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Dexmedetomidine ameliorates TNBS-induced colitis by inducing immunomodulator effect

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ARTICLE INFO

Article history:
Received 27 November 2012
Received in revised form
16 February 2013
Accepted 7 March 2013
Available online 31 March 2013

Keywords:
Inflammatory bowel diseases
Dexmedetomidine
Immune response
Cytokines

ABSTRACT

Background: Since sedatives are often administered to immune-compromised and critically ill patients, our understanding of immunomodulation by sedation will be critical. Dexmedetomidine, a selective α_2-adrenergic receptor agonist, is often used for sedation and analgesia especially in intensive care units. There are conflicting and little data concerning both the effect and the mechanism of dexmedetomidine on immune response. In our study, we aimed to investigate the effect of dexmedetomidine on immune system at two different doses (5 $\mu g.kg^{-1}$ and 30 $\mu g.kg^{-1}$) during inflammatory bowel disease by using an experimental model, which resembles both systemic and local inflammation.

Methods: The effect of dexmedetomidine on the course of inflammatory bowel disease was investigated by measuring macroscopic and microscopic parameters. We investigated pro-inflammatory Th1, Th2, and Th17 cytokine levels in serum samples to analyze systemic immune response. Following this, local immune response was investigated by measuring cytokine levels in the presence of dexmedetomidine in spleen cell culture.

Results: Dexmedetomidine administration led to amelioration of all disease associated pathological manifestations. According to our *in vitro* and *in vivo* results, dexmedetomidine shows anti-inflammatory effect by increasing IL-4 and IL-10 levels responsible from anti-inflammatory response via Th2 pathway. Moreover, we showed for the first time in the study that dexmedetomidine administration reduces IL-23, which is responsible from initiation of inflammatory response via Th17 pathway.

Conclusions: Dexmedetomidine can have beneficial effect on preoperative or postoperative inflammatory bowel disease patients in intensive care units by down-regulating inflammatory immune response not only in systemic circulation but also in tissue-specific manner.

1. Introduction

Inflammatory bowel diseases (IBD) are a chronic inflammatory disease characterized as disabilities in the gastrointestinal system accompanied by defective mucosal immune response [1,2]. Although the etiology of IBD is still unknown, this group of diseases is classified according to several factors, such as hereditary and environmental factors, effectiveness of host immune response, and the location of inflammation in gastrointestinal system. An inadequate activation of the

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http://dx.doi.org/10.1016/j.jss.2013.03.028

intestinal and systemic immune system, mainly activation of CD4 T helper 1 and T helper 17 cells, causes an imbalance reaction between pro-inflammatory and anti-inflammatory responses, which plays a critical role in the pathogenesis of disease [3–5].

The first therapeutic approach of IBD depends on the use of nonspecific anti-inflammatory agents and immunosuppressive drugs; secondly, specific monoclonal antibodies together with anti-inflammatory agents are commonly used [6–9]. In most cases, these therapeutic approaches do not modify the disease course; thus surgical applications are chosen as a third therapeutic approach [10]. However, postoperative complication rates are higher in patients with IBD than benign diseases [11–13]. In general, postoperative intra-abdominal septic complications are among them, requiring more aggressive treatment and longer hospitalization in intensive care units [14]. Owing to the high risk of infection in these patients and the frequency of systemic and local inflammation, an effective immune response is important for the health of such patients.

Dexmedetomidine, a selective α_2-adrenergic receptor agonist; is often used for sedation and analgesia. It is used to form sedation in patients receiving mechanical ventilation, in the form of infusions, which do not exceed 24-h application [15]. Owing to its effect on α_2-adrenergic receptor in the nervous system, it abolishes the secretion of catecholamines instantly in patients under stress. In addition, because of its sedative, analgesic, and hypotensive effects, its use as an adjuvant during the perioperative period is increasing [16]. The use of dexmedetomidine in sepsis patients causes an improvement in brain functions in a short time and the duration of mechanical ventilation is decreased for these patients. In addition, it suppresses pro-inflammatory cytokines, which are a part of an innate immune response in sepsis patients [17].

In our study, we aimed to investigate the effect of dexmedetomidine on systemic and local inflammatory responses and, consequently, its effect on immune system both *in vivo* and *in vitro* during inflammatory bowel disease. For this purpose, an experimental inflammatory bowel disease model was created by using 2,4,6-trinitrobenzene sulfonic acid (TNBS)+ethanol, and the effect of dexmedetomidine on the inflammatory response at two different doses (5 $\mu g\ kg^{-1}$ and 30 $\mu g\ kg^{-1}$) in both cellular and histopathologic levels was investigated.

2. Materials and methods

2.1. Mediums and chemicals

In histologic examination, formaldehyde (Merck, Darmstadt, Germany), ethanol (Sigma, St. Louis), xylene (Merck), and paraffin were used for routine tissue monitoring. Hematoxylene (Merck) and eosin Y (Merck) were used in histologic examination; number and viability of spleen cells were determined by using 0.4% Trypan Blue (Merck). Spleen cells were grown in RPMI-1640 medium (Sigma) with 50 IU penicillin and 50 $\mu g\ mL^{-1}$ streptomycin (Sigma) supplemented with 10% fetal bovine serum (Sigma). TNBS (Sigma) 5% (wt/vol) and 95% ethanol were used to induce colitis.

