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Gabapentin-induced changes of plasma cortisol level and immune status in hysterectomized women ☆,☆☆

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ABSTRACT

Aim: We have examined the effects of gabapentin (GBP) on stress-related changes of cortisol and catecholamines in patients who underwent hysterectomy because of uterine fibrinoids. Additionally, we have observed the effect of GBP on the immune status in the acute stress response to surgery.
Methods: Sixty patients scheduled for an abdominal hysterectomy were randomly assigned to the GBP administration 1 h before surgery (n = 30 pts), or to the placebo group (n = 30 pts). Blood samples were collected before and 24 h after the surgery. The intensity of pain was assessed by a visual analogue scale (VAS) every 8 h at rest. Immunomodulatory effects of GBP were determined by flow cytometry. We followed the total proportion of $CD3^+$ lymphocytes, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD19^+$ B lymphocytes, $CD16^+CD56^+CD3^-$NK cells and $CD16^+CD56^+CD3^+$ NKT cells before and 24 h after hysterectomy. The plasma cortisol and catecholamines concentration was used to estimate the level of the stress response.
Results: VAS pain score at rest was significantly lower in the GBP group than in the placebo group ($P = 0.003$). Application of GBP significantly decreased the plasma cortisol level 24 h after the operation in comparison to the placebo group ($P < 0{,}001$). We found significant positive correlation between the VAS pain score and concentration of cortisol in all patients ($P = 0.025$). GBP reduced the concentration of catecholamines ($p < 0.05$). The proportion of $CD3^+$ ($P = 0.027$) and $CD3^+CD4^+$cells ($P = 0.006$) was significantly lower in the GBP group 24 h after operation, while the contribution of $CD19^+$ ($P = 0.033$) was significantly higher.
Conclusion: Preoperative administration of GBP reduced the pain scores at rest in patients at 0, 16 and 24 h after abdominal hysterectomy. Additionally, GBP reduced the stress response and changed immune parameters in the reaction to surgery.

1. Introduction

Gabapentin (GBP) is an antiepileptic drug which exerts analgesic effects in treatment of a variety of chronic pain conditions, including post-herpetic neuralgia, diabetic neuropathy, complex regional pain syndrome, inflammatory pain, central pain, malignant pain, trigeminal neuralgia, HIV related neuropathy, and headaches [1–4]. Recently, it was shown that GBP can be applied as an analgesic drug in the preemptive analgesia and in the acute postoperative pain management. It also displays beneficial effects on postoperative pain scores and enables the reduction of analgesics consumption after a variety of surgical procedures [5–7].

The anti-nociceptive action of GBP is mostly explained, although it includes several different mechanisms. GBP is targeting the $\alpha_2\delta$ subunit of voltage-dependent calcium channels and regulates the intracellular Ca2+ current [8–10]. Also, it has been shown that GBP inhibits the evoked release of glutamate, aspartate, substance P, and calcitonin gene-related peptide (CGRP) from the spinal cord of rats [11]. Recent studies demonstrated that the ligation of α2 adrenergic receptors in the descending noradrenergic system and spino-bulbo-thalamic circuit, mediates the analgesic effects of GBP in addition to α2δ interaction [12].

Postoperative pain is not purely nociceptive, and may consist of inflammatory, neurogenic, and visceral components. Therefore, multimodal analgesic techniques, utilizing a variety of drugs acting through different analgesic mechanisms, are becoming popular [11]. An increasing number of randomized clinical trials showed the efficacy of GBP in the postoperative analgesia [13–15]. Three outcome parameters (postoperative analgesic requirements, pain score at rest, and pain score

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during activity) were significantly reduced in GBP treated group of patients compared to placebo [6,16,17]. The side-effects of GBP analgesia were also analysed, and results demonstrated no significant increase in the incidence of GBP-related adverse effects in comparison to control group.

Pre-emptive analgesia is a type of anti-nociceptive treatment which starts before surgery and shows better results in the reduction of postoperative pain than treatment which starts in the early postoperative period. As GBP has a substantial inhibitory effect on the development and establishment of chronic pain, we investigated whether the preemptive usage of GBP could reduce postoperative pain intensity and analgesic requirements in the initial 24 h after an abdominal hysterectomy.

