# Some clinical, pharmacological and immunological aspects of Crohn's disease

PhD Thesis

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## 1. Introduction

The inflammatory disease of terminal ileum was observed first in a patient /34 years old, post mortem/ and described by Morgagni in 1765. Thereafter other authors published also similar clinical symptoms, however, after the publication of Crohn, Ginzburg and Oppenheimer was called this inflammatory bowel disease as Crohn's disease /CD/.

According to our present knowledge CD has a complex etiology in which genetic, enviromental, microbiological and immunological factors can be involved. Familiar appearance, ethnical differences may also play an important role in the development of this inflammatory bowel disease. Clinically two types of inflammarory bowel disease are known: ulcreative colitis (UC) and CD. Typically CD appears in the terminal ileum, however, different symptoms can be detected in other segments of intestinal tract, as well. The symptoms of bowel inflammation can be different on the base of severity of changes: from moderate inflammation to the very severe destructive inflammation involved all layers of the intestinal tract.

CD appears usually in young patients, but it can be developed in older age, too. The highest incidence of occurence of CD was found in Ashkenazi Jewish communities. The incidence is higher in people who are living in cities and it is probably explained by the urbanization. Mortality of CD is continously decreaseing, however, the quality of life of these patients is worse than in the average population, which can be explained first of all by the appearance of severe complications.

The knowledge and information about the genetic factors became more pronounced in the last decades and it is known nowadays that the predisposition is partly determined genetically and the heredity of CD is polygenic. Determination of different genes is especially important in the study of the etiopathogenesis of CD.

The normal bacterial flora play an important role in the pathogenesis of CD and it produces a protective influence on the mucosal immunological response.

The normal intestinal bacterial flora provokes a continous antigen stimulus on the immunsystem of the intestinal mucosa and when this process is disturbed, irregular immunological responses are induced. The TNF-alfa may play an important and central role in the regulation of the systemic immunological responses.

The etiopathogenesis of CD is not known, therefore its therapy is unsolved.

Recently significant changes can be seen in the therapy of CD, e.g. introduction of antibody therapy against TNT-alfa /anti-TNF, biological therapy/. The most widely used drugs in the therapy of CD are the foolowings: Sulfasalazine, Corticosteroids, Azatioprine, Infliximab, Adalimumab and Vedulizumab.

## 2. Aim of investigations

The aim of investigations was to study some aspects of Crohn's disease and to produce an animal experimental model by which the morphological, functional/pharmacological and immunological changes can be analyzed in the intestinal bowel inflammation. Production of the intestinal bowel inflammation in experimental animals can be considered as a model of CD, therefore the influence and protection of the appearance and severity of symptoms are very important from theoretical and clinical point of view, as well. Our study was mainly focused on the following questions:

1. -lnvestigation of clinical aspects of CD and first of all the study of appearance of resistance against the pharmacotherapy and to analyse the change of vascular factors.
2. -Development of a model of CD in the small intestine of rats by administration of indomethacin and the investigation of morphological and functional/pharmacological changes produced by indomethacin-induced bowel infammation.
3. -Investigation of the elimination function of small intestine and the study the possible influence and protection of changes produced by indomethacin pretreatment.
4. -Investigation of the bile production and the elimination function of the liver after indomethacin administration and the analysis of protective effects of drugs.
5. -Immunological investigations: Measure of the level of TNF-alfa stimulated by LPS and PMA, investigation of the possibility to influence of changes induced by indomethacin.

## 3. Methods: Clinical, experimental and analytical investigations

### 3.1. Clinical investigations

#### 3.1.1. Investigation of multidrug resistance

Investigations were performed on 30 inactive Crohn s disease patients treated with mesalazine maintaince therapy. The steroid resistance was determined in living cells by P-glycoprotein (MDR protein expression) and functional MDR (Calcein – Verapamil) tests.

#### 3.1.2. Ivestigation of vascular factors

Investigations of platelet numbers and functions were carried out from the blood obtained from the cubital vein of inactive CD patients treated with maintaince mesalazine therapy. For the investigations of demage of blood vessels, determination of vWFAg was used. Determinations of soluble vascular factors /IL-6, IL-8, CD-40, tPA, MCP-1, P-selectin, sWCAM-1/ were performed by the methods described in the literature.

