Effect of 60 and 90 days of isotretinoin treatment on the structure of the small intestine mucosa in young male Wistar rats

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ABSTRACT
Isotretinoin is a substance used in cases of severe acne and acne resistant to other treatments. This skin disease affects patients of all ages and can interfere with social life, especially in adolescents. The drug acts by suppressing sebaceous gland activity and creating an inhospitable environment for *Propionibacterium acne*. The integrity of the small intestine is important for correct nutrition and patient treatment. We intended to assess the small intestine structure after treatment with 5 mg/kg isotretinoin solution and after a period without the drug, which could be considered a rest period. Young male Wistar rats (n=24) were separated into 4 groups (n=6): C: water; D0: soybean oil; D5a: 5 mg/kg; D5b: 5 mg/kg for 60 days followed by 30 days of rest period. Soybean oil was used to dilute the drug and it was offered daily by gavage. The animals were euthanized and the duodenum, jejunum and ileum were collected for analysis with light and scanning electron microscopy. The treatment stimulated tissue proliferation in the jejunum and ileum but had no significant effect in the duodenum. The results also showed a modification in goblet cell frequency in the duodenum and ileum. A further finding was that some modifications disappeared during the rest period. The protocol showed that the small intestine was somewhat altered by the treatment yet no lasting damage was caused.

KEY WORDS: small intestine; microscopy; Wistar rats; histology

Introduction

Acne is a skin disorder that can affect social relations, with skin scars often leading to low self-esteem, mainly in adolescents. Isotretinoin is currently the most effective acne treatment available, with reported long-term remission rates as high as 89%. This treatment is especially indicated in cases of resistant disease, unresponsive to other therapies (Ortonne, 1997; Sieving et al., 2001; Chia et al., 2005; Passier et al., 2006; Zane et al., 2006).

The treatment usually initiates with a daily dose of 0.5mg/kg (or higher) and can be increased to 1.0 mg/kg. A low dose of isotretinoin, such as 0.15–0.40 mg/kg, has been reported to be effective with a low incidence of severe side effects. Aiming at a total dose of 120–150 mg/kg per treatment, it may last for 3–7 months depending on the daily doses used (Passier et al., 2006; Akman et al., 2007; Sundstrom et al., 2010). About 25% of patients treated with isotretinoin have elevated triglyceride plasma levels, which in some cases may be associated with the onset of acute pancreatitis. Isotretinoin can also cause a slight decrease in plasma HDL cholesterol and increased LDL and VLDL cholesterol. Changes in serum triglycerides and cholesterol are reversible upon treatment interruption. Less frequent adverse reactions, which are reversible, include vomiting, gastrointestinal bleeding, appendicitis, gut inflammation, esophagitis, anorexia, weight loss and ulcerative colitis (Shalita et al., 1983; Bigby & Stern, 1988; Diniz et al., 2002; Charakida et al., 2004; Akman et al., 2007; Brito et al., 2010). Nonspecific symptoms include nausea, diarrhea and abdominal pain. There is also evidence that the drug may worsen the manifestations of inflammatory diseases. However, it has been administered successfully to patients with Crohn’s disease and ulcerative colitis without causing discomfort (Brito Mde et al., 2010) (10).

In the intestine, ingested food is converted into a small particle nutrient solution (McCarter & Chen, 1992; Barret, 2006). Isotretinoin absorption occurs in the intestine and the lymphatic system absorbs the esterified
chylo micron with a retinol group (Junqueira & Carneiro, 2015). Although the drug is widely used and many side effects have been described, information about the direct effect in the healthy small intestine is not available. The duodenum is the main digestion site and the jejunum and ileum seem to be the absorption sites of drugs and nutrients. Thus the aim of this study was to investigate in young male Wistar rats the structure of the duodenum, jejunum and ileum after treatment with isotretinoin and after a rest period with no exposure to this drug.

Material and methods

Experimental groups

24 male Wistar rats (Rattus novergicus) were randomly allocated to the following groups:

• C: control group with water;
• D0: control group with soybean oil, the vehicle where we dissolved the substance;
• D5a: 5 mg/kg solution for 60 days;
• D5b: 5 mg/kg solution for 60 days of treatment followed by 30 days drug free.