Many studies on the stress-associated immune dysregulation are focused on interactions between the central nervous system (CNS), endocrine and immune system in surgical patients. Neuro-endocrine modulation of the immune response is mediated by the complex network of signals within the bi-directional communication of these three systems. The hypothalamus-pituitary gland-adrenal gland axis (HPA) and sympathetic nervous system-adrenal medulla axis (SAM) are well described pathways through which immune functions can be altered [18].

After tissue damage, mast cells and macrophages are activated, neutrophils are recruited and a variety of immune mediators are released, such as histamine, prostaglandins, tumour necrosis factor α (TNF-α), interleukin -1β (IL-1β), interleukin 6 (IL-6), nitric oxide (NO), nerve growth factor (NGF) and substance P (SP) [19]. This "inflammatory soup" stimulates intracellular cascades in nociceptors, which ultimately activate afferent nerves and transmit pain stimuli to the spinal cord, brain stem and thalamus, which, finally, activate the HPA axis. Activation of the HPA axis induces a release of corticotrophin-releasing hormone (CRH) from the hypothalamus, and adrenocorticotrophic hormone (ACTH) from the pituitary gland. ACTH induces a release of cortisol from the adrenal gland, which in combination with the stress induced release of catecholamines, i.e. epinephrine (E) and norepinephrine (NE), mediates immune functions [18].

Release of E and NE affects lymphoid cells and exerts an immunomodulatory role via the α and β adrenergic receptors (ARs) [20]. Thus, ARs, expressed on immune cells, are targets of „remote control" and play a role in the signal transmission at the sympathetic-immune interface [21]. All lymphoid cells express β-ARs, with the exception of T helper 2 cells [20]. Under normal conditions the α_2-ARs are not expressed on peripheral mononuclear cells (PBMC). However, under certain pathological conditions, blood T lymphocytes can express α_2-ARs, which mediate their function by endogenous and exogenous catecholamines. Stimulation of α_2-ARs have an anti-inflammatory effect and participate in suppressive modulation of lymphocyte proliferation and cytokine production in vitro [22,23].

In the immune system, T lymphocytes play a central role in the cell mediated immunity. As excitable cells, T lymphocytes express voltage gated calcium channels of the Ca_v1 and Ca_v2 class24. The Ca_v1 channels subfamily contains an auxiliary $\alpha_2\delta$ subunit. Activation of $\alpha_2\delta$ mediates Ca^{2+} influx into the cell, as a key event of T lymphocyte activation [25].

In this study we have presented data showing effects of GBP on surgical stress-related plasma cortisol and catecholamines concentration and subsequent changes in patient's immune status, followed by the proportion of lymphocyte subpopulations in peripheral blood [8,11,12,26]. These immune effects of GBP can be indirectly related to its anti-nociceptive and cortisol-mediated actions.

2. Patients/material and methods

Sixty female patients, scheduled for abdominal hysterectomy, older than 18 years and ranging in BMI from 18 to 35 kgm^{-2} were enrolled in this study. Patients were randomly assigned into two equal groups of 30 patients. To fulfil randomization criteria, we always predicted assortment of newly admitted patient, scheduled for hysterectomy, to another group than previous one was assigned. One group received gabapentin 600 mg (GBP^+ group), (Neurontin, Pfizer, Croatia) and another group matching placebo (GBP^- group), orally, 1 h before surgery. The medications were supplied by hospital pharmacy and were administered by a nurse, not involved in the study. Patients were excluded from the study if any of the following criteria were present: inability to cooperate; allergy to any of the drugs used in the study; emergency surgery or reoperation; treatment with antidepressants; history of diabetes or epilepsy; known impaired kidney function; alcohol, drugs or both abuse; uncontrolled systemic disease (asthma, hypertension, cardiac and liver disease); and treatment with systemic glucocorticoids within 4 weeks prior to surgery. Before the operation, routine laboratory parameters were examined. The study was approved by Ethical Committee of School of Medicine Rijeka and Clinical Hospital Rijeka (Kl: 003-006/11-01/75), and written informed consent signed and dated from each participant was obtained.

2.1. Anaesthetic procedure

All patients received oxazepam 15 mg, orally in the evening before the surgery. Anaesthesia was induced with propofol 2 mg kg^{-1}, sufentanyl 2 μg kg^{-1} and rocuronium bromide 0,6 mg kg^{-1} followed by orotracheal intubation, and maintained by a propofol infusion in the dose 100 to 200 μg kg^{-1} min^{-1} and 50 % mixture of air and oxygen was applied. The lungs were mechanically ventilated and adjusted to maintain normocapnia. At the end of surgery, if necessary, the neuromuscular block was antagonized by sugamadex up to 2 mg kg^{-1}. Tracheal extubation was done when adequate spontaneous ventilation was established and the patients responded to verbal commands. Thereafter the patients were shifted to postanesthesia care unit (PACU).

