissues.
4. Circulating DCs.
5. Follicular DCs: in lymph nodes.

Langerhans cells are the most investigated cells of these cell types while studies on IDCs have been limited in number. It has been suggested that the scientific and clinical importance of IDCs has increased due to their key role in anti-cancer host responses and their potential use as biological adjuvants in tumour therapy.⁵ In recent years vaccine developers are focusing on DCs.^{7,8} These cells have an important role in immunopathogenesis and their functions include cancer, infectious diseases and immune tolerance. Therefore, the structural characteristics of these cells need to be investigated. The distribution and function of IDCs in various animal groups have been investigated by various microscopic methods and immunohistochemical analysis. Fonfria *et al.*⁹ found interdigitating cells of *Sturnus unicolor* principally in the thymic medulla and corticomedullary border and discussed their functional and phylogenetic importance in the vertebrate immune system. Alvarez¹⁰ found interdigitating cells in the thymus of *Rana perezi*. These cells were also observed in the cortex area and were found to make contact with lymphocytes. They suggested that their study supplied morphological support for the hypothesized role of interdigitating cells in antigen presentation. Marettova *et al.*¹¹ studied the distribution of S-100 protein positive structures in cat thymus by immunohistochemical methods. They tracked this protein in relation to the interdigitating cells and Hassall's corpuscles, and found a strong positive reaction to S-100 in the interdigitating cells present in the medulla zone. Duijvestijn *et al.*¹² investigated medullary IDC and cortical macrophages after irradiation-induced thymocyte necrosis in rat thymus. They studied the phagocytic activity and population progress of these cells by electron microscopy. They found that IDCs increased in number slightly and were predominantly phagocytizing in the corticomedullary region. They suggested that medullar IDC could be phagocytic but had a different developmental pathway from cortical macro-

phages. They suggest that IDCs possibly belong to mononuclear phagocytes as do the cortical macrophages. Currently, it is well known that these cells belong to the mononuclear phagocyte system. It is thought that patulin has many toxic effects, and that it triggers and leads to cell destruction. Therefore, the effects of patulin on the rat thymus IDCs have been investigated at the cellular level and the results have been evaluated from the standpoint of cell destruction. Additionally, distribution of IDCs within the thymus has been evaluated.

MATERIALS AND METHODS

Wistar albino male rats were obtained from The Production Centre of Experimental Animals in Hacettepe University, Ankara, Turkey. Patulin was administered orally to growing male rats aged 5–6 weeks. In the study, there were four groups consisting of two control and two treatment groups. A dose of $0.1 \text{ mg kg}^{-1} \text{ bw day}^{-1}$ patulin was given to rats in the treatment groups for a period of 60 or 90 days daily. All of the rats were fed standard laboratory diet and water *ad lib* during the experiment. At the end of the experiment, the rats in all experimental groups were sacrificed and the thymus was removed and processed for examination by transmission electron microscopy (TEM). The tissue specimens were fixed in 2.5% sodium phosphate-buffered glutaraldehyde, post-fixed in 1% sodium phosphate-buffered osmium tetroxide at pH 7.4, dehydrated in a graded series of ethanol and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEOL100CXII Transmission Electron Microscopy.

RESULTS

IDCs were particularly clustered at the thymic medulla and corticomedullary border. The ultrastructural study of rat thymus demonstrated that IDCs contained an indented and displaced nucleus. The cytoplasm had most of the membranous and non-membranous organelles. Near to the nucleus, there was a well-developed golgi complex, mitochondria, granular endoplasmic reticulum, free ribosomes and a small number of lysosomal structures (Figures 1 and 2). The most important cytological characteristic of these cells was their dendritic cytoplasmic extensions. It was observed that patulin changed the ultrastructural characteristics of IDCs. In the 60-day patulin-treated rat group (P-60), IDCs had irregular nuclei and the perinuclear area of the cytoplasm was surrounded by an electron-lucent zone. In some