All four groups received the treatment for 60 days but the D5b group was followed up for further 30 days without any treatment in order to elucidate the conditions right after the treatment. The rats had free access to rodent food and water and the animal house had luminosity control with 12 hours of light/dark and a temperature of about 22±1 °C. We diluted the drug in soybean oil (Tsukada et al., 2002) and offered it by daily gavage for 60 days. The 5 mg/kg concentration dosage was chosen as it is considered a little higher but still not a harmful dose.

Sample collection from the small intestine

After the treatment the groups C, D0 and D5a were euthanized with a mixture of 10 mg/kg of ketamin and 80 mg/kg of xylazine solution and the duodenum, jejunum and ileum were immediately sectioned and washed in saline solution. The preparations followed the usual procedure for light microscopy using Karnovsky’s modified fixative. The next steps were the critical point drying, followed by gold sputtering.

Mucosal morphometry

To determine the relation between villi and crypt height, we applied the formula (villi height/crypt height).

Absorptive surface

To find the assumed absorptive surface (AS) for the duodenum, we used the following formula (Hardin et al., 1999):

\[
AS (\mu m^2) = \text{villi height (\mu m)} \times \text{medium width at 50\% of villi height (\mu m)}.
\]

In the 5 μm thickness section we performed the combination of Alcian Blue (AB) pH 2.5 with Periodic Acid Schiff (PAS) (AB+PAS) (Alcian Blue pH2.5-PAS®, EasyPath) to stain the goblet cells according to their mucin type. The second technique was Reticulin (Reticulina®, EasyPath) to reveal reticulin fiber distribution and structure. The third one was the Masson Trichrome Stain (Weigert’s Iron Hematoxilin Set®, Sigma-Aldrich and Masson Trichrome Stain Kit®, Sigma-Aldrich) to show muscle and connective tissue distribution.

Goblet cell evaluation

The first step was to determine the area occupied by villi and crypts. We selected ten different fields of the same samples applying the histochemical technique combining AB+PAS pH 2.5. Using the software Image Pro Plus® (Media Cybernetics, version 4.5.0.29), we counted the goblet cells considering the type of mucin revealed in the cytoplasm. The different mucins were revealed according to their dominant group. It is thus possible to assess the frequency of cells secreting basic (PAS+, magenta color), acidic (AB+, blue color) and a mixture thereof (AB+PAS+, purple color).

Scanning electron microscopy

In order to assess the surface structure, we collected fragments to be observed by scanning electron microscopy. The fragments were completely dehydrated (ascending ethanol series of 70 to 100%), after fixation with a modified Karnovsky fixative. The next steps were the critical point of drying, followed by gold sputtering.

Ethical permissions

This experiment followed the established ethical standards in accordance to the animal protection laws of Brazil. The Ethics Committee of Animal Use of the State University of Campinas (CEUA/Unicamp/ protocol #2831-1) approved the research technique.

Statistical analysis

Considering sample size, we applied the Kruskal-Wallis statistical test followed by Dunn’s post test. Data are presented as mean± standard deviation. The tests considered p<0.05 statistically significant and were performed with Minitab® 16 program (LEAD Technologies, Inc. Charlotte, North Carolina).
Results

The treatment did not cause obvious signs of damage to the health of the rats. Gavage was performed without difficulty and, as shown in Table 1, all groups had body mass gain during the treatment.

The morphometric data indicate that the duodenum parameters did not alter with the treatment. The stereology of goblet cells showed that the effects were dosage-dependent and that the rest or recovery period was sufficient since the parameters were comparable to those of the controls. We found diminished jejunal wall thickness in the D5a group in relation to the control D0. Crypt evaluation showed that their height decreased in the recovery group D5b in relation to control D0. Considering the villus data, the treatment did not affect their height but it could have affected the assumed absorption surface because these measurements were smaller in the D5b group in relation to D5a. Stereology and morphometry of the cell types indicated that goblet

Table 1. Body weight, stereological and morphometric analysis of the duodenum, jejunum and ileum mucosa after 60 days of treatment with isotretinoin and a recovery period in young male Wistar rats.