2.2. Determination of pain intensity by visual analogue pain scale (VAS)

All patients were instructed for the use of the VAS, ranging from 0 to 10 (0 = no pain, 10 = worst pain imaginable). After initial assessment, a senior resident, who was not the part of the anaesthesia team, recorded the pain score at 0, 8 h, 16 h, and 24 h, postoperatively on a VAS 0–10 scale, at rest. From the data of all 30 patients in each group, we calculated average pain score per the time point, that we further compared in statistical analysis. Patients received diclofenac sodium 75 mg and paracetamol 1000 mg on demand. The total rescue analgesic requirement in the first 24 h was recorded.

2.3. Collection of blood samples and cell isolation

All patients assigned for elective abdominal hysterectomy were classified as ASA physical status I and II. Prior to operation and 24 h postoperatively, peripheral blood samples were drawn in vacutainers with heparin. Mononuclear cells were isolated by Ficoll Hypaque (delta = 1,077) density gradient centrifuge. Cells were collected, washed twice, counted, and resuspended in RPMI 1640 culture medium.

2.4. Immunofluoroscence analysis

Immunomodulatory effects of pre-emptive usage of GBP were determined by flow cytometry. We followed proportion of peripheral blood $CD3^+$ lymphocytes, helper $CD3^+CD4^+$ and cytotoxic $CD3^+CD8^+$ T lymphocytes, CD 19^+ B lymphocytes, natural killer (NK) cell ($CD16^+CD56^+CD3^-$)and NKT cell ($CD16^+CD56^+CD3^+$) before the abdominal hysterectomy and 24 hours after surgery (BD Simulset, IMK-Lymphocyte Kit). Immunophenotyping was performed on the BD FaxCalibur flow cytometer. The percentage of positive cells

was analyzed by BD CellQuest software (Becton Dickinson, San Jose, CA).

2.5. Detection of plasma cortisol concentration

The stress response was determined by the cortisol in two samples of plasma, obtained at the 9 h in the morning preoperatively and next day at the same time point, postoperatively. Plasma cortisol concentrations were measured on Elecsys 2010 analyser by the Cobas Cortisol assay (Roche Diagnostics GmbH, Manheim, Germany). The Roche Cobas assay is electrochemiluminecsence immunoassay (ECLIA), based on the antibody competition principle, for the in vitro quantitative determination of cortisol in human serum, plasma, urine, and saliva. In studies with the Elecsys Cortisol assay, the following values were determined using samples from healthy individuals (5th–95th percentile): morning hours 7–10 a.m.: 171–536 nmol/L, n = 144; afternoon hours 4–8 p.m.: 64 327 nmol/L, n = 135 (Roche Diagnostics GmbH, Manheim, Germany).

2.6. Detection of epinephrine (E) and norepinephrine(NE) concentration in plasma

For determination of serum E and NE, blood samples were obtained 30 min after completion of the anaesthetic procedure, from patient laying at rest. Blood was collected from an indwelling catheter in a peripheral arm vein directly in chilled tubes containing EGTA and reduced glutathione for determination of cateholamines in plasma (Kabevette® N, Kabe Labortechnik GmbH). Plasma levels of cateholamines were measured on high pressure liquid chromatography (HPLC Prominence, Shimadzu GmbH) with an electrochemical detector CLC 100 (Chromsystems GmbH, Germany) using a commercially available HPLC kit and a reverse phase analytical column for HPLC analysis of catecholamines in plasma (Chromsystems GmbH, Germany).

2.7. Statistical analysis

Statistical analysis was performed by Statistica for Windows, release 11.0 (Stasoft, INC., Tulsa, OK, USA). The normality of distribution of all parameters was checked by Kolmogorov-Smirnov test with Lillieforse correction and the data were presented as the median (5th–95th) percentile or with the mean ± standard deviation (SD), depending to the normality of distribution. Differences between dependent groups were performed by the Student t-test or Wilcoxon test. We used repeated measures ANOVA to identify differences in VAS between the groups and different postoperative time points. Tukey's test was used as post-hoc test. The correlations analysis was performed by Pearson or Sperman correlation coefficient, what depended on the normality of the data distribution. Multiple regression analysis was used to determine the influence of age, GBP administration and proportions of lymphocyte subpopulations on cortisol levels measured 24 hours after operation. All statistical values were considered significant at the P level of 0.05.

