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Bethametasone Prevents Plasmatic Glutamine Precipitation: An *In-Vivo* Study

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Abstract

Glutamine (Gln) is an amino acid which plays an important regulatory role in many physiological functions and during acute or chronic human pathologies.

The primary purpose of this study was to investigate potential changes of glutamine levels in the plasma of women that needed minor gynaecological surgery. The changes in glutamine levels were then evaluated in a group of patients pre-medicated with Bethametasone. In this study 46 patients were subdivided into two groups. All of them were anesthetized with loco-regional administration of mepivacaine 0.2%. Subsequently, to the first group was given placebo, whereas the second group of women was pre-medicated with Bethametasone 4 mg (i.v.).

We report that in 34 patients out of 46, the minor gynaecologic surgery caused a significant decrease of glutamine (>20%) associated with an increase of postoperative pain. Interestingly, 10 out of 12 patients received Betamethasone, had increased levels of glutamine (>25%) and the use of analgesic drugs in the postoperative time was not required.

In conclusion, in this study we found that minor gynaecological surgery causes a significant decrease of plasma level of glutamine that is associated with increase of postoperative pain. In contrast, the premedication with Betamethasone increases the plasma levels of glutamine, correlating with a good control of postoperative pain.

Keywords: Glutamine (Gln); Bethametasone; Ginecological surgery

Introduction

Glutamine is one of the most abundant non-essential amino acid of human body with a role in numerous biological functions including the immune system responses [1-4], glucose metabolism [5-8], lipogenesis [9], muscular skeletal tropism [10-11], cell cycle regulation and apoptosis [12] etc.

In the body, once synthetized, Gln is converted to glutamic acid (or glutamate) and glutathione [13]. The glutamic acid is the main excitatory neurotransmitter in the central nervous system. It plays a pivotal role in regulating key neurological functions including the neuronal excitability, synaptic plasticity, long term potentiation and depolarization etc. [14-17]. On the other hand, the glutathione possesses important anti-oxidant proprieties that counteract oxidative stress [13,18-22].

Many studies have shown positive effects of glutamine on some painful syndromes and particularly it can prevent pain in patients suffering from stomatitis, or mucositis of gastrointestinal tract caused by chemotherapy and radiotherapy [23], as well as on some forms of arthralgia and myalgia caused by paclitaxel [24].

Glutamine is also a marker of physical stress, in fact its plasmatic reduction increases the inflammatory cytokines released during surgery.

Based on these considerations, the supplementation of glutamine showed significant beneficial effects in several human disorders. In fact, it has been widely reported that the Gln reinforces the activity of the immune-system [25], reduces the synthesis of pro-inflammatory cytokine, induces the expression of heat shock proteins, prevents the cell death [26,27] and ameliorates the brain functions [28]. Moreover, in patients that require critical care glutamine supplementation reduced inflammatory systemic syndrome (SIRS) and the risk of sepsis [29-33].

According to what we have said and to its biologic properties, even its depletion, caused by surgical and/or anaesthesia stress, could be contrasted by preventive glutamine administration, reducing recovery times and post-operative complications, as we have observed in numerous studies on patients operated for colon-rectal cancer [17,34,35].

In spite of its importance, the potential changes of plasma Gln levels caused by gynaecological surgeries as well as the type of anesthesia carried out, has not yet been established.

Therefore the aim of this study is to evaluate the potential changes in the plasma level of glutamine in patients undergoing minor gynaecological surgery, in local anesthesia with paracervical block [36], premedicated or not with Bethametasone.

Materials and Methods

Clinical and surgical procedures

After approval by the local ethics committee n. 318/2008 and signed informed consent, at the gynaecological day surgery of the Second University of Naples, for this study we have recruited 46 women, aged 18–63 with ASA physical status I–II, needed gynecologic or obstetric surgery that were randomly assigned, by a computer-generated randomization list, to the betamethasone treatment (group B) or placebo (group P).

The patients belonging to the group P, after premedication with Atropine (0.5-0.8 mg/Kg) and Fentanyl (0.5-0.1 mg/Kg) was administered placebo; whereas the group B of patients received bethametasone (4 mg).