2.2. Animals

BALB/c mice, male, 5–6 wk old, weighing 16–20 g, were used in all experiments. Mice were kept under specific pathogen-free environment. All experiments were approved by the Ethics Committee of Inonu University Experimental Animals Production and Investigation Centre (Ethic Report Number: 2010/28).

2.3. Dexmedetomidine preparation and application

Stock dexmedetomidine (Precedex, Dexmedetomidine HCl 1638) was at 200 μg/2 mL concentration. Dexmedetomidine was administered at two different doses, 5 $\mu g\ kg^{-1}$ and 30 $\mu g\ kg^{-1}$, in phosphate buffered saline [(150 mM NaCl, 30 mM KCl, 15 mM Na_2HPO_4, 2 mM KH_2PO_4, pH7.4)], intraperitoneally. These doses were decided according to the data of our preliminary experiments based on similar studies. Dexmedetomidine administration started at first day after the application of TNBS+ethanol and continued daily for 6 d.

2.4. Induction of colitis and experimental groups

Experimental model of acute inflammatory bowel disease was induced in BALB/c mice by the method as described previously [18]. Briefly, TNBS colitis was induced by intra-rectal installation of TNBS (Sigma) 5% (wt/vol), mixed with an equal volume of ethanol, into anesthetized mice, 150 μL/mouse. Mice in the control group were administered only 50% ethanol. Mice that died in the first 2 d were considered as treatment casualties and were excluded from all calculations or presentations.

Experimental groups were designed and named as follow; no induction as 'naive group,' mice that were administered only ethanol as 'ethanol group,' mice that were administered TNBS+ethanol as 'colitis group TNBS-EtOH.' In a group of animals after induction of colitis 5 $\mu g\ kg^{-1}$ dexmedetomidine was administered 'TNBS+DEX5.' After induction of colitis 30 $\mu g\ kg^{-1}$ dexmedetomidine was administered 'TNBS+DEX30.' Each treatment group included 10 to 20 mice.

2.5. Assessment of colitis

Weight and survival were monitored daily. Six days after TNBS induction, mice were killed, and microscopic colonic damage was evaluated. Proximal, medial, and distal portions of colon were fixed in 10% phosphate-buffered formalin. Paraffin embedded sections were stained with hematoxylin and eosin. The degree of histologic damage and inflammation was graded in a blinded fashion by an expert histologist according to our previous schedule [19]. The following manifestations were included in the evaluations: the amount of inflammation (0, none; 1, mild; 2, moderate; 3, severe; and 4, accumulation of inflammatory cells in the gut lumen), distribution of lesions (0, none; 1, focal; 2, multifocal; 3, nearly diffuse; and 4, diffuse), depth of inflammation and layers involved (0, none; 1, mucosa only; 2, mucosa and submucosa;

3, limited transmural involvement; and 4, transmural), and nature of mucosal changes (0, none; 2, more degeneration; and 3, more necrosis). The overall histologic score was the sum of the four manifestations (maximal score of 15). The average scores of proximal, medial, and distal regions of each colon samples were presented.

2.6. Cell isolation and cell culture

Spleens were collected under aseptic conditions. Spleens were minced with a scalpel in order to isolate mononuclear cells in a single cell layer. Number and viability of isolated cells were examined in 0.4% Trypan blue. Cells were at more than 95% viability in all *in vitro* experiments. In order to examine the *in vitro* effect of dexmedetomidine on cytokine levels, 2×10^6 cells mL^{-1} were layered in 24-well plates under sterile conditions. Thereafter, dexmedetomidine was added to appropriate wells at 10 ng mL^{-1} and 100 ng mL^{-1} concentrations. Cells were incubated at 37°C and 5% CO_2 conditions for 24 and 48 h. At the end of the incubation time, cell culture supernatants were collected from each well and samples were stored at −80°C until the analysis of cytokines.

2.7. Analysis of cytokine levels

Blood samples were obtained by cardiac puncture of anesthetized mice. Serum samples were collected after centrifugation and were stored at −80°C until analyzed. Proinflammatory (interleukin [IL]-1α, IL-6, tumor necrosis factor [TNF]-α), Th1 (IL-2, interferon [IFN]-γ), Th2 (IL-4, IL-5, IL-10), and Th17 (IL-17, IL-23) cytokine concentrations were analyzed in each serum sample of mice for all groups and cell culture supernatants. Proinflammatory, Th1, Th2, and IL-17 cytokines were determined by using commercially available fluorescent bead immunoassay, FlowCytomix mouse Th1/Th2 10 plex kit (BenderMed Systems, GmbH, Vienna, Austria) according to the manufacturer's instructions. In brief, serum samples and cell culture supernatants or serial diluted standards were suspended with fluorescent beads and coated with monoclonal antibodies specific to distinct cytokines, together with cytokine-specific biotin-conjugated monoclonal antibodies. Following 2-h incubation, beads were washed twice and incubated with pyhcoerythrin-labeled streptavidin. Forward and side scatter voltages were adjusted with specific setup beads; 675 nm (fluorescent 4) detector was used for bead quantification. Standard curves were determined for each cytokine from a range of 27–20.000 pg/mL. Samples and standards were analyzed at FACSCanto II flow cytometry (BD BioSciences, New Jersey). The concentrations assessed by using FlowCytomix Pro 2.2 software (BenderMed Systems, GmnH, Vienna, Austria). For each analysis 10000 beads were collected.

IL-23 concentrations in serum and cell culture supernatants were analyzed by using mouse IL-23 ELISA kit (BenderMed Systems) according to manufacturer's instructions.