3. Results

Analysis of age, BMI and duration of anaesthesia procedure revealed no statistically significant difference between groups of patients (data not showed).

Effects of GBP and abdominal hysterectomy on standard hematological parameters

Table 1. shows the laboratory data of both groups of patients before and 24 hours after the hysterectomy. All patients were assigned to a GBP group (GBP^+) or placebo group (GBP^-). There was no clinically relevant difference between groups before the operation in any of the observed parameters. Surgery alone induced changes in hematologic parameters, glucose and potassium, although in both groups (GBP^+ and GBP^-) equally.

3.1. GBP reduced the VAS pain score in hysterectomized patients

Analysis of the postoperative VAS pain score at rest every 8 hours during first 24 hours postoperatively, showed a similar course of pain intensity in both groups. The VAS score ascended during first 8 hours post operation and declined afterwards. However, we showed that GBP significantly decreased subjective sense of pain immediately after surgery, 16 and 24 hours p.o. (Fig. 1.).

3.2. GBP reduced the surgery-related cortisol response

The difference in the plasma cortisol concentration between preoperative time point and postoperative (24 h) was calculated for every patient in particular and average values of two groups were compared. GBP reduced significantly stress-related cortisol secretion (Table 2). To present better the effect of GBP we calculated the relative change of the cortisol level (Conc. after − Conc. before / Conc. before) which revealed a significantly higher average increase of cortisol concentration ($p < 0.001$) in patients who didn't receive GBP (Fig. 2).

3.3. Correlation between cortisol level and VAS pain score

We correlated the calculated average of VAS pain score, obtained 24 h postoperatively, from all patients in the study (GBP^+ and GBP^-) with the relative change of plasma cortisol, obtained at the same time point. We found a significant positive correlation between the cortisol level and VAS pain score considering the data from all patients ($r = 0.304$, $p = 0.018$). Thus, we concluded that the pain-related stress is inducing cortisol secretion (Fig. 3.).

Table 1
Patients laboratory data before and 24 h after operation.

Parameter	GBP + (n = 30)			GBP − (n = 30)		Statistics
	Beforeoperation	24 hoursafter operation	P	Before operation	24 hours after operation	P
E ($\times 10^{12}$ /L)	4.2 ± 0,4	3.7 ± 0.4	<0.001*	4.0 ± 0.4	3.6 ± 0.4	<0.001*
Hgb (g/L)	123.8 ± 15.2	106.5 (80–124)	<0.001*	116.2 ± 16.6	101.7 ± 15.9	<0.001*
Htc (/L)	0.38 ± 0.04	0.31 ± 0.03	<0.001*	0.37 ± 0.04	0.32 ± 0.03	<0.001*
L ($\times 10^9$ /L)	6.3 (3.6–10.2)	11.9 (6.9–17.9)	<0.001*	6.0 (3.6–8.0)	10.0 (6.9–16.3)	<0.001*
urea (g/L)	4.05 ± 1.03	4.09 ± 1.32	0.771	4.01 ± 0.84	4.01 ± 1.09	0.781
creatinin (mmol/L)	62 (55–98)	65 (52–90)	0.250	60 (51–72)	61 (54–73)	0.465
glucose (mmol/L)	5.33 ± 0.47	5.72 ± 0.74	0.023*	4.93 ± 0.76	5.64 ± 1.40	<0.001*
sodium (mmol/L)	140.4 ± 3,5	139.1 ± 3,4	0.075	138.3 ± 2,8	136.7 ± 2.7	0.056
potassium (mmol/L)	4.4 (4.0–4.9)	4.0 (3.8–4.5)	<0.001*	4.2 (3.9–5.1)	4.0 (3.6–5.3)	0.005*