| Groups                              | C control with water | D0 control with soybean oil | D5a 1 mg/kg of isotretinoin | D5b 5 mg/kg of isotretinoin and a recovery period |
|-------------------------------------|----------------------|-----------------------------|-----------------------------|--------------------------------------------------|
| Initial body weight (g)             | 192.82±13.96         | 193.5±10.17                 | 193.46±15.24                | 192.78±3.8                                       |
| Final body weight (g)               | 468.43±40.15         | 459.44±33.34                | 466.68±22.54                | 474.25±41.73                                     |
| **Duodenum**                        |                      |                             |                             |                                                  |
| Duodenum Wall Thickness (μm)        | 955.47±529.22        | 733.07±118                  | 761.52±168.47               | 679.24±122.47                                    |
| Mucosal area (mm²)                  | 2.93±0.12            | 2.85±0.22                   | 2.83±0.38                   | 2.97±0.10                                        |
| Assumed Absorption surface (mm²)    | 0.04±0.01            | 0.046±0.01                  | 0.05±0.006                  | 0.039±0.005                                      |
| Villus Height (μm)                  | 419.61±98.46         | 442.19±85.19                | 449.80±70.16                | 388.97±59.29                                     |
| Crypt height (μm)                   | 223.40±139.74        | 169.32±28.33                | 162.96±34.75                | 148.44±20.04                                     |
| Villus height: Crypt height ratio   | 2.19±0.79            | 2.63±0.42                   | 2.79±0.34                   | 2.64±0.37                                        |
| Goblet cells- Units/mm²             | 559.80±134.54        | 657.83±5.45                 | 426.48±62.44                | 663.99±163.81                                    |
| Goblet cells- PAS+/mm² (%)          | 97.30±1.17           | 98.96±0.27                  | 99.39±0.35                  | 99.10±0.24                                       |
| Goblet cells- PAS+AB+/mm² (%)       | 2.70±1.17           | 1.04±0.27                  | 0.61±0.35                   | 0.90±0.24                                        |
| **Jejunum**                         |                      |                             |                             |                                                  |
| Jejunum Wall Thickness (μm)         | 734.69±40.26         | 717.88±82.68                | 803.28±71.50                | 760.91±56.81                                     |
| Mucosal area (mm²)                  | 3.12±0.31            | 2.97±0.28                   | 2.87±0.26                   | 2.89±0.36                                        |
| Assumed Absorption surface (mm²)    | 0.49±0.07            | 0.46±0.12                   | 0.55±0.063                  | 0.45±0.09                                        |
| Villus Height (μm)                  | 468.00±21.22         | 442.38±80.42                | 502.61±28.14                | 475.49±46.85                                     |
| Crypt height (μm)                   | 176.73±18.24         | 180.95±29.98                | 176.98±13.91                | 155.29±9.38                                      |
| Villus height:Crypt height ratio    | 2.67±0.25            | 2.53±0.72                   | 2.85±0.25                   | 3.08±0.40                                        |
| Goblet cells- Units/mm²             | 62.46±13.02          | 76.01±1.60                  | 70.35±10.09                 | 81.74±6.51                                       |
| Goblet cells- PAS+/mm² (%)          | 99.18±0.46           | 99.54±0.20                  | 99.66±0.32                  | 99.27±0.23                                       |
| Goblet cells- PAS+AB+/mm² (%)       | 0.81±0.46            | 0.46±0.20                   | 0.34±0.33                   | 0.73±0.23                                        |
| **Ileum**                           |                      |                             |                             |                                                  |
| Ileum Wall Thickness(μm)            | 580.05±68.33         | 618.25±76.57                | 583.54±42.53                | 585.65±41.75                                     |
| Mucosal area (mm²)                  | 0.25±0.01            | 0.24±0.06                   | 0.28±0.02                  | 0.29±0.03                                        |
| Assumed Absorption surface (mm²)    | 0.30±0.04            | 0.30±0.06                   | 0.32±0.06                  | 0.27±0.03                                        |
| Villus Height (μm)                  | 333.89±7.51          | 313.65±30.98                | 308.31±27.95                | 296.88±25.04                                     |
| Crypt height (μm)                   | 145.47±19.39         | 137.61±19.87                | 171.69±11.26                | 156.31±12.41                                     |
| Villus height:Crypt height ratio    | 2.33±0.27            | 2.30±0.28                  | 1.80±0.11                  | 1.91±0.18                                         |
| Goblet cells- Units/mm²             | 236.8±19.35          | 234.52±61.06                | 192.8±21.80                | 245.4±9.35                                        |
| Goblet cells- PAS+/mm² (%)          | 4.20±1.86            | 1.73±0.96                   | 0.60±0.39                  | 1.41±0.19                                         |
| Goblet cells- PAS+AB+/mm² (%)       | 98.2±0.76            | 99.2±0.45                   | 99.7±0.18                  | 99.42±0.02                                         |