2.8. Statistical analysis

Microscopic scores analysis were performed by using Student *t*-test. The survival rate differences between groups were compared and analyzed by using Kaplan–Meier test. Analysis of differences in cytokine levels in different groups was analyzed by using Student *t*-test. All statistical analyses were performed by using the SPSS v. 10.0 software program (SPSS Inc, Chicago, IL). The level of significance was set as $P < 0.05$.

3. Results

At first step of our investigation we formed an acute IBD in BALB/c mice by using TNBS-EtOH. By the help of this model, we were able to investigate the effect of dexmedetomidine on inflammation and inflammatory response at two different doses, both *in vivo* and *in vitro*. We created five different groups according to the aim of our investigation. Those groups with their abbreviations were as follow: healthy group- Naive-; IBD formed group- TNBS-; following IBD forming mice treated with 5 μg kg^{-1} dexmedetomidine via intraperitoneal route- TNBS + Dex5, following IBD forming mice treated with 30 μg kg^{-1} dexmedetomidine via i.p route- TNBS + Dex30-. In addition to all those groups, a group of mice were treated only with ethanol (ETOH as carrier molecule) to prove that ethanol is not the major molecule that causes disease. Only body weight loss was followed in this group. First, we analyzed the effect of dexmedetomidine on disease parameters in IBD such as survival rate, weight loss, histologic changes in comparison with TNBS group. Second, cytokine levels were investigated in serum samples to understand the route of immune response in systemic circulation. Finally, after isolation of spleen cells, cytokine levels were investigated in the presence and absence of dexmedetomidine in culture supernatants.

3.1. Macroscopic and microscopic evaluation

The occurrence of IBD and the effect of dexmedetomidine on macroscopic parameters of disease were investigated by following weight loss and mortality on experimental animals throughout 6 d. As shown in Figure 1A, we observed average 17% weight loss for the first 3 d in colitic mice. Interestingly, dexmedetomidine administration prevented weight loss. This effect was significant only in TNBS + Dex30 group at first 3 d after disease induction ($P = 0.0039$, $P = 0.0064$, $P = 0.036$, respectively). Although the weight loss in TNBS + Dex5 group was less than TNBS group at first 3 d of disease, this reduction was not statistically significant ($P > 0.05$).

Mortality rate was followed in all groups for 6 d. We observed 15% mortality in TNBS group starting at d 4 of disease (Fig. 1B). Mortality ratio for TNBS + Dex5 group was 15% that has started 2 d after disease induction and this ratio did not increase in following days. Interestingly, there was no mortality in TNBS + Dex5 and TNBS + Dex30 groups for 6 d. In order to check whether dexmedetomidine itself causes any weight loss and mortality or not, we analyzed the same parameters on a group of animals in which only 5 μg (Dex5) or 30 μg (Dex30) dexmedetomidine was administered. Neither weight loss nor mortality has been observed in Dex5 and Dex30 groups (Fig. 1A and Fig. 2J–K, Fig. 2L–M, respectively).

Dexmedetomidine was effective in preventing the microscopic colonic damage induced by TNBS colitis (Fig. 2A–I). A reduction of 1.30-fold in histologic score was obtained in TNBS + Dex5 group, and 2.43-fold in TNBS + Dex30 group in

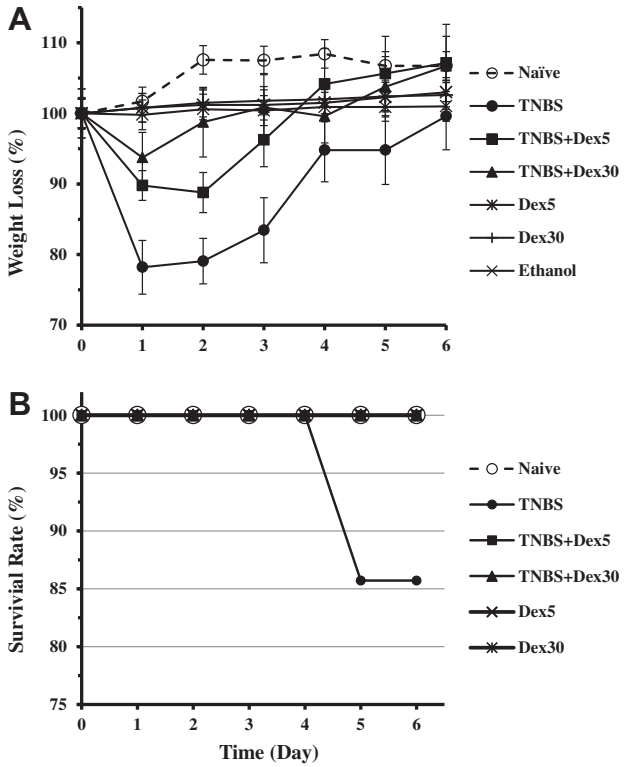

Fig. 1 – The effect of dexmedetomidine on TNBS-induced colitis in BALB/c mice. (A) Weight changes in percentage. (B) Survival rates. Mice with TNBS-induced colitis that were administered dexmedetomidine at two different doses via intraperitoneal route were compared with colitic mice and naive mice or mice that were inoculated with 50% ethanol alone. Mice that died in the first 2 d were considered as treatment casualties and were excluded from all calculations or presentations. The weights of mice in TNBS + Dex30 group were significantly higher than those of colitic mice ($P < 0.05$). Data are presented as percentage of weight loss ± SD of two similar groups.

comparison with TNBS colitis group. However, reduction only in TNBS + Dex30 group was statistically significant ($P < 0.001$) (Fig. 2A).