* indicated significant difference

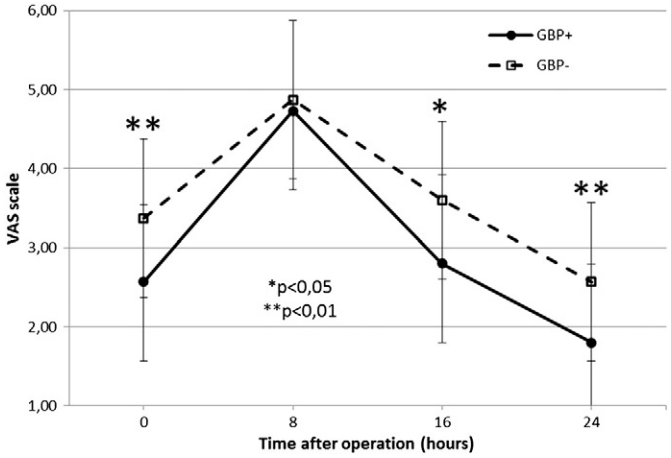

Fig. 1. Effect of GBP on VAS score during the early course after operation in comparison to control group of patients (mean, 95%CI).

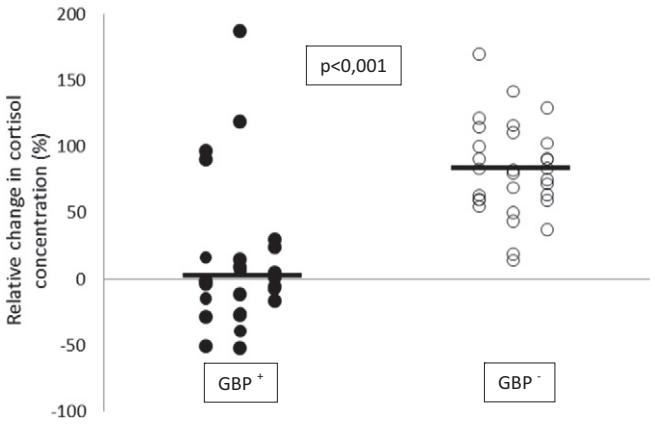

Fig. 2. Cortisol levels in GBP$^+$ and GBP$^-$ groups of patients, 24 h after operation. GBP lowered the cortisol secretion, thus the cortisol concentration did not change as much as in the GBP $^-$ group.

The analgesics consumption during the first 24 postoperative hours (diclofenac 75 mg, paracetamol 1000 mg and diclofenac 75 mg + paracetamol 1000 mg) was not significantly different between GBP$^+$ and GBP$^-$ groups (data not shown).

3.4. The effect of GBP on the catecholamine secretion

Catecholamine concentration was determined in the plasma of hysterectomized patients, obtained at rest in the early period after ending of all anaesthetic procedures. GBP significantly reduced catecholamine level in comparison to placebo group (Fig. 4.)

3.5. GBP changed the proportions of CD3$^+$, CD3$^+$CD4$^+$, CD16$^+$56$^+$CD3$^-$ and CD19$^+$ cells

We analysed the proportions of lymphocyte subsets in GBP$^+$ and GBP$^-$ groups of patients before and 24 h after surgery. Hysterectomy alone lowered the proportion of the CD16$^+$56$^+$CD3$^-$ NK cells in both groups of patients, while decrease in the proportion of CD19$^+$ B lymphocytes was noticed only in the hysterectomized patients receiving GBP (Table 3.). We found no difference between the groups preoperatively. However, 24 h after the operation the contribution of CD3$^+$ and CD3$^+$CD4$^+$ subsets in the total lymphocyte population was lower in the GBP$^+$ group in comparison to the GBP$^-$ group of patients (67. 5 ± 8.1 vs.71.6 ± 6.5, p = 0.027) and (42.7 ± 8.2 vs. 48.2 ± 6.7; P = 0.006), respectively. The proportion of CD19$^+$ cells was significantly lower in the GBP$^-$ group compared to GBP$^+$ group (15.2 ± 5.6 vs. 12.6 ± 3.2; P = 0.033) (Table 3).

3.6. Multiple regression analysis of predictors for the cortisol level change

Finally, multiple regression analysis was performed to find out the influence of predictors for the cortisol level change and proportions of lymphocyte subpopulations 24 hours after the operation (Table 4.). As predictors we used GBP administration, age, preoperative (initial) cortisol level and proportions of CD3$^+$ cells, CD3$^+$CD4$^+$ and CD3$^+$CD8$^+$cells.