### 3.2. Pharmacological and pharmacokinetic investigations

#### 3.2.1. Animal experiments

Male Wistar rats /weighing 220-250g/ were used in the animal experiments. The animals were unesthetized with urethane /1.2 g/kg i.p./. The abdominal wall was opened on the midline with a longitudinal incision and a 10 cm long middle segment of small intestine was cannulated in vivo. The segment was flushed with warm /37°C/ isotonic buffer solution, then it was made empty by blowing through 4-5 ml air. The intestinal segment was perfused with the isotonic perfusion medium in a recirculation mode with a speed of 13ml/ min. The pefusion medium contained PNP (p-nitrophenol) in a concentration of 500µM. The solution coming out from distal end of the isolated intestinal segment was collected in a reservoir, from where it was

continously recirculated to the intestinal lumen with a peristaltic pump. Samples /250µl/ was obtained from the perfusion medium coming out from the intestinal segment. The initial perfusion volume was 15 ml and the experiment lasted 90 minutes. The temperature of the perfusion medium was maintained constant at 37°C. When the biliary excretion was investigated, the bile duct was cannulated with a PE-10 tubing and the bile was collected in 15 min periods. The samples were stored in refrigerator /-20°C/ until analysis. The animals were fasted 16-20 hours prior to the experiments, water was given ad libitum.

Inflammatory bowel disease /model of CD/ was induced by indomethacin /lx10 mg/kg subcutaneously on day 1, day 2 and day 3/. Steroids (Prednisolone, 0.5mg/kg orally Methylprednisolone, 1.78-3.75 mg/kg i.p.), Pentoxifylline (40mg/kg i.p.), Mesalazine (125-1000 mg/kg) and Pro-Gastro (42.86 mg/kg) by bastric tube were administered once a day for three days. Experiments were carried out at day 4. The evaluation of severity of macroscopic changes was performed by a scoring system /Tamaki score/. For microscopic analysis the sections of segments of small intestine were routinely fixed and stained by hematoxylin eosin.

3.2.2. Analytical procedures

The samples obtained from the intestinal perfusion medium and from bile were analyzed and quantified by the HPLC-methods which was developed in our related experiments. Summarized shortly: the mobil phase consisted of methanol and distilled water /50:50 v/v %/ containing 0.01 M tetrabutyl ammonium bromide to determine the metabolites. The samples were vortexed and centifugated at 3000g for 10 minutes before separation. The flow rate of the eluent was 1.2 ml/min. The volume of the samples was 20 µl and the detection was effected at 290nm, because this wavelength was optimal for the simultaneous determination of PNP, PNP-G (PNP-glucuronide) and PNP-S (PNP- sulfate). At the bile samples the mobil phase consisted of methanol and citrate buffer pH 6.2 /47:53 v/v%/ containing 0.03M tetrabutyl ammonium bromide. The samples were prepared by mixing 200µl cold methanol containing 3.125Mp-ethylphenol as internal standard and 50 µl bile. After vortexing the samples were centrifuged by 10000 g for 10 minutes and 20µl supernatants were injected to the HPLC. The samples were stored at -20°C and before

the analysis the temperature of samples was allowed to rise ambient temperature.

The HPLC system consisted of a Varian 2010 pump, a Rheodyne 7725i injection valve, an UV-detector 308 with a Powerchrom 280 data collecting and integrating unit and software. A Nucleosil 100 $C_{18}$ reversed phase column /250mm x 4.6mm ID, 10 μm partiele size/ was employed for the separation of metabolites, and at the bile samples Teknokroma TR-C-160K1 guard column was applied also.