3.2. *Effect of dexmedetomidine treatment on systemic cytokine network*

In this part of our investigation, we examined the effect of parenteral administration of dexmedetomidine on cytokine levels in systemic circulation in the presence or absence of inflammation. Accordingly we measured the pro-inflammatory (IL-1α, TNF-α, IL-6), Th1 (IFN-γ, IL-2, GM-CSF), Th2 (IL-4, IL-5, IL-10), Th17 (IL-17, IL-23) cytokine levels in serum samples of mice. Our first observation was the significant elevation of pro-inflammatory cytokines TNF-α and IL-6 in TNBS group in comparison with naive group ($P < 0.05$). Administration of dexmedetomidine in the presence of inflammation did not cause reduction on pro-inflammatory cytokines levels (Fig. 3A–C). On the contrary, administration of dexmedetomidine significantly increased IL-6 and TNF-α levels at different concentrations ($P < 0.05$) (Fig. 3B and C). However, this was not in a dose dependent manner.

Another interesting data in Figure 3 was about IL-2 level, which is one of the growth factor of T cells. Administration of dexmedetomidine increased IL-2 level significantly at TNBS + Dex30 group in comparison with TNBS and TNBS + Dex5 ($P < 0.001$ in both comparisons) (Fig. 3D).

A group of major cytokines IFN-γ, IL-17 and IL-23 are produced and secreted by Th1 and Th17 T cells during an inflammation, respectively (Fig. 4A). All three cytokines level increased significantly in TNBS group in comparison with naive group ($P < 0.05$). Administration of dexmedetomidine did not reduce IFN-γ and IL-17 level at both concentrations. The most prominent data in that group was the significant reduction on IL-23 level in TNBS + Dex5 and TNBS + Dex30 group in comparison TNBS group ($P = 0.037$ and $P = 0.025$, respectively). Boosting on dexmedetomidine concentration did not affect on that reduction.

Another intriguing result on that analyze was about IL-4 and IL-10 levels which are responsible from anti-inflammatory T cell response (Fig. 4B). IL-4 was significantly elevated only in TNBS + Dex5 group in comparison with TNBS group ($P = 0.014$). This elevation was also significant in comparison with TNBS + Dex30 ($P < 0.05$). More than that, administration of dexmedetomidine in the presence of inflammation increased IL-10 level significantly. IL-10 level was significantly high in TNBS + Dex5 and TNBS + Dex30 groups in comparison with the naive group ($P < 0.001$).

3.3. *Effect of dexmedetomidine on mononuclear cell activation*

We cultured spleen cells in the presence of 10 and 100 ng dexmedetomidine for 24 or 48 h to understand the effect of dexmedetomidine on inflammatory Th1, Th17, and anti-inflammatory Th2 cytokines secretion. In case of Th1 inflammatory cytokine levels, there were no significant differences on IFNγ levels between groups (Fig. 5A). Instead, interestingly, IL-17 level was significantly high not only at TNBS group but also at TNBS + Dex5 and TNBS + Dex30 in comparison with naive group ($P < 0.05$) (Fig. 5B). On the other hand, IL-23, which has a critical role on production of IL-17, was significantly low in TNBS + Dex5 and TNBS + Dex30 groups in comparison with TNBS group in the presence and absence of dexmedetomidine ($P < 0.001$) (Fig. 5C). However, this reduction did not increase in parallel with dexmedetomidine concentration.

Another prominent manifestation was observed on levels of anti-inflammatory Th2 type cytokines. Neither IL-4 nor IL-10 was detected in TNBS group. On the other hand, dexmedetomidine treatment at both concentrations kept IL-4 and IL-10 at naive level (Fig. 6A and B).

4. Discussion

In our study, we investigated the effect of dexmedetomidine on immunopathogenesis of inflammatory bowel disease to understand whether or not dexmedetomidine can down-regulate