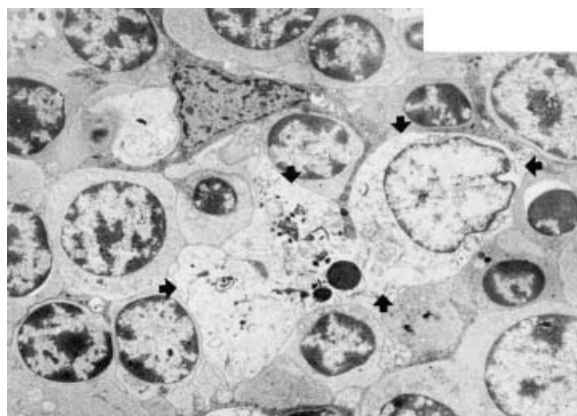


Figure 1. A typical IDC. Electron micrograph of a typical IDC (arrows) with clear cytoplasm, surrounded by thymocytes from control group rat thymus. X4350

samples, there was loss of cristae in mitochondria and chromatin margination and lysis in the nucleus. It was noted that organelles had become localized near to the nucleus (Figure 3). In the 90-day patulin-treated (P-90), some groups had a nucleus that was irregular and centrally localized. Additionally, an increase in vesicular structures in the cytoplasm was noted (Figure 4). In other P-90 groups, IDCs contained displaced and smaller nuclei with peripheral condensation of nuclear chromatin. IDCs had a perinuclear area of cytoplasm, containing most of the membranous organelles, surrounded by a peripheral electron-lucent zone. In particular, various sizes of apoptotic bodies presented a well-defined ultrastructural aspect

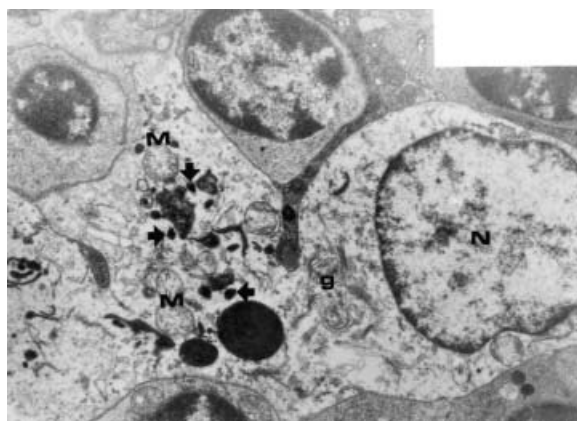


Figure 2. Detail of Figure 1. The cytoplasm contains a well-developed golgi complex (g) near to the nucleus (N), mitochondria (M) and a small number of lysosomal structures (arrows). X8700

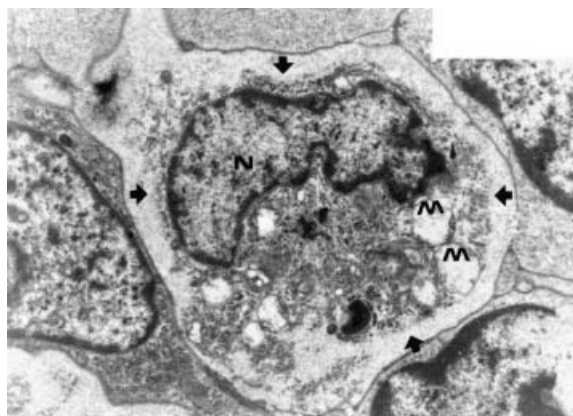


Figure 3. IDC in the thymus of P-60 rats. Ultrastructural features of the IDC of P-60-treated groups: irregular nucleus (N), loss of cristae in mitochondria (M) and a peripheral electron-lucent zone (arrows). X10 800

(Figure 5). It was observed that the applied dose of patulin influenced the results. P-90-treated rats had more cell destruction than P-60-treated rats. In the P-90-treated samples, it is thought that the applied dose caused apoptotic body formation and led to cell apoptosis.

