The role of insulin-like growth factor-I and polyamines in developing rat small intestine

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Abstract

IGF-I, Insulin like growth factor-I is a multi-functional polypeptide which organizes the proliferation and differentiation of various cell types and has a metabolic activity similar to insulin. We know that IGF-I receptors localize in the gastrointestinal tract. Polyamines play an important role in the gastrointestinal growth which connect with IGF-I. DFMO, α-difluoromethylornithine is an irreversible inhibitor of ODC, ornithine decarboxylase, the rate-limiting enzyme in the synthesis of polyamines. In the IGF-I-promoted growth the role of polyamines mainly investigate *in vitro* systems, however, *in vivo* studies are necessary to clarify the subject. Our aim in this study is to examine the *in vivo* role of IGF-I as a stimulant in the growth of developing rat's small intestine, and to clarify the role of polyamines in the period of growth promoted by IGF-I. In this study, animals were divided into 2 groups. DFMO (500 mg/kg/day) was administered for 10 days to 9 rats forming the experimental group. Sterile 0.9% NaCl was injected at the same volume as for the experimental animals into 8 rats forming the control group. Tissue samples were taken from the rats for histological, immunohistochemical and biochemical analyses on the 10th day. In the group given DFMO, the expansion and compression of the villi, as well as a decrease in the PAS-positive reaction intensity of brush border and in the number of goblet cells were observed. Immunohistochemical assessments showed that the number of PCNA, ODC and IGF-I positive cells in the DFMO-treated group decreased significantly, compared with the control group. Biochemical investigations showed that DNA values in the small intestinal tissue decreased insignificantly in the DFMO treated group as compared to the control one. We concluded that application of DFMO in the developing rat small intestine inhibited polyamine synthesis and IGF-I synthesis and partially prevented growth of the intestine.

Keywords: Polyamine, α-difluoromethylornithine, insulin-like growth factor-I, ornithine decarboxylase
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Gelişen sıçan ince bağırsağında insülin benzeri büyüme faktörü I'in ve poliaminlerin rolü

Özet

İnsülin benzeri büyüme faktörü I (IGFI), çeşitli hücre tiplerinde ve dokularda hücre çoğalmasını ve farklılaşmasını düzenleyen, insülin gibi metabolik aktiviteye sahip olan çok fonksiyonlu bir polipeptiddir. IGF-I reseptörlerinin gastrointestinal kanalda bulunduğu bilinmektedir. α-Difluoromethlornitine (DFMO), intraselüler poliamin sentezinin anahtar enzimi olan ornithine dekarboksilaz'ın (ODC) spesifik bir inhibitörüdür. Poliaminler, IGF-I ile ilgili gastrointestinal büyümede önemli bir rol oynarlar. IGF-I'in teşvik ettiği büyümede poliaminlerin rolü eses olarak *in vitro* sistemlerde araştırılmıştır, bununla beraber, konuya açıklık getirmek için in vivo çalışmalar gereklidir. İmmunohistokimyasal düzeyde yaptığımız, ayrıca biyokimyasal olarak da

desteklediğimiz bu çalışmada, gelişen sıçan ince bağırsağında, in vivo olarak IGF- I'in büyümeyi uyarıcı etkisini araştırmak ve IGF-I'in uyardığı büyüme süresince poliaminlerin rolüne açıklık getirmek amaçlanmıştır. Bu çalışmada, hayvanlar iki grupta toplandı. Deney grubunu oluşturan 9 sıçana 500 mg/kg DFMO (Difluoromethylornithine) 10 gün süre ile enjekte edildi. Kontrol grubundaki 8 sıçana ise aynı doz ve sürede fizyolojik su enjeksiyonu yapıldı. Histolojik, immunohistokimyasal ve biyokimyasal analizler için 10. günde sıçanlardan doku örnekleri alındı. Difluoromethylornitine (DFMO) verilen grupta villuslarda genişleme ve basıklaşma, çizgili kenardaki ve goblet hücrelerindeki PAS pozitif reaksiyonun şiddetinde ve goblet hücrelerinin sayısında azalma gözlendi. İmmunohistokimyasal değerlendirmeler, DFMO verilen grupta PCNA, ODC ve IGF-I pozitif hücrelerin sayısında kontrol grup ile kıyaslandığında anlamlı bir azalma olduğunu gösterdi. Biyokimyasal incelemeler ince bağırsak dokusundaki DNA değerlerinin DFMO verilen grupta kontrol grup ile kıyaslandığında anlamlı olmayan bir şekilde azaldığını gösterdi. Sonuç olarak, DFMO'nun gelişmekte olan sıçan ince bağırsağında poliamin sentezini ve IGF-I sentezini inhibe ettiğini ve bağırsak büyümesinin kısmen engellendiğini söyleyebiliriz.