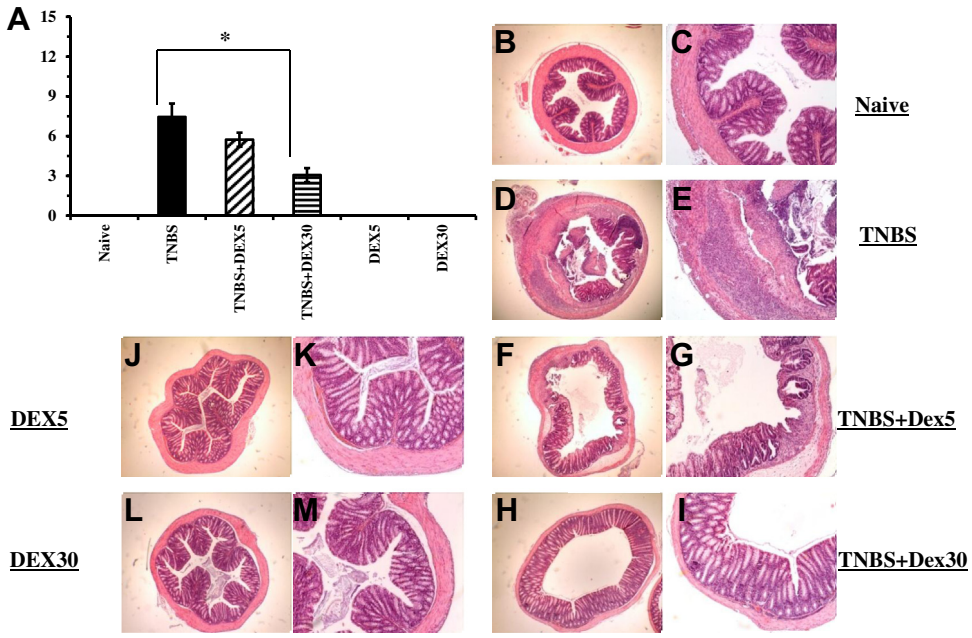

Fig. 2 – Proximal, medial, and distal parts of colons were examined after hematoxylin and eosin staining 6 d after experimental colitis induction. After scoring proximal, medial, and distal parts of each colon, the difference on scoring is presented as mean values ±SD as shown in (A). *Indicates significant difference of histological scores between TNBS group and TNBS + Dex30 groups ($P < 0.05$). Normal colon (B) (×4) and (C) (×10); (D) (×4) and (E) (×10) show mucosal ulceration, intense transmural inflammation, extensive ulceration, and disruption of intestine architecture in the colon of TNBS-induced colitis mouse. (F) (×4) and (G) (×10) demonstrate histologic view of a representative colon of a colitic mouse treated with 5 μg dexmedetomidine via i.p route with limited amount of mononuclear cell infiltration in submucosa, and normal glandular structure. (H) (×4) and (I) (×10) demonstrate the colon of a colitic mouse treated with 30 μg dexmedetomidine via i.p route; resembles almost normal sections, preserved mucosa, and submucosa, and normal glandular structure. (J) (×4), (K) (×10) and (L) (×4), (M) (×10) demonstrate the colon of naïve mice treated only with 5 and 30 μg dexmedetomidine, respectively. (Color version of figure is available online.)

the inflammatory immune response during inflammatory bowel disease. Accordingly, we analyzed histologic alterations and the route of immune response on spleen cells and on systemic circulation by measuring inflammatory and anti-inflammatory cytokines levels. For this purpose, we used as an inflammatory model an experimental bowel disease model, which resembles Crohn's disease in pathology and immune response in human. We chose this model since it induces inflammatory immune response not only locally but also systemically according to our previous observations [20,21].

After inducing inflammatory bowel disease in mice, dexmedetomidine was administered intraperitoneally at 5 μg kg^{-1} or 30 μg kg^{-1} concentration. In clinical applications, dexmedetomidine is recommended to be used at 1 μg kg^{-1} as a loading starting dose in adults and followed by 0.2–0.7 μg $kg^{-1}h^{-1}$ continuous infusion. Although it has been authorized for use in short-term sedation, long-term usage has also been reported in studies [22]. In our study, we chose to use intraperitoneal injections of dexmedetomidine rather than intravenous infusion, which could be one of the limitations of this study. However, the systemic effects with intraperitoneal injections of dexmedetomidine have also been shown to be effective in several studies [23–25]. We administered dexmedetomidine at 5 and 30 μg kg^{-1} concentrations for 6 d. We decided to use those concentrations according to our preliminary data and the observations belonging to other groups [24,26,27].

For the first time, in this study, we investigated the anti-inflammatory effect of dexmedetomidine and its mechanism in an experimental inflammatory bowel disease model. In our study, we observed reduction on weight loss, no mortality, and histopathologic parameters depending on the concentration of dexmedetomidine. Especially minimal weight loss, no mortality, and similar histopathologic appearance with naive group were remarkable at 30 μg kg^{-1} concentration. In addition, we should remind that dexmedetomidine treatment without TNBS induction did not result in any histological damage at two different doses.

We should emphasize that according to literature, the expected mortality remains ~20% to 25% through 7 d with an expected chronic disease phase of 30% in this model. In the TNBS colitis model, disease severity will increase over the initial 7 d and then enter a variety of stages (i.e., mice will die, recover, or enter into a chronic phase). In the latter stage, severity will remain constant and the weight of the mice will plateau with no further loss observed [28]. In our experiment, we observed nearly 15% mortality through 7 d, which is close to the values of literature.

According to all these macroscopic and microscopic manifestations we believe that dexmedetomidine affects one or more steps of an inflammation cascade. However, in