### 3.3. Immunological investigations

#### 3.3.1. Isolation and stimulation of mononuclear cells

Mononuclear cells were separated from the anticoagulated blood samples by Ficoll-Paque (Sigma- Aldrich) gradient centrifugation. The isolated cells were washed with PBS (phosphate buffered saline) then added to 6-well cell culture plate at the concentration of $10^6$ cell/ml in RPMI-1640 medium (1% L-Glutamine-Penicillin-Streptomycin) (Sigma-Aldrich) containing 10% FCS (fetal calf serum). The mononuclear cells were stimulated with 25 ng/ml PMA (phorbol myristate acetate) and 1μg/ml ionomycin or 1μg/ml LPS (Sigma-Aldrich) for 24 hours. Then the cells were centrifuged (l000rpm – l0min) and supernatants were collected and stored at -20°C.

#### 3.3.2. TNF-alfa determined by ELISA

Concentration of TNFa in supernatants were determined with commercially available sandwich ELISA kit (rat TNF-alpha modul set ELISA kittel; Bender Medsystems) according to the protocol suggested by the manufacturer. 96-well polystyrene plates (Nunc Maxisorp; Thermos scientific) were coated with the capture antibody diluted in PBS (phosphate buffered saline) at 4°C overnight. Following saturation of non-specific binding sites with PBS containing 0.5% BSA (bovine serum albumin, Sigma- Aldrich) and 0.05 Tween-20 (Sigma-Aldrich) for 2 hours supernatants at a dilution of 1:2 and recombinant TNFa standards at the concentration of 2500-39 pg/ml with biotinylated detection antibody were incubated for 2 hours on a shaker at 200rpm at room temperature. Following washing (PBS

conatinig 0.05% Tween-20) the plate was incubated with HRPO conjugated (horseraddish peroxidase) streptavidin for another 2 hours. The reaction was developed with TMB (tetramethylbenzidine), stopped after 15 minutes with 4N sulphuric acid and the optical density was measured on an iEMS MF microphotometer (Thermo Labsystem) at 450 nm. The concentration of TNF-α in the supernatants was calculated using the standard curve.

## 4. Calculations, statistical analysis

The luminal appearance of PNP-G and PNP-S was calculated on the base of their luminal concentrations and the actual volume of perfusion solution. The original volume of perfusion medium was 15 ml which was modified by the obtained samples and the absorptive and resorptive actions of the perfused intestinal segment, therefore the actual volume of perfusion solution was calculated and corrected by these changes. The biliary excretion of PNP and its metabolites was calculated as the product of biliary volume and the biliary concentration of compoounds.

Data show the mean values and S.E., n: means the number of experiments or determinations. Data were analyzed by one-way ANOVA, the difference amoung different groups was detremined by Student s t-test.

## 5. Results

### 5.1. Clinical investigations

#### 5.1.1. Investigation of multidrug resistance

These investigations are very important for the adequate therapy of CD. They were carried out on 30 inactive CD patients, who were treated by Mesalazine maintaince therapy. Several factors, e.g. demand on higher dose of steroids, an early requirement of introduction of immunosuppressive therapy, demand on anti-TNF therapy and occurence of frequent relapses indicate the development of steroid resistance. 18 from our patients were treated continously with steroids and 9 patients from this group had already surgical interventions, 6 patients were treated with

Immuran and 3 of them had surgical operations despite the Immuran therapy. High predisposition to chemoresistance was observed at 5 patients, who were C/C homozygotes.

### 5.1.2. Investigation of vascular factors on inactive CD patients

It was found in these studies that the platelet function, the percentage ratio of ADP-induced and activated platelets and the factor von Willebrand /indicator of demage of blood vessels/ remained in the normal range, no significant differences were observed from the control values.

During the investigations of the following soluble vascular factors were determined: levels of sCD40L /one of the markers in the blood for thrombocyte activation/, IL-8 and IL-6 /interleukins/, concentration of MCP-1 /monocyte chemotactic protein/, level of sVCAM-1 /vascular cell adhesion molecule-1/, P-selectin /cell adhesive molecule/ and tPA /tissue plasminogen activator/. It was found that the level of sCD40L, MCP-1, IL-8 and IL-6 in 40 percent, the level of tPA in 30 percent of patients were elevated, however, the value of P-selectin was lower in all patients compared to the control values. The level of the best parameter of vascular demage /sVCAM-1/ was enhanced consequently and significantl in all patients investigated, which indicates that the role of this factor can be especially important in the development of CD.