Anahtar Kelimeler: Poliamin, α-difluorometilornitin, insulin-benzeri büyüme faktörü-I, ornitin dekarboksilaz

Introduction

Growth hormone causes the production of various small proteins called somatomedins in liver, and to a smaller extent in other tissues. Diverse effects of the somatomedins on growth resemble those depicted by insulin. Because of these similarities, they are also known as "insulin-like growth factors". The most important somatomedin of all is somatomedin C, an insulin-like growth factor-I (IGF-I). Some researchers suggested that growth-related effects of growth hormone are rather indirect, and mediated by IGF-I and some other somatomedins. Human IGF-I shows itself to be the mediator of proliferation depicted by growth hormone, and increases growth on the gastrointestinal channel. Regulation of the human intestinal mucous membrane is very complex, and depends on various factors (Li et al. 2001). Yet, the factors which increase proliferation and differentiation of human intestinal mucous membrane are not well known, and the possible roles of IGF-I are of recent interest. IGF-I is known to exhibit trophic effects in various tissues (MacDonald et al. 1993). After exogenous IGF-I injection, a significant increase in the weight and protein content of the intestines was detected (Höpfner et al. 2002). IGF-I was shown to stimulate the proliferation of intestinal epithelial cells (Kojima et al. 1998; Olanrevaju et al. 1992).

Polyamines, the organic cations found in all living cells, are essential for cell proliferation and differentiation. Concentrations of these low-molecular-weight polycationic compounds increase throughout the rapid proliferation period (Buts 1998; Loser et al. 1999; Farriol et al. 2000; Peulen et al. 2001). Polyamines are important mediators of cell differentiation and synthesis of macromolecules such as DNA, RNA and proteins (Bardocz 1996; Facchiano et al. 2001; Cochon et al. 2002). Polyamine metabolism is shown to have a primary effect on normal and adaptive pancreatic growth in rats (Höpfner et al. 2002). As secondary messengers are polyamines, putrescine, spermidine and spermine that also act as mediators for hormones and growth factors (Bardocz 1996). In order to satisfy requirements of the cells in the proliferation period and for protein synthesis, concentration of polyamines is regulated by various enzymes among which ODC, ornithine decarboxylase, is of particular importance. ODC is catalyzer decarboxylation of ornithine to putrescine. This is the first step of the synthesis of polyamines (Buts 1998). ODC, as a key enzyme in the control of polyamine synthesis, has important roles in intestinal growth (Luk 1990; Lin et al. 2002) and also affects the growth of other mammalian tissues (Slotkin et al. 1984). ODC activity probably plays primary roles on mucosal

hyperplasia by stimulating proliferation of the crypt cells.

α-Difluoromethylornitine (DFMO) is the specific inhibitor of ODC, a key enzyme of intracellular polyamine synthesis (Slotkin et al. 1984; Yang et al. 1984; Kojima et al. 1998; Schipper et al. 1999; Thyssen et al. 2002). It showed that DFMO inhibits pancreas growth significantly. DFMO is also a strong inhibitor of adaptive growth initiated by polyamine synthesis in the cell (Höpfner et al. 2002). Due to the effect of DFMO, ODC activities of intestinal mucosa inhibit and suppress intestinal adaptation (Yang et al. 1984). Studies on inhibitors of polyamine biosynthesis proved that polyamines are required for differentiation of the animal cells (Höpfner et al. 2002).

This means polyamines play important roles on IGF-I-related gastrointestinal growth and the trophic effect induced by IGF-I in intestines, depends on polyamines (Höpfner et al. 2002). To our knowledge, immunohistochemical studies demonstrating the effects of IGF-I and polyamines on rat intestines were not yet undertaken. The reason for selecting intestines as the subject is its importance as a highly-proliferating organ. This study aims at the immunohistochemical and biochemical determination of the role of IGF-I that involves in the differentiation of rat intestines, on growth stimulation as well as the roles of polyamines on growth stimulation process *in vivo*. It was determined that ODC expression varies with age as the tissues grow and differentiate (Schipper et al. 1999). Therefore, developing animals were chosen because polyamine synthesis would also vary. Moreover, small intestines in growing animals exhibit higher mitotic activity.

Materials and Methods

In this study, we used 10-days old Sprague-Dawley male rats, weighing 12-19 grams obtained from DETAE, Istanbul University Institute for Experimental Medicine. Animals were kept under controlled laboratory conditions during the study. Animals used for experiments were split into two groups. Nine rats from the first group received a daily subcutaneous injection of 500 mg/kg DFMO for ten days. The remaining eight rats were similarly injected with physiological saline solution to serve as the control experiment.