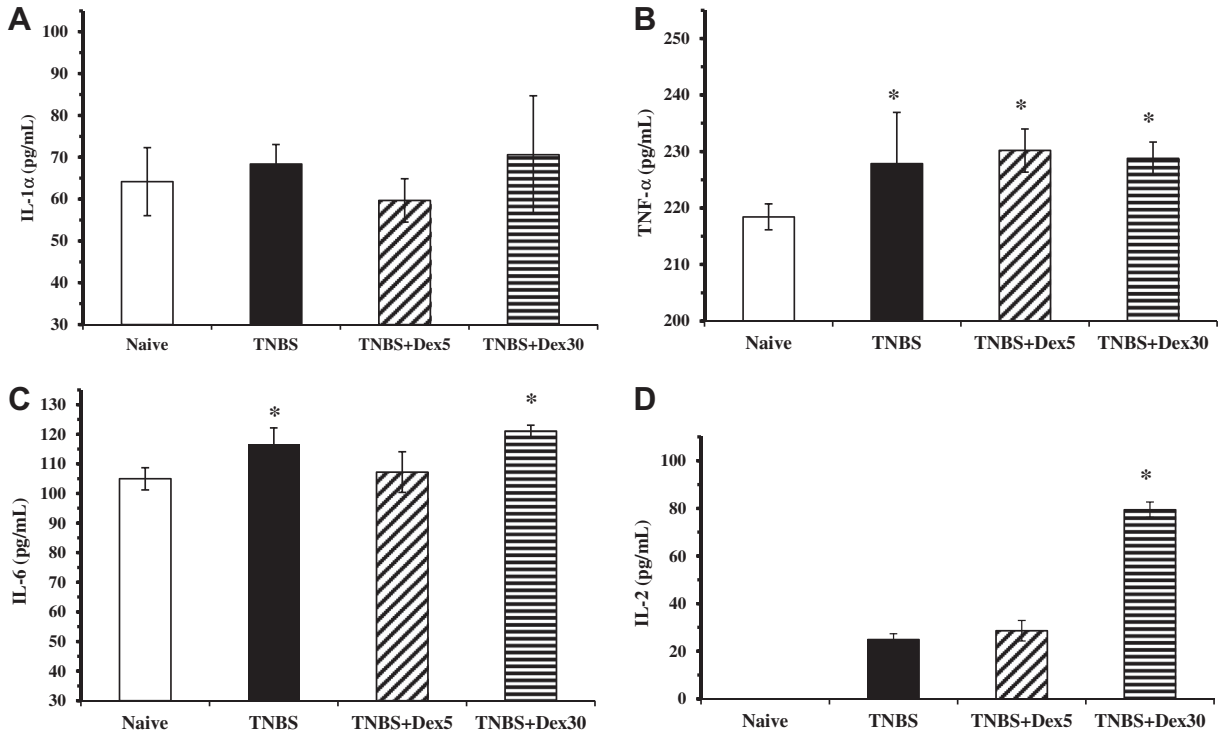

Fig. 3 – Peripheral blood was collected from each mouse by cardiac vein puncturing method six days after colitis induction. Proinflammatory cytokines IL-1α (A), TNF-α (B), IL-6 (C), and T cell growth factor IL-2 (D) concentrations were investigated in each serum samples. Data are presented as mean value ± SD of two similar experiments. *Indicates statistical significance between related groups.

separate studies, it has been observed that dexmedetomidine shows its anti-inflammatory effect by reducing pro-inflammatory cytokines IL-1, TNF-α and, in some cases, IL-6 in sepsis patients and in experimental model of sepsis, which is one of the most aggressive inflammation model [17,27,29,30]. It is obvious that dexmedetomidine has beneficial effects on sepsis patients in comparison with other sedatives such as benzodiazepines according to the outcomes of delirium and other clinical parameters [31]. However, in contrast, we did not detect a reduction on pro-inflammatory cytokines levels. This may be because the TNBS model is not a model as serious as a sepsis model; therefore, we may miss the threshold level for dexmedetomidine to down-regulate pro-inflammatory cytokines. On the other hand, the advantage of our model is to detect T-cell-dependent cytokine levels together with pro-inflammatory cytokines. Furthermore, it should be noted that TNF-α and IL-6 levels were significantly higher in comparison with naïve group in our model.

It might be considered that pro-inflammatory cytokines are reduced at the end d 6 and might be too late to detect high levels of those cytokines. However, according to literature and our experiences, TNBS colitis remains an easy and highly reproducible animal model of intestinal inflammation. Disease induction occurs rapidly and appears 4 to 7 d after intra-rectal administration of the TNBS-hapten reagent [28]. Microscopic analysis reveals the occurrence of a transmural colitis within this time. In several of our experiments, we measured significant elevation on pro-inflammatory cytokines, especially on TNF-α, even 7 d after colitis induction [18–20].

In case of pro-inflammatory cytokines, another point that should be underlined is the reverse correlation between the mortality rate and pro-inflammatory cytokine levels. In our data, while proinflammatory cytokines, TNF-α, and IL-6 levels increased significantly in TNBS and TNBS + dexmedetomidine groups in comparison with naïve group, mortality rate in those groups did not increase. First of all we should emphasize that BALB/c is one of the susceptible strains to TNBS-induced colitis. For this model highly susceptible strain is SJL mice [28]. According to our experiences, the morbidity and mortality rates in colitis animal models vary according to the animal model that is chosen. Variable TNF-α levels have been observed in TNBS-Colitis model, 2.5% DSS model, and also IL-10 deficient transgenic mice C3H/HeJBir IL-10−/− model. Moreover, TNF-α levels never correlate with the rate of morbidity and mortality levels in these models [18,19].

In this study, for the first time, we evaluate that anti-inflammatory effect of dexmedetomidine caused by significant variations on levels of two different cytokines, functionally contrast to each other during inflammatory bowel disease. One of them was an elevation on IL-10, a cytokine belonging to Th2 type cytokine with anti-inflammatory effect, and the other one was reduction on IL-23, a cytokine belongs to Th17 type cytokine with inflammatory effect. Although reduction on colonic damage correlated with elevation on dexmedetomidine dose according to macroscopic and microscopic evaluations, we could not detect a similar harmony between levels of anti-inflammatory cytokines and dexmedetomidine dose.