## 5.2. Pharmacological and pharmacokinetic investigations

### 5.2.1. Development of CD model, morphological changes

Bowel inflammation -as a model of CD- was produced by indomethacin /lxl0 mg/kg s.c. for 3 days/. Appearance and severity of macroscopic morphological symptoms of the inflammation were investigated and evalueted by a generally used scoring system /Tamaki score/. It was found that indomethacin provoked characteristic changes /hyperemia, petechia, bleeding, lesions, ulcerations/ in the intestinal tract. Alterations produced by indomethacin can be depressed by steroids /e.g. methylprednisolone/ and non-steroidal /e.g. mesalazine/ antiinflammatory

agents, as well.

On the base of histological changes of indomethacin-induced bowel inflammation it was found that indomethacin provoked a deep ulcer, even the inner isoproprial muscular layer was also involved and a nonspecific inflammation could be seen in the fat tissue beneath. Mesalazine and methylprednisolone produced protective actions, the effect was more pronounced after mesalazine administration.

### 5.2.2. Elimination function of small intestine

It was found that the disappearence of PNP from the luminal perfusion solution was slower than in control rats, therefore the PNP level was higher in the luminal perfusion medium. This finding indicates a slower absorption rate or an enhanced blood to lumen flux of PNP due to an increased permeability of the small intestine in this direction. It is interesting to note that mesalazine corrected this effect of indomethacin.

Indomethacin depressed definitely the luminal appearance of PNP-G and PNP-S which means a negative effect on the intestinal metabolic and excretory function, however, this decreasing effect of indomethacin was not influenced significantly by mesalazine.

### 5.2.3. Study of the elimination function of the liver

Indomethacin depressed the biliary flow, which could be compensated partly by pentoxifylline and totally by mesalazine. Indomethacin pretreatment inhibited significantly the biliary excretion of PNP and its metabolites /PNP-G and PNP-S/, as well. Mesalazine produced a protective action in all cases, that is, it stimulated definitely the indomethacin-depressed biliary excretion rate of PNP, PNP-G and PNP-S, however, the control level of biliary excretion was reached only in the case of PNP-S. These results indicate that it the intestinal inflammation can be detected changes not only in the intestinal tract, but in the liver, as well. Moreover, the alterations in the liver can be protected partially or totally in the liver, but not in the small intestine.

### 5.2.4. Effect of pentoxifylline on the elimination function of the small intestine and in the liver

According to our experimental results pentoxifillyne was unable to protect the indomethacin-induced decrease of the luminal appearance of PNP-G and PNP-S, furthermore, the indomethacin- induced depression of the biliary excretion of PNP and its metabolites was also unchanged after pentoxifylline administration.

## 5.3. Immunological investigations

Indomethacin increased considerably the LPS- and PMA- induced level of TNF-α. This increased level of TNF-α was depressed by mesalazine or Pro-Gastro and by their combination, as well. However, no significant difference was found between the protective actions of mesalazine and Pro-Gastro, furthermore Pro-Gastro was unable to stimulate the effect of mesalazine after the administration of their combination.

# 6. Discussion – Conclusions

We have found a relationship between the development of chemoresistance and the dosages of administered steroids. All patients with an average and high level of chemoresistance had a higher number of anamnestic relapses than the controls. Our investigations indicate that more than one factors are needed for the clinical laboratory demonstration of multidrug resistance /genetic, protein expression, MDR function/. In pathological conditions the increased expression of MDR does not mean necessarily the enhanced function. Correlation between the biological behavior of patients, the effectivity of therapy and the status of MDR can be different on the base of individual genetic constellation and clinical symptoms of patients. In present investigations it was found that the number and functions of platelets was not different from the control range, which can be explained probably by the drug /mesalazine/ treatment of inactive CD patients. According to the results of our investigations regarding soluble vascular factors of CD patients and carried out by flow cytometry, no differences were found from control values. Similarly to these results, the values of other vascular factors remained also in control range with

exception of value of vascular adhesive molecule /sVCAM.l/, which was consequently higher than the control. More recently other authors found and published similar results and on this base nowadays there is a new theory according which the microvascular endothel cells play an important role in the start of inflammatory processes.