The regression model closely predicts (r = -0,568, P < 0.001) the influence of GBP administration on the change of cortisol plasma level 24 h after the operation. The initial cortisol concentration level is also an important predictor, because it had more than 20% of influence while other included parameters were not important. Interestingly, multiple regression analysis of the change in proportion of CD3$^+$ cells, CD3$^+$CD4$^+$ and CD19$^+$ cells 24 hours p.o. did not depict the cortisol level as an important predictor (cortisol as a predictor influence was <1%, data not shown).

4. Discussion

The results of our prospective study demonstrated that pre-emptive administration of GBP 600 mg one hour before the operation significantly reduces VAS pain scores at rest and induces better pain relief during the first 24 h after abdominal hysterectomy. Patients receiving GBP had significantly lower VAS pain scores determined at 0, 16, and 24 h after operation. The lower value of VAS pain score indicates better pain relief. Many studies reported gabapentin to be beneficial in postoperative pain relief, especially after abdominal hysterectomy in doses varying from 300 mg up to1200 mg [11]. Pandey CK et al. investigated the effect of different preoperative GBP doses of 300 mg, 600 mg, 900 mg, or 1200 mg, and found no additional analgesic effect of GBP at doses over 600 mg [27]. Higher doses may increase incidence of side effects, whereas lower doses have inappropriate and insufficient effects [28,29].

Many clinical trials emphasised the beneficial role of GBP in acute postoperative pain control [5–7,9,30,31]. It reduces pain scores and analgesic requirement on first postoperative day [32]. In animal models of nociception it was shown that GBP reduces hypersensitivity induced by nerve injury, inflammation, and postoperative pain [33–35]. Hyperalegesia surrounding the postoperative wound, as well as experimental heat-induced hyperalgesia are caused by the central neuronal sensitization that contributes to the development of chronic pain [36]. Up to 30% of female patients suffer from chronic pain after abdominal hysterectomy [37]. Drugs like GBP could have a significant role in the treatment of postoperative pain, because in

Table 2
GBP administration decreases cortisol concentration after hysterectomy.

Parameter	Before operation		After 24 hours		Statistics
	GBP + (n = 30)	GBP − (n = 30)	GBP + (n = 30)	GBP − (n = 30)	P
Cortisol	438.0 ± 135.2	356.4 ± 79.1	458.0 ± 178.6	642.0 ± 173.3	0.001*

* indicated significant difference

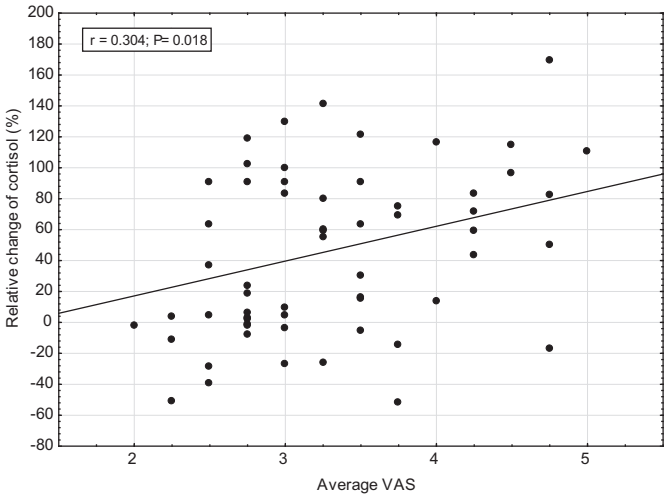

Fig. 3. Correlation between cortisol levels and calculated average VAS pain score in all patients 24 h after operation.

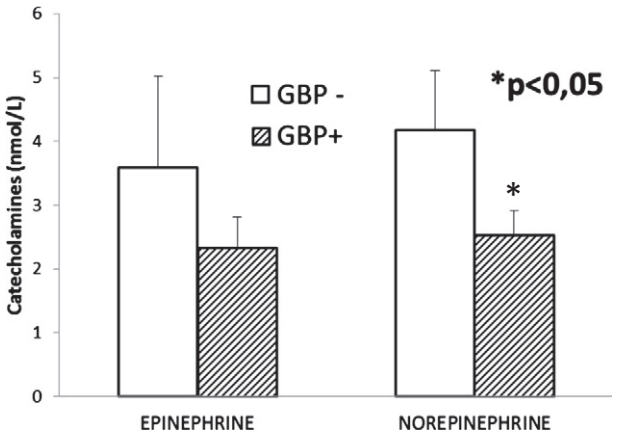

Fig. 4. GBP lowered stress-related plasma catecholamines (epinephrine and norepinephrine) concentration in hysterectomized patients.