Mean ± standard deviation. Averages in the same row followed by different letter differ by the Kruskal-Wallis test followed by Dunn post test at a 5% significance level.
cell frequency increased in group D5b in relation to C, while the frequency of subtypes showed no statistical difference among the groups (Table 1 and Figure 1).

Observing all data obtained for the ileum segment, we found indication of tissue recovery as well as indications of direct effect of the treatment. The crypt height was higher in the D5a group in relation to control groups. On the other hand, villus height was higher in the D5b group in relation to the control, indicating that even after the end of the treatment, the drug, which is dose dependent, was

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Figure 1. Light microscopy and scanning electron microscopy images of duodenum, jejunum and ileum mucosa. **a–f**: images of duodenum in control group (Group C). Image **a** shows the expected structure in light microscopy. V: villus; C: crypt; M: muscle; L: lumen. This image was chosen as a model to show the structure of the two regions, jejunum and ileum. Images **b** and **c**: duodenum in control group obtained using scanning electron microscopy. V: villus; C: crypt; M: muscle; L: lumen. We chose this image as a model to show the structure found in the other two regions, jejunum and ileum. Image **d**: using the histochemical technique of Alcian Blue pH 2.5 combined with PAS. In purple (large arrow), the goblet cells secreting neutral mucin and in pink (small arrow) the goblet cells secreting basic mucin. **e**: Masson’s Trichrome technique. The connective tissue is stained in blue (*) and the normal structure of epithelium and muscle in red. **f**: results after Reticulin technique. The arrow indicates reticulin fibers, seen throughout the connective tissue, providing support for the organ. **g**: duodenum in group D5a; **h**: duodenum in group D5b; **i**: jejunum in group D5a; **j**: jejunum in group D5b; **k**: ileum in group D5a; **l**: ileum in group D5b. Images **a–f**: hematoxylin-eosin staining; Bar: 100 μm.
still active until its complete elimination, interfering with this parameter. For the goblet cells, we also found some indication of recovery in the D5b group after treatment and a drug-free period. We found no AB+goblet cells. The frequency of PAS+goblet cells was smaller in the D5a group in relation to both controls. Further, the frequency of AB+PAS+goblet cells was higher in the D5a group in relation to both controls. Since we found no difference in the D5b group in relation to D5a or control groups, we can assume that this represents a recuperation. The mucosa area considered in this analysis was higher in the D5b group in relation to C. The total number of goblet cells was smaller in the D5a group than in D5b and higher in relation to the C group.

Goblet cells occurred throughout the mucosa, more concentrated at the base of the crypt and villus and diminishing in the direction to the apex. Cells secreting acidic mucins (AB+PAS+) were not found and secretory cells with basic mucins (PAS+) were more frequent in the crypts, reducing in number as the villi were reached, where they occurred rarely or not at all. Regarding secreting Goblet cells with mucin composed of a mixture of basic and acidic mucin (AB’PAS’), these were infrequent in the crypts and increased to predominate in the villi. This pattern applied to all groups, indicating that the proposed treatment did not change the basic distribution of secretory cell types.

Reticulin histochemistry is based on silver impregnation of collagen fibers. The fibers were found throughout the connective tissue, forming a framework. This distribution was consistent for the different groups (Figure 1). In the samples stained with Masson’s Trichrome technique, the connective tissue appeared in blue. All groups showed the same distribution pattern of connective and muscle tissue labeling.

Scanning electron microscopy performed for small samples of the different groups showed the expected morphology, already described in the literature. We found villi extending into the lumen and intact absorptive epithelium with microvilli on the surface. The crypt below the villi was observed and connective tissue was found just below this tissue. The muscle layer seals the wall of the intestinal segment. This pattern occurred in all groups.