Histological Methods

Jejunum pieces were removed from the intestines of both animal groups under the ether anesthesic and were fixed with Bouin fixative for 18 hours. They were passed through an increasing alcohol solution series and the tissue samples were embedded in paraffin. Sections of 5 micrometer thickness were cut from the blocks and were treated with Masson's triple stain (Masson) and Periodic Acid Schiff (PAS). Stained sections were examined in an Olympus CX41 bright light microscope.

Immunohistochemical Methods

PCNA, nucleus antigens, IGF-I receptors and immunolocalization of ODC in the proliferating cells were determined using "Streptavidin-Biotin-Peroxidase" method.

Immunohistochemical Determination of the PCNA in Proliferating Cells

Jejunum tissue pieces were fixed with Bouin fixative treated with consecutive alcohol solution series of increasing alcohol concentration. Tissue samples were then embedded into paraffin. Sections of 4 micrometer thickness were cut from the blocks and placed on Poly-L-lysine covered slides. Following deparaffinization and rehydration, samples on slides were placed in distilled water. Antigen healing was then done by boiling the sections in a 700 W microwave oven for 10 minutes in 10mM citrate buffer (pH 6.0). Slides were washed with PBS, phospate buffered salt solution (pH 7.4). In order to permeabilize the membranes, specimens were incubated for 10 minutes with 0.3% Triton X-100 solution prepared in distilled water. Slides were kept in 3% H_2O_2 solution at room temperature in a

humidified environment for 10 minutes to inhibit endogenous peroxidase activity. The following procedures were completed using an UltraTek HRP Anti-Polyvalent Lab PACK (ScyTek Lab.) kit. To avoid non-specific binding in tissues, blocking solution was applied for 10 minutes. Afterwards, samples were incubated at room temperature for 30 minutes in the presence of PCNA-specific antibody (PCNA Ab-1 (Clone PC10) Rat Monoclonal Antibody NeoMarkers MS-106-P1) containing 0.3% Triton X-100, and diluted (1:50) with PBS solution. Negative controls received PBS instead of the antibody. Following the biotinylated secondary antibody treatment, slides were washed with PBS and treated with streptavidin-peroxidase conjugate, and washed once more with PBS. For the color reaction, slides were treated with aminoethylcarbazole (AEC) for 90 seconds. Following the development of the specific immune reaction, further reaction was terminated by placing the slides in distilled water. In order to observe the tissue morphology, slides were counterstained with Mayer's haematoxilin. Slides were then sealed with a sealing solution that is compatible with AEC. PCNA-positive epithelial cells of Lieberkühn crypts from treated and control groups were counted on an Olympus CX41 light microscope. Approximately 1000 cells were counted in a 6.25×10^{-2} mm^2 slide area at 400X magnification. The number of the proliferating crypt cells was determined using the following formula:

$$\text{Crypt cell proliferation index} = \frac{\text{PCNA positive crypt cell count}}{\text{Total crypt cell count}} \times 100$$

Immunohistochemical Detection of ODC

Immunolocalization and the amount of ODC were detected by "Streptavidin-Biotin-Peroxidase" method. This method is similar to the detection of PCNA's immunolocalization described above. The only difference was that the slides were boiled in a 700 W microwave oven in the presence of 10 mM citrate buffer (pH 6.0) for 20 minutes for antigen healing before rehydration and distilled water wash. A 3% H_2O_2 solution was added onto the slides and incubated for 20 minutes to avoid endogenous peroxidase activity. Blocking solution was applied on slides for 20 minutes to avoid non-specific binding, and they were then incubated for 23 hours at +4 ^{0}C with ODC-specific primary antibody (ODC Ab-1 (Clone MP16-2) Mice Monoclonal Antibody, NeoMarkers MS-464-P) which was prepared by dilution (1:50) in PBS containing 0.3% Triton X-100. For the color reaction, AEC chromogen was applied on slides for 7 minutes. ODC-positive epithelial cells of Lieberkuhn kripts and villi in the treated and control groups were examined. Approximately 1000 cells were counted on Olympus CX41 model light microscope in an area of 6.25×10^{-2} mm^2, at 400X magnification. The number of the ODC positive cells was calculated as follows:

$$\text{Number of ODC-positive epithelial cells} = \frac{\text{ODC-positive epithelial cell count}}{\text{Total number of epithelial cells}} \times 100$$

Immunohistochemical Detection of IGF-I

Immunolocalization and concentration of insulin-like growth factor-I was detected by "Streptavidin-Biotin-Peroxidase" method. This method is similar to that of PCNA immunolocalization described above. Slides were incubated with the IGF-I specific primary antibody (IGF-1 Rabbit Monoclonal Antibody, NeoMarkers RB-9240-P) that was prepared by dilution in PBS (1:50) that contained 0.3% Triton X-100, at room temperature for 30 minutes. For the color reactions, the slides were treated with AEC choromogen for 4 minutes.