combination with other analgesics it produces a synergistic effect which enables the reduction of total analgesic consumption [29,30,32]. The exact mechanism of analgesic action of GBP is not yet known, but experimental data suggest that target of drug's action is $\alpha_2\delta$ subunit of voltage-gated calcium channels and regulation of Ca^{2+} current. It also inhibits substance P, CGRP and glutamate release on the level of spinal cord [36]. Recent studies depicted the descending noradrenergic system, spinal α2 adrenergic receptors and intact spino-bulbo-thalamic circuit as mediators of the analgesic effects of GBP in addition to $\alpha_2\delta$ interaction [12].

The response to general anaesthesia and surgery varies from minor to widespread changes in metabolic, endocrinal and biochemical reactions. Various neuroendocrine hormones and inflammatory mediators are involved in stress response to surgery. These well-known changes are related to the activation of sympathetic nervous system-adrenal medulla axis (SAM-axis) and the hypothalamic-pituitary-adrenal axis (HPA-axis). Via these activation pathways the release of stress hormones, such as catecholamines, adrenocorticotropic hormone (ACTH) and cortisol were regulated [38].

The secretion of cortisol is the largest in the beginning of surgical procedure and its level depends on the intensity of stress and surgical trauma [39,40]. A fourfold rise in the cortisol level was observed 30 minutes after the skin incision of the lower abdominal surgery in patients under general anesthesia [41]. We tested total cortisol concentration 1 h before surgery and 24 h after abdominal hysterectomy. Our results showed the statistically significant reduction in the plasma cortisol level in the GBP^+ group compared to placebo group (Table 2., Fig. 2.). We concluded that GBP suppresses the stress response to surgical stress and trauma. This conclusion was supported by our findings of positive correlation between cortisol levels in plasma and VAS pain scores at rest (Fig. 3). Thus, the probably mechanism of GBP-related suppression of cortisol release is its peripheral antinociceptive action and activation of supraspinal brain areas involved in nociceptive processing [36]. Also, it has been published that GBP administration is associated with significantly higher level of sedation as a result of its GABAergic actions [11,42].

Modulation of the immune response by the CNS is mediated through the complex network of signals that involves bi-directional communication between the nervous, endocrine and immune system. Activation of HPA axis induces release of CRH from hypothalamus and release of cortisol from the adrenal glands. Cortisol exerts anti-inflammatory and immunomodulatory actions. Like other glucocorticoids, cortisol inhibits accumulation of macrophages and neutrophils in the site of the tissue damage, and blocks the release of a variety of immune mediators, especially prostaglandins [40].

As mentioned before, activation of SAM pathway results in the release of catecholamines [18]. E and NE affect lymphoid cells *via* α and β adrenergic receptors (ARs) which play a key role in signal transmission at the sympathetic-immune interface [20]. All lymphoid cells express β-ARs, with the exception of T helper 2 cells [20]. The α_2-ARs may be expressed and activated under certain pathologic conditions, and suppress peripheral blood T lymphocyte functions by endogenous and exogenous catecholamines. Activated α_2-ARs mediate an anti-inflammatory effects and participate in suppression of lymphocyte proliferation and cytokine production *in vitro* [22,23]. T lymphocytes express four Ca_v1 channels and four Ca_v2 channels [20,24,43]. The Ca_v1 channel subfamily contains an auxiliary $\alpha_2\delta$ subunit. Activation of $\alpha_2\delta$ subunit mediates Ca^{2+} influx into the cell and T lymphocyte activation [20]. GBP binds to the auxiliary $\alpha_2\delta$ subunit of Ca_v1 Ca^{2+} expressed on T lymphocytes and modulates immunologic response [8,11,12,26]. By binding to auxiliary subunit of $\alpha_2\delta$ Ca_v1 Ca^{2+} channels, GBP suppresses intracellular calcium influx and omits lymphocyte activation and proliferation.