In these animal experiments the morphological and functional /pharmacological/ changes were studied in the indomethacin- induced inflammatory bowel disease /rat model of Crohn's disease/. It was found that indomethacin produced characteristic morphological /macroscopic and microscopic/ alterations in the small intestine which are basically similar to those of Crohn's diseaese. The morphological changes could be depressed by steroidal /methylprednisolone/ and non-steroidal /mesalazine/ antiinflammatory agents, as well.

The functional/pharmacological alterations in the inflammatory bowel disease were also investigated. The disappearance of xenobiotics /e.g. PNP/ from the intestinal lumen depends first of all on the absorption /permeability/ and the elimination /metabolism and excretion/ processes in the intestinal tract. It was found that the diappearance of PNP from the intestinal lumen was decreased by indomethacin, because greater amount of PNP remained in the luminal perfusion solution in the indomethacin-pretreated rats than in controls. The intestinal metabolism of PNP /luminal appearance of PNP-metabolites/ was decreased, however, this depression can not explain the definitely greater difference in the amount of disappearance of PNP from the luminal solution. Therefore these results theoretically suggest a smaller absorption rate or a greater blood to lumen flux of PNP in the indomethacin-pretreated rats. More precisely our results indicate an enhanced blood to lumen permeability, that is, a stimulated flux of PNP from blood into the intestinai lumen. These results suggest that the intestinal permeability can be changed in both direction, that is from lumen to blood and from blood to lumen, as well. It is interesting to note that the depressing effect of indomethacin in the luminal appearance of PNP-G and PNP-S could not be protected by mesalazine, whereas the changes of disappearance of PNP from the luminal solution in the indomethacin- pretreated rats was compensated by administration of mesalazine. These results suggest that mesalazine can produce independent effects on the intestinal elimination of PNP and on the intestinal permeability.

The hepatic elimination of PNP metabolites was considerably inhibited by indomethacin similarly to the luminal appearance of PNP-G and PNP-S. However, the depressed biliary excretion rate of PNP-G and PNP-S was compensated by mesalazine which shows a sharp contrast with the intestinal elimination of these metabolites. Moreover, the biliary excretion of the mother compound /PNP/ in a non-metabolized form was also diminished by indomethacin, which means that indomethacin was able to influence not only the metabolism of PNP, however, the biliary excretion of the mother compound, as well.

These results show that in the indomethacin-induced bowel inflammation severe changes occur in the intestinal tract, however, alterations can be detected in other organs /e.g. in the liver/, as well. Furthermore, the changes in the small intestine and in the liver can be partly similar, but differences can be detected, too.

In the etiopathogenesis of CD several factors, e.g. bacterial infections may play a role, therefore the effect of pentoxifylline, a strong antibacterial agent was also investigated on the inflammatory symptoms produced by indomethacin. It was found that in our experimental conditions pentoxifylline was unable to eliminate morphological alterations produced by indomethacin, furthermore given in combination it did not stimulate the effect of mesalazine. Similar findings were observed on the pharmacological changes, as well. Investigations with other antibiotics produced various effects, which can be explained by different experimental conditions and by different susceptibility of microorganisms. Immunological investigations indicate that the LPS- and PMA- stimulated level of TNF-alfa measured in indomethacin-pretreated rats was significantly decreased by mesalazine. Similar effect was found after Pro-Gastro administration, too.

Further investigations are needed to clarify the mechanism of action of indomethacin in the intestinal and hepatic elimination processes and the differences in the protective effects produced by mesalazine and other agents.