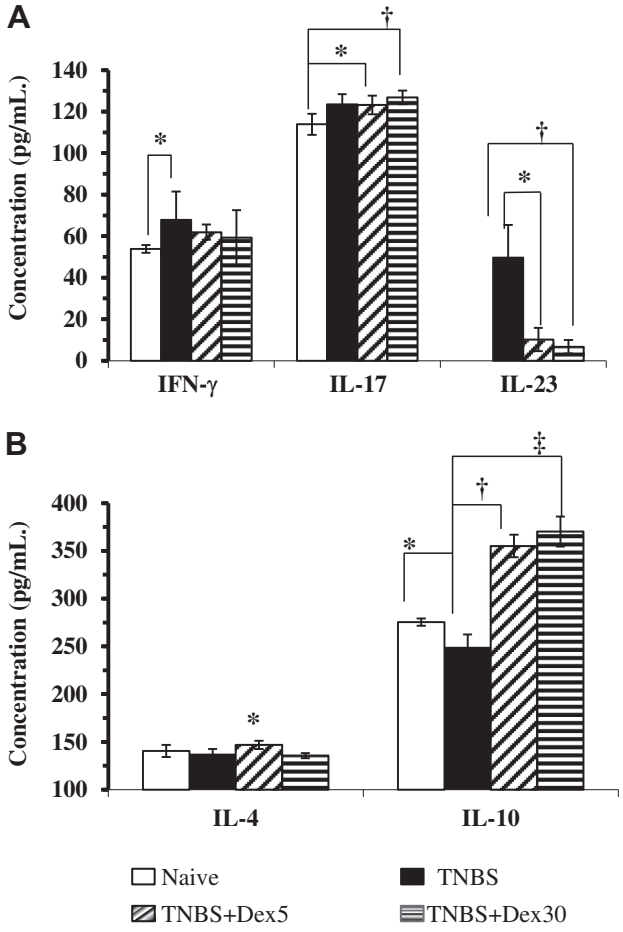

Fig. 4 – Th1 cytokines IFN-γ, Th17 cytokines IL-17, IL-23 (A) and Th2 cytokines IL-4, IL-10 levels (B) were measured in serum samples of each animal in all groups. Data are presented as mean value ± SD of two similar experiments. *, †, ‡ indicate the statistical differences between groups.

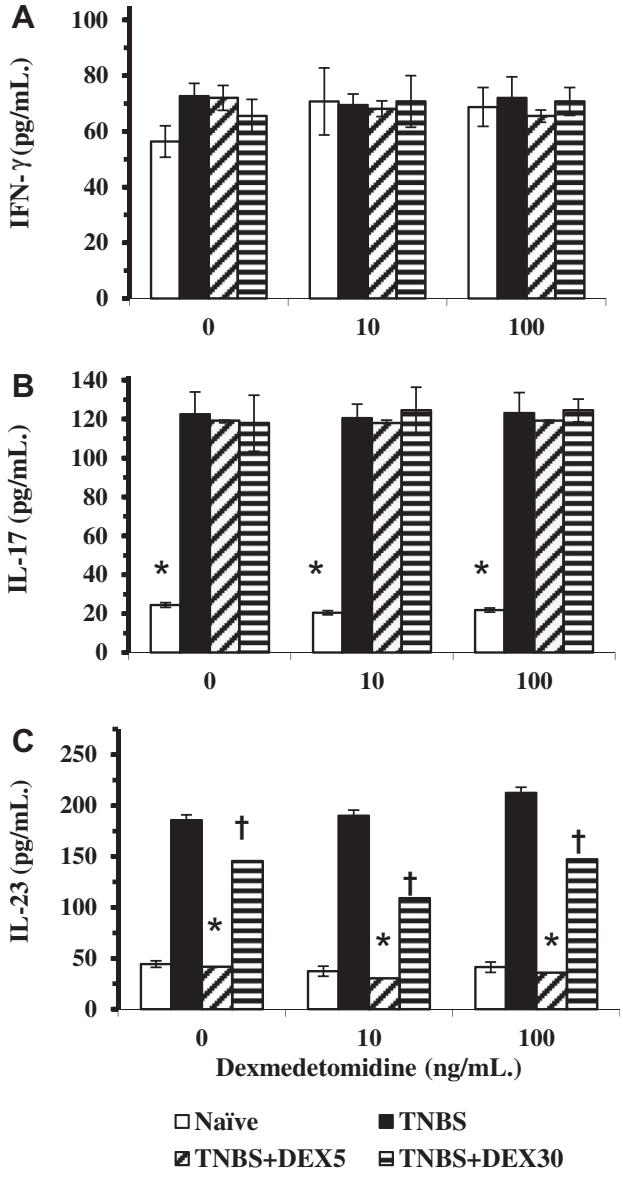

Fig. 5 – Th1 (IFN-γ) (A) and Th17 (IL-17 and IL-23) (B and C) cytokines levels after incubation of spleen cells in the absence or presence of 10 or 100 ng dexmedetomidine, *in vitro*. Spleen cells were isolated from each animal and pooled separately for each group. Data are presented as mean value ± SD of two similar experiments; * and † indicate the statistical differences between groups.