The number of the IGF-I positive cells of lamina propria in the DFMO-treated group were compared with that of the control group. The number of the IGF-I positive cells was calculated as follows: IGF-I positive lamina propria cells were evaluated in 7 slides per rats prepared from both treated and control groups.

Antibody-labeled lamina propria cells were counted using the Olympus CX41 microscope in an area of $6.25x10^{-2}$ mm^2, at 400X magnification.

The number of the IGF-I positive cells of lamina propria in the DFMO-treated group were compared with that of the control group.

Biochemical Methods

Intestinal tissue pieces were kept in 0.9% physiological saline solution at -86 ^oC freezer until the treatment. Before the treatment, frozen samples were subsequently relocated into – 20 ^oC, and to +4 ^oC and then thawed. Samples were homogenized in a glass homogenizator following weighing, and 10% suspensions were prepared in physiological saline solution. The amount of DNA in the homogenized tissues was detected by means of spectroscopy using diphenylamine method (Plummer 1978). All the solutions used were prepared with ultrapure water.

Statistical Analyses

Data obtained from biochemical methods and microscopic examinations were assessed via Student's T test using Prism software (Graph Pad, California, USA). Value of $P<0.05$ considered as statistically meaningful.

Results

Histological Results

In the histological aspect sections were obtained from the control group animals that had received only physiological saline solution, jejunums exhibited usual histological properties. Villi in this group were smooth and long (Fig. 1). Villi of DFMO-treated group, however, appeared significantly broadened and flattened (Fig. 2). The intensity of PAS positive reactions in the brush border and goblet cells of treated rats was lower than that in the control group. A significant decrease in goblet cell count was observed in the treated group (Figs 3, 4).

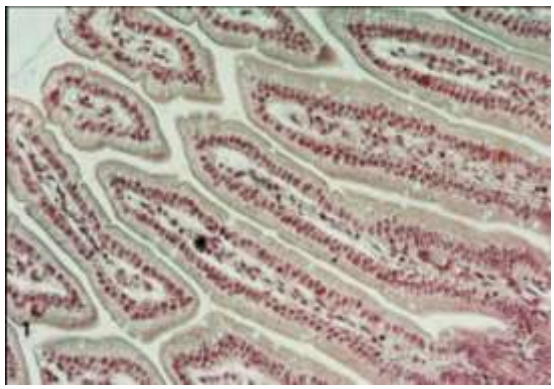

Figure 1. Control group. Masson's tri dye. X270. Fine and tall villi (✸).

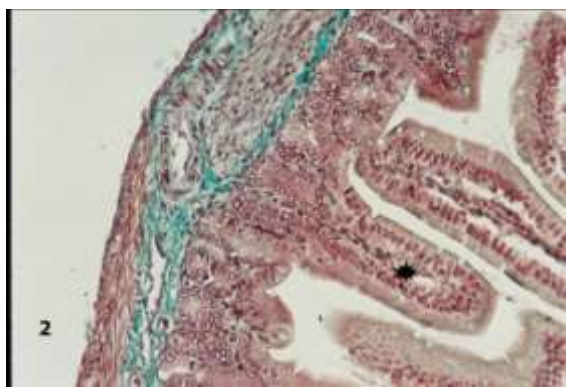

Figure 2. DFMO- administrated group. Masson's tri dye. X270. Broaden and flatten in villi (✸).

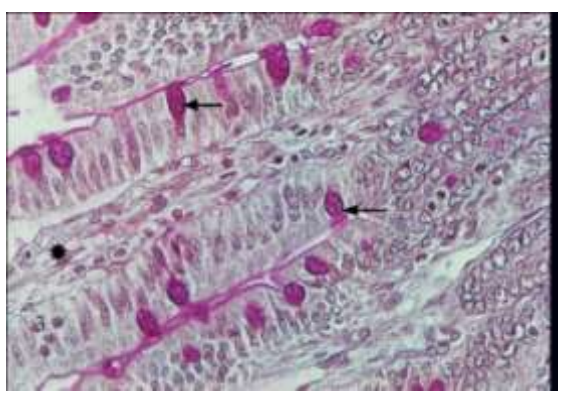

Figure 3. Control group. PAS. X540. Fine and tall villi (✸), strong PAS (+) reaction in Goblet cells(→) and the brush border (▸).