DISCUSSION

There are many studies on the medical effects of patulin at acute, chronic and cellular levels. Edema, ulceration, hyperemia and epithelial cell degeneration are examples of acute symptom^{13–15} while neurotoxicity, immunotoxicity, carcinogenic effects are some

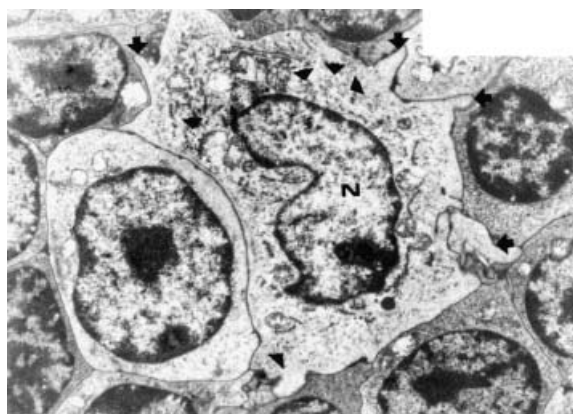


Figure 4. IDC in the thymus of P-90 rats. In the thymus of P-90-treated rats, IDC is shown with cytoplasmic extension (arrows) in close apposition with neighbouring thymocytes and irregular nucleus (N) and increased amount of vesicular structures (arrow-heads) in the cytoplasm. X7200

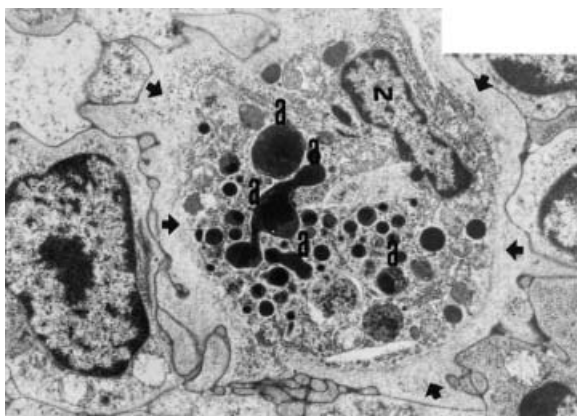


Figure 5. Apoptotic IDC in the thymus of P-90 rats. In the 90-day patulin-treated (P-90) rat thymus, a large number of apoptotic bodies (a), a peripheral electron-lucent zone (arrows) are recognizable in the cytoplasm of the IDC. Note that the nucleus (N) has become smaller and has been pushed into a corner X8700

chronic symptoms.^{16,17} At the cellular level, the effects include plasma membrane disruption, protein synthesis inhibition, transcription and translation disruption and DNA synthesis inhibition.^{15,18–20} There are few studies on cell destruction caused by patulin. In this study, the effects of patulin on IDCs of the thymus were investigated by electron microscopy. Plasma membrane disruption found by Riley and Showker¹⁹ was not observed in our study. Plasma membranes of cells were intact. However, nuclear and cytoplasmic organelle degenerations and apoptotic body formations were noted. Fonfria *et al.*⁹ had observed the following in the interdigitating cells of the thymus of *S. unicolor*: (a) irregular nucleus, (b) a perinuclear cytoplasm containing many organelles with membranes and (c) cytoplasm surrounded by a peripheral electron-lucent zone. In our study, the cytoplasm of the two patulin-treated groups had electron-lucent zones near to the nucleus; while the control group did not have such features. When we investigated the impact of different doses of patulin on apoptosis; we found that P-90 treatment led to massive apoptosis and increase in apoptotic bodies. In conclusion, patulin led to degeneration of IDCs and triggered apoptosis especially in P-90-treated groups. It can be said that patulin induces apoptosis in rat thymus. The distribution of IDCs in thymus has been the object of this present study. It has been found that IDCs become concentrated in the medullary zone and corticomedullary border in conformity with previous reports.^{9–12} We rarely found IDCs in the cortex area as reported by

Alvarez. In conclusion, IDCs can act as Antigen-presenting cells (APCs) to help start an immune response. Currently, DC studies are the focus of many cancer vaccines. It can be suggested that the toxic effect of patulin derives from its induction of cell destruction. However, the toxicity mechanism at the cellular level seems very complex. We believe that further studies at the cellular level may show that patulin triggers cell destruction and leads to cell death. IDCs treated with patulin may be damaged and they could fail to function so leading to negative consequences for the immune system. Patulin could be a risk for the immune system and therefore its intake dose should be controlled.

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