Discussion

Many publications can be found concerning the role of isotretinoin in cancer and its effects on the nervous system of patients (Hardin et al., 1999; Cisneros et al., 2005; Ferguson et al., 2005; Brenner & McCaffery, 2008), but its direct effects on the structure of the gastrointestinal tract is rarely discussed (Thomazini & Dolder, 2017). The exposure to isotretinoin did not cause any obvious signs of damage to the rat’s health although there was a trend to reduced food and water intake. In a previous study (Cisneros et al., 2005), the authors observed a reduction in food intake linked to consumption of isotretinoin. In our study, since the consumption of food and water was reduced in both groups receiving the drug and in the control groups, this reduction cannot be directly linked to the consumption of the drug, but it does follow a trend already suggested in the literature (Cisneros et al., 2005; Brenner & McCaffery, 2008).

Intestinal mucosa should provide appropriate morphological and functional characteristics, since the absorption processes are dependent on epithelium integrity. Numerous infectious or noninfectious agents can damage the intestinal mucosa and compromise the digestive processes. Inflammatory bowel disease is attributed to the use of some substances, including isotretinoin (O’Reilly et al., 2006; Passier et al., 2006; Reddy et al., 2006). Treatment with the substance could cause inflammatory diseases, but these symptoms disappeared at the end of the medication period (Passier et al., 2006).

The gastrointestinal tract has primarily a mechanical function. The overall rating of each histological section of the small intestine regions stained with haematoxylin-eosin revealed the general morphology corresponding to that already described in the literature (Lu et al., 2005; Barret, 2006; Shale et al., 2009). The absorptive capacity of the gut is initially proportional to the number of villi present (Kierszenbaum & Tres, 2011). A desirable ratio describes crypts that are shallower when compared to the villi and this situation is found in all groups. The optimum ratio of villus height: crypt height varies, and the accepted ratios are 3:1 to 5:1, but ratios of 2:1, 1.8:2:1 and 1:1 have also been found and are accepted as normal (Pelcano et al., 2003). In this study, the average found for the groups followed the trend of approximately 2:1, in agreement with descriptions proposed in the literature.

Crypts consist predominantly of increased goblet cells, Paneth cells in the base, enteroneoendocrine cells along the structure and some enterocytes. In the villi, the enterocytes with some goblet cells predominate. We found a normalization of goblet cell frequency after treatment interruption. This result is in accordance with the alteration found for D5a in the duodenum and jejunum.

Mucus is viscoelastic, gel-like and its primary function is to protect the mucosal cell surface from acids and peptidases. In addition, it serves as a lubricant for the passage of solids and as a barrier to antigens, bacteria and viruses (Shirazi et al., 2000; Walker & Talley, 2011; Chawla et al., 2013; Kim & Khan, 2013). The diet and the use of drugs can alter the distribution and frequency of goblet cells and it is related to the maintenance of bacterial colonization and microbiota (Frankel et al., 1995; Deplancke & Gaskins, 2001; Azevedo et al., 2007; Vieira-Lopes et al., 2014). The modifications concerning goblet cells were concentrated in the duodenum and ileum and the results are interesting, considering the typical goblet cell type in both regions. In the duodenum the tendency of increase in PAS+ cell during the treatment and a decrease of the same cell after treatment interruption indicates a potential of bolus hydration, linked to this type of mucin. In the ileum, the opposite situation was observed, with a decrease of PAS+ cell during the treatment and an increase after this phase. In the ileum, we had an increase in PAS+AB+ goblet cells. This result indicates a potential of microbiota.
maintained by PAS+AB+goblet cell secretion (Frankel et al., 1995; Deplancke & Gaskins, 2001; Azevedo et al., 2007; Vieira-Lopes et al., 2014). The difference found in these proportions is related to the treatment, considering the reversal observed after its interruption, but the alteration is not sufficient to cause loss of the small intestine function. Considering the three segments, duodenum, jejunum and ileum, we observed that compared to the duodenum, the jejunum and ileum were the two regions most sensitive to treatment with isotretinoin, with the ileum being the most strongly affected. The activity potential seems not to be modified, although in the jejunum we observed an increased wall thickness during the treatment and its reduction after the interruption. In the ileum we observed a tendency of continuing increase of the wall thickness throughout the experimental period.