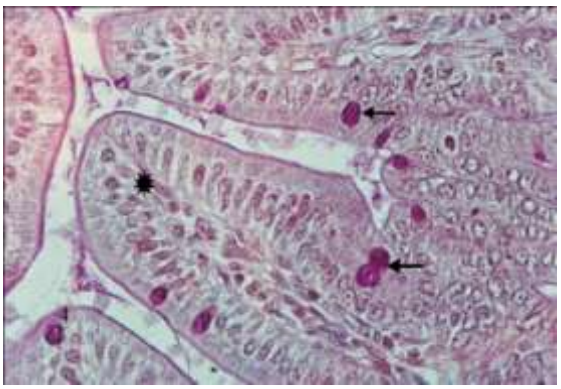

Figure 4. DFMO-administrated group. PAS. X540. Broaden and flatten in villi (✸), decrease in PAS (+) reaction in Goblet cells(➔) and the brush border (‣).

Immunohistochemical Results of PCNA in Proliferating Cells

A significant amount of immunoreactivity was observed microscopically on crypt cell slides (Fig. 5). The numbers of PCNA-positive crypt cells as well as their staining intensities were decreased in the DFMO-treated group, when compared to the control group (Fig. 6). No immunoreactivity was observed on negative control slides treated with PBS instead of PCNA antibody.

Statistical assessments demonstrated that the number of PCNA-positive cells decreased meaningfully in the DFMO-treated group, in comparison to the control group (*P<0.05) (Fig. 7).

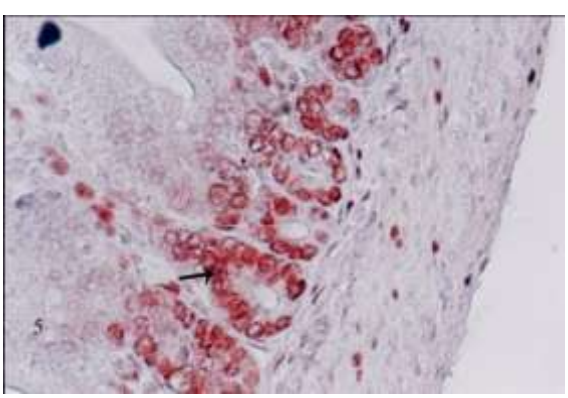

Figure 5. Control group. X540. Intense PCNA immunreactivity in lieberkühn crypt (➔).

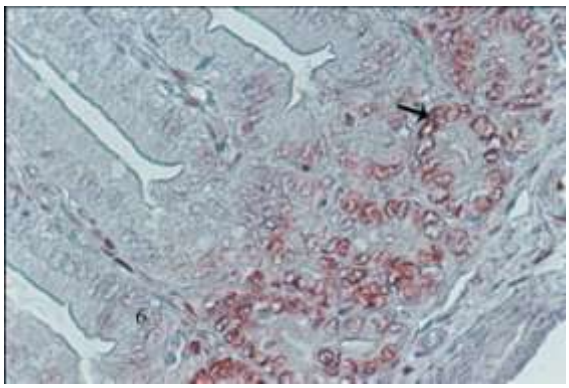

Figure 6. DFMO-administrated group. Decrease in the number of PCNA-positive crypt cells as well as their staining intensities (➔).

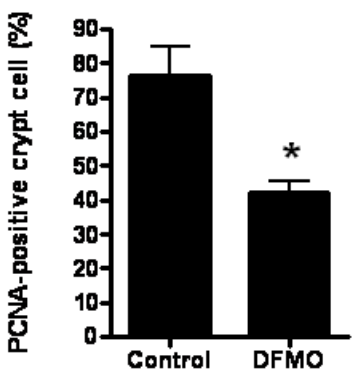

Figure 7. Proliferation index of crypt cells determined by PCNA immunohistochemistry. Mean values ± standard deviation calculated for both groups: Control: 76.58 ± 8.08, DFMO: 42.31 ± 3.09. *P< 0.05.

Immunohistochemical Results for ODC

Microscopy results showed considerable immunoreactivity on the slides prepared from the intestinal crypt cells of the control group (Figs 8, 9). The number of the ODC-positive cells as well as their staining intensity appeared to decrease on slides prepared from DFMO-treated group (Figs 10, 11). No reactions were observed on slides prepared from negative controls treated with PBS instead of ODC-antibody.

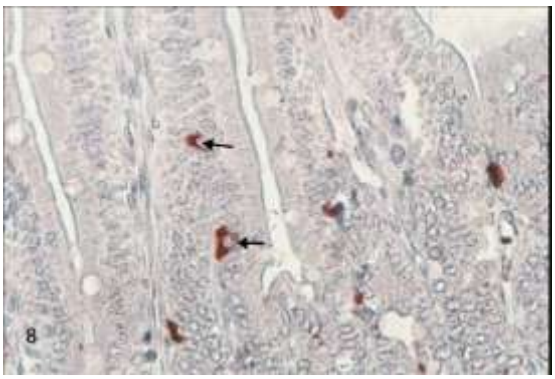

Figure 8. Control group.X540. ODC immunreactivity in villi epithelium cell (➔).