It was shown that catecholamines inhibit selectively Th1 and stimulate Th2 functions. They suppress type 1 cytokines (IL-12, TNFα, IFNγ, etc.) release and stimulate type 2 cytokines such as IL-10 and IL-6, although catecholamines do not affect Th2 cells directly [20]. However, those actions are mostly mediated by β ARs, since norepinephrine via α2-ARs, can augment TNF-α production, although such an effect is present only in some local transient responses and is based on macrophage functions, suggesting different action of cathecolamines on systemic and local immune reactions [20]. Furthermore, catecholamines can interfere with secretion of chemokines and affect distribution of the immune cells in the body [44].

Todd RD and all found that GBP can inhibit catecholamine release in concentration dependent manner from adrenal medulla. They used adrenal chromaffin cells as a model to investigate a secretion of adrenal chatecolamines in acute stress response. They found that GBP reduces the number of vesicles undergo exocytosis and adrenal stress hormone release [45].

We analysed the proportions of lymphocyte subpopulations in GBP^+ and GBP^- groups of patients. Preoperatively, no difference was found between the groups. However, 24 h after the operation the contribution of $CD3^+$ and $CD3^+CD\ 4^+$ subsets in the total lymphocyte population was lower in the GBP^+ group in comparison to the GBP^- group of patients. Consecutively, the proportion of $CD19^+$ cells was significantly higher in the GBP^+ group compared to placebo.

We hypothesised that GBP caused changes in proportions of lymphocytes by influencing the redistribution of the cells within the body.

Table 3
The proportion of peripheral blood lymphocyte subpopulations in the GBP$^+$ and GBP$^-$ group before and 24 h after the operation.

	Before operation			After 24 hours				
	GBP + (n = 30)	GBP − (n = 30)	P	GBP + (n = 30)	GBP − (n = 30)	P	P′	P″
CD 3	70.2 ± 8.2	70.1 ± 6.9	0.766	67. 5 ± 8.1	71.6 ± 6.5	0.027*	0.126	0.482
CD 4	44.7 ± 8.9	47.3 ± 6.1	0.198	42.7 ± 8.2	48.2 ± 6.7	0.006*	0.290	0.647
CD 8	23.4 (16.4–32.4)	22.5 (13.9–36.5)	0.228	22.3 (12.8–40.1)	23.0 (14.9–34.9)	0.976	0.739	0.675
CD 19	8.9 ± 3.9	10.8 ± 5.1	0.117	15.2 ± 5.6	12.6 ± 3.2	0.033*	0.001*	0.120
NK	12.7 (6.2–31.9)	11.54 (5.3–22.2)	0.478	9.9 (5.4–19.7)	9. 6 (4.8–16.9)	0.478	0.038*	0.018*
NKT	3.7 (1.9–7.8)	3.5 (1. 8–14.0)	0.918	3.9 (1.2–7.2)	4.28 (1.2–9. 8)	0.784	0.873	0.972

p = calculated p value between GBP + and GBP- groups
p′ = calculated p value within the group of GBP + patients before and after surgery
p″ = calculated p value within the group of GBP- patients before and after surgery
* indicated significant difference

Table 4
Multiple regression analysis for plasma level of cortisol 24 h after operation.

Predictors	β	SE_β	*P*	r	Part of contribution
Gabapentin	−0.543	0.127	<0.001	−0.568	30.8
Age	0.110	0.119	0.272	0.157	1.7
Initial cortisol	0.439	0.119	<0.001	0.462	20.3
$CD3^+$	−0.343	0.264	0.191	−0.169	5.8
$CD3^+CD4^+$	0.273	0.1375	0.178	0.108	2.9
$CD3^+CD8^+$	0.215	0.124	0.214	0.148	3.2

β -regression coefficient
SE_β-standard error of regression coefficient
r-coefficient of correlation

By binding to the $\alpha_2\delta$ subunit of the Ca_v1 channels and α2 ARs, expressed on activated T lymphocytes, GBP can alter chemokine and cytokine release. Recently it was shown that intrathecally administered GBP possess an anti-allodynic effect in an SNL rat model of neuropathic pain. The effect could be explained by a GBP-induced reduction in the expression of spinal pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 and upregulation of anti-inflammatory cytokine IL-10 [46]. Furthermore, GBP can reduce the chemokine release at the site of surgical tissue injury and induce changes in the PMNC body distribution, what we observed as changes of T lymphocyte subpopulations proportions in the blood of hysterectomized women.

In conclusion we want to point out that GBP, a drug widely used for treatment of perioperative pain, can interfere with stress-related hormones and immune functions.

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