The evaluation of the reticulin fibers and connective tissue distribution showed the distribution following the expected structure, as described in the literature (Lu et al., 2005; Barret, 2006), with fibers throughout the submucosa, and particularly in the connective tissue surrounding the villi and crypts.

Conclusions

We hypothesized that the jejunum and ileum are the most sensitive regions for this protocol with retinoid treatment, since they are the main absorption sites for these substances. Goblet cell type frequency was altered as an adaptive effect of this protocol. In general, the segments showed some adaptive condition for the treatment and no signs of damage to the small intestine were found with this protocol.

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Chawla G, Gupta P, Koradia V, Bansal AK. (2013). Gatrotentetion. A means to address regional variability in intestinal drug absorption. Pharmaceutical Technology July: 50–68.

Cisneros FJ, Gough BJ, Patton RE, Ferguson SA. (2005). Serum levels of albumin, triglycerides, total protein and glucose in rats are altered after oral treatment with low doses of 13-cis-retinoic acid or all-trans-retinoic acid. J Appl Toxicol 25: 470–478.

Deplancke B, Gaskins HR. (2001). Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am J Clin Nutr 73: 1135–1141S.

Diniz DGA, Lima EM, Filho NRA. (2002). Isotretinoina: perfis farmacologicos, farmacocineticos e analitico. Revista Brasileira de Ciências Farmacêuticas 38(4): 415–430.

Ferguson SA, Cisneros FJ, Gough B, Hanig JP, Berry KJ. (2005). Chronic oral treatment with 13-cis-retinoic acid (isotretinoin) or all-trans-retinoic acid does not affect depression-like behaviors in rats. Toxicol Sci 87: 451–459.

Frankel W, Zhang W, Singh A, Bain A, Satcchithanandam S, Kurfeld D, Rombeau J. (1995). Fiber: effect on bacterial translocation and intestinal mucin content. World J Surg 19: 144–148; discussion 148–149.

Hardin JA, Chung B, O’Loughlin E, V Gall DG. (1999). The effect of epidermal growth factor on brush border surface area and function in the distal remnant following resection in the rabbit. Gut 44: 26–32.

Charakida A, Mouser PE, Chu AC. (2004). Safety and side effects of the acne drug, oral isotretinoin. Expert Opin Drug Saf 3: 119–129.

Chia CY, Lane W, Chibnall J, Allen A, Siegfried E. (2005). Isotretinoin therapy and mood changes in adolescents with moderate to severe acne: a cohort study. Arch Dermatol 141: 557–560.

Junqueira LC, Canteiro J. Histologia Básica. Rio de Janeiro: Guanabara Koogan. 2015.

Kierszenbaum A, Tres L. Histology and Cell Biology: An Introduction to Pathology. 752p. Saunders, 2011.

Kim JJ, Khan WI. (2013). Goblet cells and mucins: role in innate defense in enteric infections. Pathogens 2: 55–70.

Lu X, Zhao J, Gregersen H. (2005). Small intestinal morphometric and biomechanical changes during physiological growth in rats. J Biomech 38: 417–426.

McCarter TL, Chen YK. (1992). Marked hyperplasia and pancreatitis associated with isotretinoin therapy. Am J Gastroenterol 87: 1835–1838.

Nankervis R, Davis SS, Day NH, Shaw PN. (1995). Effect of lipid vehicle on intestinal lymphatic transport of isotretinoin in the rat. The International Journal of Pharmaceutics 119(2): 173–181.

O’Reilly KC, Shumake J, Gonzalez-Lima F, Lane MA, Bailey SJ. (2006). Chronic administration of 13-cis-retinoic acid increases depression-related behavior in mice. Neuropsychopharmacology 31: 1919–1927.

Ortonne JP. (1997). Oral isotretinoin treatment policy. Do we all agree? Dermatol 195 Suppl 1: 34–37; discussion 38–40.

Passier JL, Srivastava N, van Puijenbroek EP. (2006). Isotretinoin-induced inflammatory bowel disease. Neth J Med 64: 52–54.