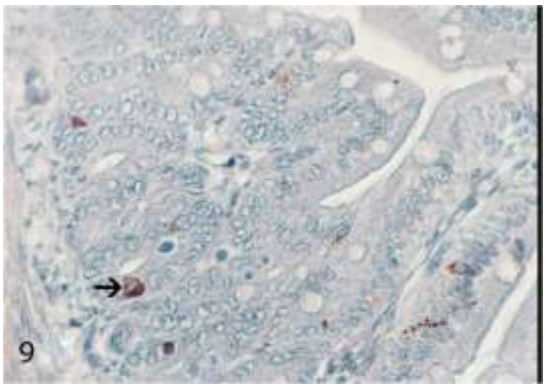

Figure 9. Control group.X540. ODC immunreactivity in lieberkühn crypt (➧).

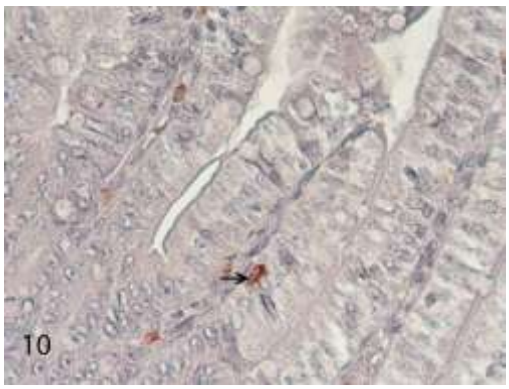

Figure 10. DFMO- administrated group. X540. Decrease in the number of ODC-positive villi epithelium cells as well as their staining intensities (➔).

When compared to slides from the control group, statistical evaluations demonstrated a

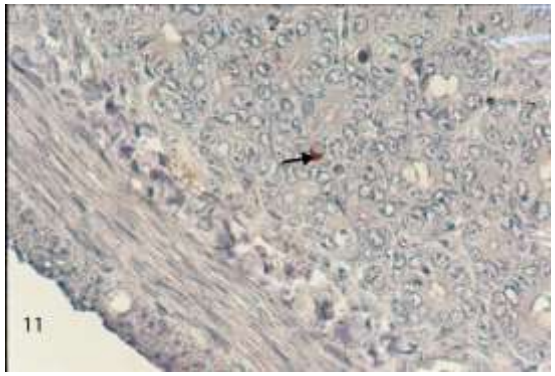

Figure 11. DFMO- administrated group. X540. Decrease in the number of ODC-positive crypt cells as well as their staining intensities (➔).

significant decrease in the number of ODC-positive cells on slides prepared from DFMO-treated group (**P<0.01) (Fig. 12).

Immunohistochemical Results of Insuline-like Growth Factor-I (IGF-I)

An average IGF-I immunoreactivity was observed on microscopic examinations of the intestinal lamina propria slides prepared from the control group (Fig. 13). The number of the IGF-I positive cells decreased in DFMO-treated group (Fig. 14). No immunoreaction was observed on the negative control slides which were treated with PBS instead of IGF-I antibody.

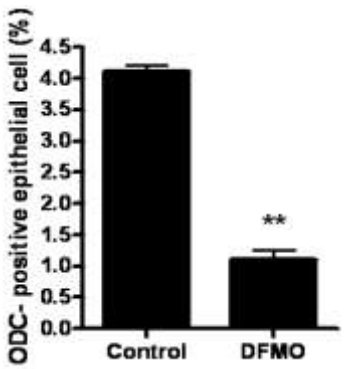

Figure 12. ODC-positive epithelial cell index determined by ODC immunohistochemistry. Mean values ± standard deviation calculated for both groups: Control: 4.1± 0.08, DFMO: 1.1±0.15. **P< 0.01.

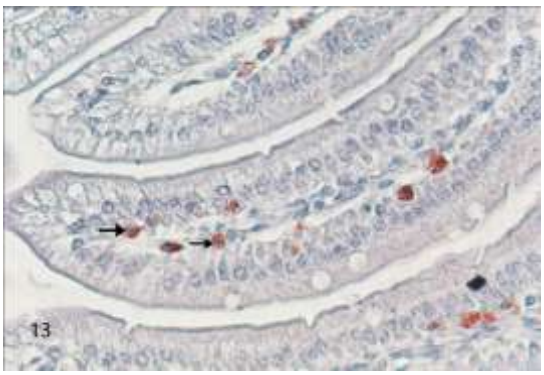

Figure 13. Control group. X540. IGF-I positive lamina propria cells (➔).