7. Summary and new results

1. -Complex investigations of genetic factors, protein expression and MDR-1 functions are needed for the clinical laboratory demonstration of MDR-1. At our patients it was found a relationship between the steroid resistance and the dosages of administrated steroids.
2. -During the analysis of vascular factors it was found that the these factors can be changed separately or together, as well. Only the sVCAM-1 was changed consequently. The elevation of sVCAM-1 level was also demonstrated by newer data of other authors, and according to the newest theory this parameter is a crucial factor and indicator of inflammatory processes.
3. -In our animal experiments the bowel inflammation produced by indomethacin can be evalueted and followed by a scoring system on the base of the appearance and severity of morphological symptoms, therefore it can be considered as a model of Crohn's disease. We have found using this model that both steroid /methylprednisolone/ and non-steroid /mesalazine/ therapy can produce protective effects in the bowel inflammation.
4. -Indomethacin decreased the diappearance from the intestinal lumen, reduced the intestinal formation and excretion of PNP- metabolites (PNP-G, PNP-S). These data suggest that the permeability from blood to lumen was increased. Indomethacin produced depressive effect on the liver function, too. However, mesalazine produced a protective effect on the biliary excretion, but it had no effect on the intestinal elimination.
5. -Influence of bacterial status by probiotic /Pro-Gastro/ or antibiotic /pentoxifylline/ agents produced some protective effects, however, they these drugs were unable to increase the effect of mesalazine.
6. -lt was found on the base of immunological studies that the TNF- alfa level stimulated by LPS or PMA was increased by indomethacin, which was depressed by mesalazine or Pro-Gastro. However, Pro-Gastro given in combination was unable to increase the action of mesalazine.

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9. Publications

1. Simon H., Kecskés M.: Vérlemezeke funkció, aktiváltsági állapot és v.WFAg vizsgálata inaktív Crohn betegekben. Honvédorvos 150/3,206-2013 (1998)

2. Simon H.: Vastagbél diverticulosis és szövődményei. Granum VIII/5, 45-46 (2005)

3. Bojcsev S., Almási A., Simon H., Perjési P., Fischer E.: Investigation of drug metabolism in various segments of small intestine in the rat. Acta Physiol. Hung. 100, 115-123 (2013) (I.F: 0,747)

4. Almási A., Bojcsev S., Fischer T., Simon H., Perjési P., Fischer E.: Metabolic enzyme activities and drug excretion in the small intestine and in the liver in the rat. Acta Physiol. Hung. 100, 478-488 (2013) (I.F:0,747)

5. Simon H., Fischer T., Almási A., Fischer E.: Effect of Mesalazine on the morphological and functional changes in the indomethacin-induced inflammatory bowel disease (rat model of Cronh's disease). POR (közlésre elfogadva) (I.F.:1,855)

10. Scientific presentations (Lectures and Posters)

1. Simon H.: Gasztrointesztinális vérzések diagnosztikája (Lecture). Pécsi Honvédkórház Tudományos Tanács Orvosfóruma, Pécs, 1990.

2. 2. Simon H.: Ulcus duodeni esetismertetése és endoszkópia élő bemutató (Lecture). Gasztroenterológia a házi orvosi gyakorlatban POTE-Medicom Glaxo Szimpózium, Pécs, 1955.

3. Tolnai Z., Simon H., Deák G.: Amoebas colitis (Lecture). Magyar Gasztroenterológiai Társaság Nagygyűlése Videosectio Balatonaliga, 1995.

4. Simon H.: A Crohn-betegség kezelése és gondozása betegklub keretében (Lecture). M. H. Központi Katonai Kórház Tudományos ülése, Budapest, 1995.

5. Simon H.: PPI szerepe a fekély kezelésében (Lecture). Baranyai Orvosklub, Pécs, 1998.

6. Simon H.: Vérlemezke funkció, aktiváltsági állapot és vWFAg szint vizsgálata Crohn- betegekben (Lecture). M.H. Belgyógyászainak Összevont VIII. Tudományos Konferenciája, Budapest, 2000.

7. Lukács M., Simon H.: Hasi panaszokkal, melaenával és reccuráló purpurákkal kezelt beteg esete (Lecture). PTE ÁOK Tudományos Szakosztály ülés. Pécs, 2000.

8. Lukács M., Nagy Zs., Papp F, Simon H.: Végbélcarcinoma és purpura: Koincidencia vagy következmény? (Poster). Magyar Belgyógyász Társaság Nagygyűlése, Budapest, 2000.