Pelciano ERL, Souza PA de, Souza HBA de, Oba A, Norkus EA, Kodawara LM, et al. (2003). Morfometria e ultra-estrutura da mucosa intestinal de frangos de corte alimentados com dietas contendo diferentes probióticos. Rev Port Ciênc Vet 98(547): 125–134.

Reddy D, Siegel CA, Sands BE, Kane S. (2006). Possible association between isotretinoin and inflammatory bowel disease. Am J Gastroenterol 101: 1569–1573.

Shale M, Kaplin GG, Panaccone R, Ghosh S. (2009). Isotretinoin and intestinal inflammation: what gastroenterologists need to know. Gut 58: 737–741.

Shalita AR, Cunningham WJ, Leyden JJ, Pochi PE, Strauss JS. (1983). Isotretinoin treatment of acne and related disorders: an update. J Am Acad Dermatol 9: 629–638.

Shirazi T, Longman RJ, Corfield AP, Probert CS. (2000). Mucins and inflammatory bowel disease. Postgrad Med J 76: 473–478.

Sieving PA, Chaudhry P, Kondo M, Provenzano M, Wu D, Carlson TJ, Bush RA, Thompson DA. (2001). Inhibition of the visual cycle in vivo by 13-cis retinoic acid (isotretinoin) or all-trans-retinoic acid. Proc Natl Acad Sci U S A 98: 1835–1840.

Sundstrom A, Alfredsson L, Sjolin-Forberg G, Gerdner B, Bergman U, Jokinen J. (2010). Association of suicide attempts with acne and treatment with isotretinoin: retrospective Swedish cohort study. BMJ 341: c3812.

Thomazini BF, Dolder MA. (2017). Dose dependent treatment with isotretinoin induces more changes in the ileum than in the duodenum and jejunum in Wistar rats. Tissue Cell 49(2 Pt B): 203–208.

REFERENCES

Akman A, Durusoy C, Senturk M, Koc CK, Soyurtdu D, Alpsoy E. (2007). Treatment of acne with intermittent and conventional isotretinoin: a randomized, controlled multicenter study. Arch Dermatol Res 299: 467–473.

Azevedo JF de, Hermes C, Manzano MA, Araújo EL de A, Sant ´Ana D de MG. (2007). Análise morfométrica da parede intestinal do ileo de ratos submetidos a intensa carência de proteínas. Arq Clínico Vet Zool Unipar 10(2): 85–89.

Barret KE. Gastrointestinal Physiology. Lange Medical Books: The McGraw Hill Companies. 2006. 294p.

Bigby M, Stern RS. (1988). Adverse reactions to isotretinoin. A report from the Adverse Drug Reaction Reporting System. J Am Acad Dermatol 18: 543–552.

Brenner JD, McCaffrey P. (2008). The neurobiology of retinoid acid in affective disorders. Prog NeuroPsychopharmacol Biol Psychiatry 32: 315–331.

Brito MdE, Sant’Anna IP, Galindo JC, Rosendo LH, Santos JB. (2010). Evaluation of clinical adverse effects and laboratory alterations in patients with acne vulgaris treated with oral isotretinoin. An Bras Dermatol 85: 331–337.

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Tsukada M, Schroder M, Seltmann H, Orfanos CE, Zouboulis CC. (2002). High albumin levels restrict the kinetics of 13-cis retinoic acid uptake and intracellular isomerization to all-trans retinoic acid and inhibit its anti-proliferative effect on SZ95 sebocytes. J Invest Dermatol 119: 182–185.

Vieira-Lopes DA, Nascimento AA, Sales A, Ventura A, Novelli IA, Sousa BM, Pinheiro NL. (2014). Histologia e histoquímica do tubo digestório de Phrynops geoffroanus (Testudines, Chelidae). Acta Amazon 44(1): 135–142.

Walker MM, Talley NJ. (2011). Clinical value of duodenal biopsies—beyond the diagnosis of coeliac disease. Pathol Res Pract 207: 538–544.

Zane LT, Leyden WA, Marqueling AL, Manos MM. (2006). A population-based analysis of laboratory abnormalities during isotretinoin therapy for acne vulgaris. Arch Dermatol 142: 1016–1022.