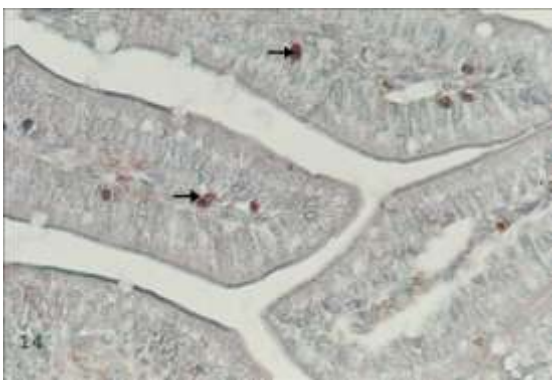

Figure 14. DFMO-administrated group. X540. Decrease in IGF-I positive lamina propria cells (➔).

Statistical evaluations showed that the number of the IGF-I positive cells decreased significantly on slides prepared from DFMO-treated group when compared to the controls (**P<0.01) (Fig. 15).

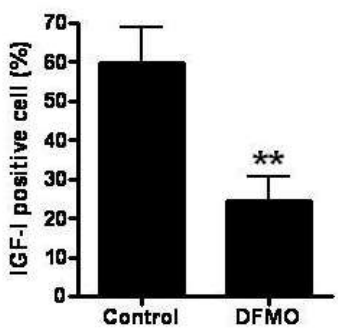

Figure 15. IGF-I-positive cell index determined by IGF-I immunohistochemistry. Mean values ± standard deviation calculated for both groups: Control: 59.6 ± 9.17, DFMO: 24.4 ± 6.12. **P< 0.01.

Biochemical Results

DNA contents of intestinal tissue homogenates showed that in comparison to the data obtained from the group injected physiological saline solution, the amount of DNA in intestinal homogenates of the DFMO-treated group decreased to a statistically insignificant degree (P › 0.05) (Fig. 16).

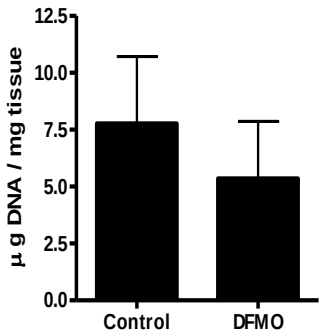

Figure 16. DNA analysis by diphenylamine method. Mean values ± standard deviation calculated for both groups: Control: 7.7 ± 2.93, DFMO: 5.3 ± 2.51. P › 0.05.

Discussion

Human Insulin-like growth factor is a small proinsulin-like peptide and is both structurally and functionally a member of the growth factor family. Its effects are mostly local, and it stimulates growth of some specific tissues. These factors are partially dependent on the growth hormone, and they mediate most of the anabolic effects of growth hormone (Keleşand Türkeli 2005; Çolak 2007). Despite its name, growth hormone does not directly stimulate growth; instead, it stimulates secretion of insulin-like growth factor from hepatocytes. Physicians measure IGF-I levels in blood in order to determine functionality of the growth hormone.

IGF-I stimulates growth of soft tissues and bone tissue, and regulates proliferation and differentiation of various cell types (Al Haj Ali et al. 2003; Çolak 2007). Significant amounts of IGF-I can be found in circulation throughout the postnatal phase. It binds tightly to a transporting protein produced in response to the growth hormone. Consequently, IGF-I slowly

crosses the blood–tissue barrier and imposes its effects on target tissues via membrane receptors. IGF-I receptors are located in the gastrointestinal channel and have stimulating effects on the development of the gastrointestinal channel (Olanrevaju et al. 1992). In rats, IGF-I receptors are distributed throughout the intestinal mucous membranes. It was shown that IGF-I causes significant weight gain in the intestines due to intestinal hyperplasia, spleen hyperplasia and hypertrophy. It is claimed that the trophic effect of IGF-I is governed by polyamines in the intestines and the spleen (Höpfner et al. 2002).

Natural polyamines which contain spermidine, spermine and putrescine are required for cell proliferation and as the cellular polyamine levels drop, the growth of intestinal mucous membranes becomes inhibited, or vice versa (Li et al. 2001). Polyamines and ODC (the enzyme that controls polyamine synthesis), play important roles in the development of intestines and cell growth processes (Schipper et al. 1999; Luk 1990). ODC affects normal mucous membrane growth and cell differentiation by regulating polyamine biosynthesis. Injection of DFMO, prevents the increase in ODC and polyamine levels, therefore suppresses intestinal growth (Luk 1990; Schipper and Verhofstad 2002). This result also demonstrates the role of polyamines on intestinal growth. DFMO inhibits *in vivo,* the ODC activity which is involved in embryogenesis, pancreatic and hepatic growth. It was also suggested that an increase of ODC activity and polyamine synthesis play important roles on human cell growth (Dowling et al. 1985; Luk and Yang 1987; Luk 1990). In the current study, ODC expression was investigated by means of immunohistochemistry to better understand the inhibitory effects of DFMO on ODC activity and thus, polyamine synthesis. DFMO inhibits the polyamine synthesizing enzyme, ODC, and therefore, the immunoreactivity of ODC in villus epithelia and crypts of control groups significantly decreases after DFMO administration.