9. Simon H.: Vascularis faktorok szerepe a Crohn-betegség etiológiájában. M. H. Központi Katonai Kórház Tudományos ülése (Lecture). Budapest, 2001.

10. Simon H.: Egy Crohn-beteg története (Lecture). PTE ÁOK Tudományos Szakosztály Ülés, Pécs, 2001.

11. Lukács M. Simon H., Pakodi F., Tornóczky T., Ezer P., Kelemen D., Kövér E.: A gyomor submucosus daganatai (Poster). Magyar Gasztroenterológiai Társaság Nagygyűlése, Budapest, 2002.

12. Simon H.: A NSAID-ok gasztrointesztinális mellékhatásai (Lecture), MSD Szimpózium, Pécs, 2002.

13. Simon H., Fischer E.: Mesalazin és prednizolon hatása az indometacinnal kíváltott intesztinális gyulladásra patkányban (Poster). Membrán Transzport Konferencia, Sümeg, 2004.

14. Simon H., Fischer E., Beró T., Simon D.: Probiotikum, mesalazin és prednizolon hatása az indometacinnal kiváltott bélgyulladásra (Lecture). Magyar Gasztroenterológiai Társaság Nagygyűlése, Szeged, 2004.

15. Simon H., Magyarlaki T., Beró T.: Funkcionális multidrug-rezisztencia (MDR) vizsgálata Crohn-betegségben (Lecture). MBT Dunántúli Belgyógyász Vándorgyűlés, Bükkfürdő, 2005.

16. Simon H., Fischer E., Beró T.: Mesalazin és prednisolon hatása az indometacinnal kiváltott bélgyulladásra és az intesztinális permeabilítás változása patkányban (Poster). Magyar Gasztroenterológiai Társaság Nagygyűlése, Balatonaliga, 2005.

17. Simon H., Fischer E.: Vaszkuláris faktorok szerepe a Crohn-betegségben (Lecture). MBT Dunántúli Belgyógyász Vándorgyűlés, Sopron, 2006.

18. Simon H, Simon D, Pálinkás L, Fischer E: Az indometacinnal kiváltott gyulladás kialakulása és befolyásolhatósága patkány vékonybélben (Poster). Membrán Transzport Konferencia, Sümeg, 2006.

19. Simon H., Fischer E., Berki T., Beró T.: Vaszkuláris faktorok vizsgálata inaktív 5-ASA-t szedő Crohn-betegekben (Lecture). Magyar Belgyógyász Társaság Északkelet-magyarországi Szakcsoport Tudományos Ülése, Eger, 2006.

20. H. Simon, E. Fischer, T. Beró, D. Simon: Effect of probiotics, mesalazin and prednisolon on indomethacin produced bowel inflammation in rats (Poster). Magyar Gasztroenterológiai Társaság, Visegrád, 2007.

21. Horvát Gy., Simon H., Kiss Zs.: A monolobáris Caroli betegség (Lecture). Magyar Belgyógyász Társaság Északkelet-magyarországi Szakcsoport Tudományos Ülése, Kazincbarcika, 2010.

22. Bojcsev S., Almási A., Simon H., Perjési P., Fischer E.: A metabolikus aktivitás vizsgálata a vékonybél különböző szegmentjeiben (Poster). Membrán-Transzport Konferencia, Sümeg, 2012.

23. Fischer T., Almási A., Bojcsev S., Simon H., Fischer E., Perjési P.: A gyógyszer-metabolizmus vizsgálata a vékonybélben perfúziós és recirculációs módszerrel (Poster). Membrán-Transzport Konferencia, Sümeg, 2015.

24. Simon H., Fischer T., Almási A., Perjési P., Fischer E.: A vékonybél xenobiotikumokat eliminálló tevékenysége Crohn -betegség modelben patkányban (Poster). Membrán–Transzport Konferencia, Sümeg, 2016.

25. Fischer T., Almási A., Bojcsev Sz., Perjési P., Fischer E., Simon H.: A máj metebolikus és exkréciós tevékenysége Crohn-betegség modellben patkányban (Poster). Membrán–Transzport Konferencia, Sümeg, 2016.