Anti-inflammatory effect of IL-10 has already been shown in several studies [32,33]. It shows this effect by inhibiting the activation of dendritic cells and macrophages. In a way, it has a negative feedback role on innate and adaptive immune response. IL-17 is a cytokine that is secreted by Th17 lymphocyte group. IL-23 plays an essential role in driving intestinal pathology in experimental models of both T cell-dependent and innate colitis. Moreover, single nucleotide polymorphism analysis on IL-23R gene has shown that IL-23 is associated with either susceptibility or resistance to inflammatory bowel disease in humans [34–36]. IL-23 is responsible from both the clonal expansion of Th17 lymphocyte group, one of the main actor of Th1 type autoimmunity, and the development of Th1 type lymphocyte group by sharing p40 subunit of with IL-12 [33,36]. Consequently, it has been shown that in several models of colitis, IL-23 is the major driver of intestinal inflammation [37]. Interestingly, we observed reduction on IL-23, on the other hand no change on IL-17 level, not only in serum samples but also in cellular manner. Low level of IL-23 at two different doses of dexmedetomidine in serum samples suggests a suppressive effect of IL-10 on IL-23 production. Our hypothesis depends on the ability of IL-23 to inhibit the accumulation of intestinal Foxp3+ regulatory T (Treg) cells, a population of CD4+ T cells of importance for maintaining homeostasis in intestine [38]. In this regard, Ahern *et al.* has proven that IL-23 takes role on reducing T cell IL-10 production [39]. Chaudry and co-workers determined suppressive role of regulatory T cells (Tr1) on function of IL-23 via IL-10. They concluded that as a result of that effect, inflammatory response in mucosa of colons reduced significantly [40]. However, we need more detailed studies to prove this kind of effect of dexmedetomidine during an intestinal

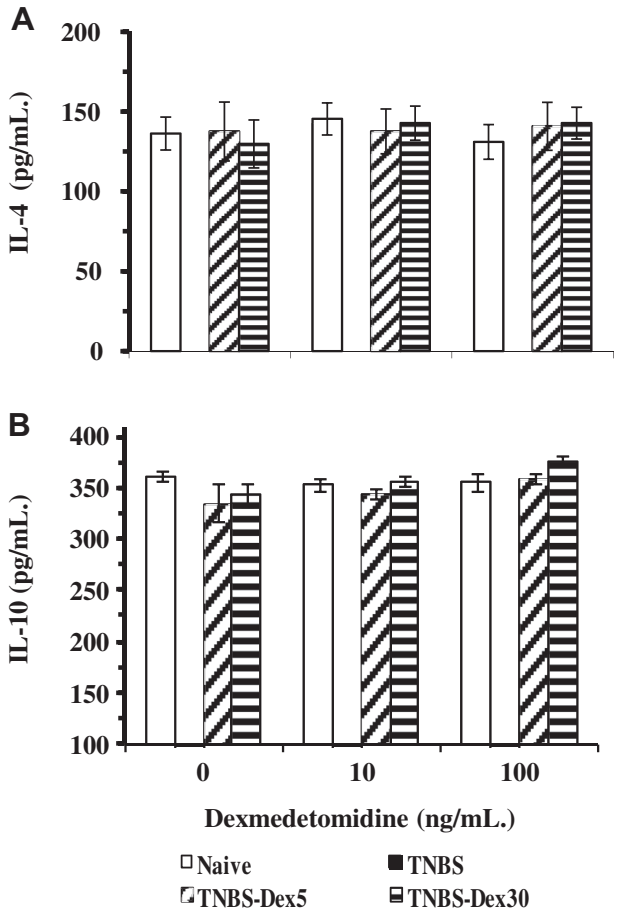

Fig. 6 – Th2 cytokines IL-4 (A) and IL-10 (B) levels after incubation of spleen cells in the absence or presence of 10 or 100 ng dexmedetomidine, *in vitro*. Spleen cells were isolated from each animal and pooled separately for each group. Data are presented as mean value ± SD of two similar experiments.

inflammation. In order to prove the role of dexmedetomidine on clonal expansion and activation of Tr1 cells during IBD, immunophenotyping studies can be performed as a future direction.

Similar to our observation, Inada and co-workers determined that dexmedetomidine switches Th1 type inflammatory response toward to Th2 type anti-inflammatory response [25]. According to our *in vivo* data, we found an elevation on IL-10 and IL-4 levels in systemic circulation. Furthermore, in our *in vitro* data, stable amounts of IL-10 and IL-4, and low amount of IL-23 were detected in cell culture supernatants of dexmedetomidine treated groups. All these data remark an effect of IL-10 on IL-23 or inflammatory response. However, IL-23 is not the only cytokine that directs the inflammatory response. At this point, levels of IL-12 and IFN-γ are also important and especially IL-12 level should be investigated in similar studies. In past studies, it has been proven that Th1 cell activation is down-regulated by two different factors called internal and external. In internal pathway, IL-10 secreted by Th2 cells and in the case of external pathway, IL-10 secreted by regulatory T cells (Tr1) are the key components [32,40]. In our case, Tr1 cells may play major role in immunomodulatory effect of dexmedetomidine. At this point, more investigations such as identification of Tr1 cells and secretion of TGF-β1 and IL-35 should be done.

First time in our study we showed the immunomodulator effect of dexmedetomidine during an inflammation in the gut. This effect was observed not only in damaged tissue but also in systemic circulation. Furthermore, we determined that immunomodulator mechanism of dexmedetomidine depends on T lymphocyte cytokine network switch toward to anti-inflammatory response by elevation of IL-10 and reduction on IL-23 level. In order to prove that immunomodulatory mechanism of dexmedetomidine, comprehensive investigations involving α-2-adrenergic receptor agonists and anti-IL10 antibodies are needed.

Acknowledgment

This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) in short-term R&D Funding Programme (110S243).

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