It was stated that polyamines play important roles on IGF-I-related gastrointestinal growth (Höpfner et al. 2002; Schipper et al. 1999). However, to our knowledge, no immunohistochemical data on this subject is reported. In the current study, the role of IGF-I on the regulation of gastrointestinal growth was demonstrated using IGF-I immunoreactivity. Al Haj et al. (2003) previously demonstrated IGF-I reactivity of cells in lamina propria in jejunum of *Camel dromedarius*. In our study, IGF-I reactivity was observed only in lamina propria of jejunum. The trophic effect of IGF-I in the intestines is dependent on polyamines and a specific effective inhibitor of ODC. The trophic effects observed after exogenous IGF-I administration could be eliminated if IGF-I were administered along with DFMO. Therefore, a direct relationship between IGF-I-mediated growth process and polyamine metabolism is established (Höpfner et al. 2002). According to the results of the current study, the decrease in the amount of IGF-I-immunoreactive cells observed following the DFMO injection suggests that this decrease is due to the inhibition of polyamine synthesis by DFMO.

Intestinal cells have a short proliferation cycle and strong growth capability (MacDonald et al. 1993). Inhibition of epithelium proliferation is one of the major reasons for damaging the integrity of intestinal mucous membrane (Xu et al. 2005). DFMO exhibits inhibitory effects on cell proliferation in liver, stomach, pancreas, intestines and colon by inhibiting ODC (Schipper and Verhofstad 2002). Nuclear PCNA reactivity is an appropriate indicator for cell proliferation (Guo et al. 1993). In our study we investigated immunohistochemically the expression of PCNA, a protein that helps DNA polymerase, in order to determine its role on inhibition of ODC by DFMO injection, and thus, the inhibition of polyamine synthesis. It was found that when compared to the control group, in the jejunum crypts of the animals treated with DFMO, inhibition of cell proliferation was more pronounced.

It was shown via various experimental models that DFMO inhibits intestinal growth (Luk 1990). This effect is probably due to the inhibition of ODC activity by DFMO which suppresses growth, regeneration and differentiation of epithelial cells (Alarcon et al. 1987). We know DFMO causes intestinal villus atrophy (Yarrington et al. 1983; Alarcon et al. 1987). In the current work, we observed a broadening and flattening of intestinal villi from DFMO treated animals, which implies that these changes could result in villus atrophy.

Numerous studies exist on the effects of DFMO on proliferative parameters such as DNA (Alarcon et al. 1987; Luk 1990). IGF-I starts DNA synthesis (Spencer et al. 1988) and thus, regulates propagation and differentiation of cells of various tissues (Steeb et al. 1995). It means polyamines can regulate nucleic acid synthesis as well (Wang et al. 1991). Höpfner et al. (2002) showed that IGF-I causes a meaningful decrease in DNA content when it was administered with DFMO. The oral administration of DFMO to the weaned rats led to a decrease of DNA content of the intestinal cells (Alarcon et al. 1987). The decrease in ODC activity and DNA synthesis levels are in harmony with the decrease in DNA, RNA and protein contents (Wang et al. 1991). Here, a decrease in DNA content in the intestinal cells of DFMO-treated animals was found. This result did not appear to be statistically meaningful, and confirmed the results of previous studies.

Finally, in evaluation the immunohistochemical and biochemical data showed the administered DFMO doses inhibited ODC which catalyses biosynthesis of polyamines that play important roles on cell growth, proliferation and differentiation. Therefore, inactivation of ODC caused an inhibition of IGF-I synthesis which has a stimulating effect on polyamine synthesis and the development of the gastrointestinal channel. Consequently, intestinal development was also prevented to a certain extent. Inhibition of polyamine biosynthesis causes decreasing IGF-I synthesis, therefore, a decrease in intestinal development is observed, giving support to the roles polyamines play in IGF-I mediated intestinal growth. In future studies, an investigation of the synthesis of IGF-I in damaged liver tissues is planned, and if the synthesis does not occur, the roles of polyamines on intestinal growth in the presence of exogenously introduced IGF-I is expected to be clarified.

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