All patients were anaesthetized by para-cervical block by loco-regional injection of mepivacaine 0.2% (10 ml). During the paracervical block, the Lee-Frankenauser ganglion, where the sensory visceral nerve fibers of the uterus, cervix, and part of the bladder converge, was blocked. A 22-G, 10-cm long needle, was introduced only 5–7 mm into the submucosa of the lateral fornices to reduce the risk of intravascular injection. After controlled aspiration, the local anesthetic was injected (5 mL for each fornix).

After paracervical block and before surgery, we slowly administered a bolus of propofol (0.5–0.7 mg/kg) to women in both groups, according to the type of surgery and individual needs.

Patients spontaneously breathed and in all of them the main vital parameters (ECG, heart rate, arterial blood pressure and oxygen saturation) were monitored.

In all patients the pain score was measured by VAS (Visual Analogical Scale) method (0 reflects no pain and 100 unbearable pain) at the end of surgery, after one hour and at discharge, by an anesthesiologist who was unaware of drugs administered.

In case of postoperative pain, 1 g of paracetamol was administered i.v.

The patient's degree of satisfaction was evaluated on discharge with a score of 0–4: 0=poor, 1=fair, 2=good, 3=very good, and 4=excellent.

In each patient, the plasma levels of glutamine was measured by the blood analysis before (Sample A) and after surgery (Sample B), in both cases in a haemachrome test tube EDTA as anti-coagulant, to measure out glutamine with high-performance liquid chromatography and creatinine.

Data analysis

Sample size calculation was performed using a statistical power analysis: an estimated 40 patients were needed to result in a 90% chance of detecting a difference between groups in pain incidence respect glutamina deplection during surgical procedures conducted under conscious sedation with peripheral block, with an error of 5%, a difference from baseline of 15%, and a standard deviation of 0.5.

The levels of Gln in the blood sample A and B of each patient (see method section) were compared by use of matched Student's t tests and expressed in % ± SEM. Statistically significant differences were accepted when the p value was ≤ 0.05 (Iannotti et al. 2013).

Results

Patients' characteristics were similar in both groups. Subjects were randomized in group B (N=23) and Group P (N=23). No statistical differences were observed for age, weight, ASA physical status, or parity (Table 1).

In all patients the renal function did not showed anomalies.

Hospitalization never exceeded the expected duration. There were no significant changes in the monitored parameters.

No significant side effects or complications appeared. We did not administered oxygen beyond expected time.

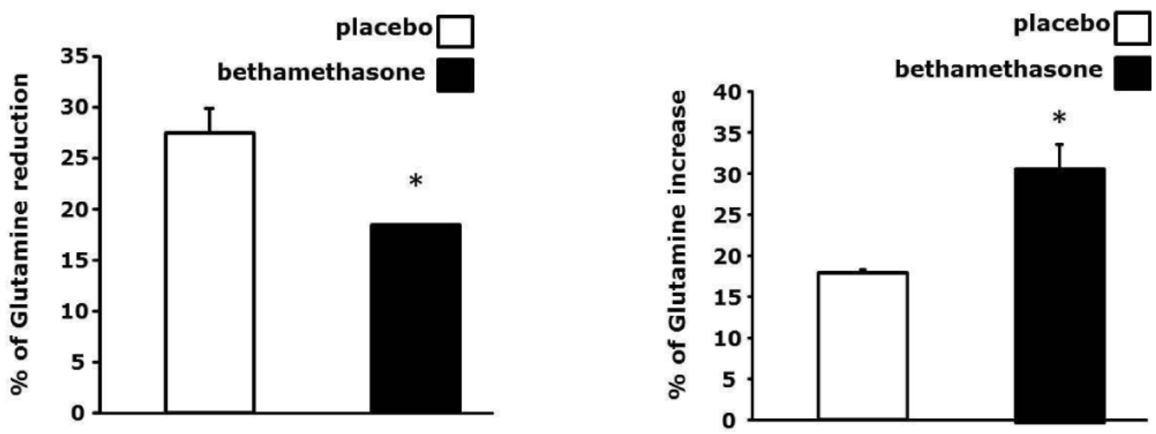

Figure 1: Plasmatic levels of glutamine from in patients pre-medicated or not with bethametasone. Plasmatic levels of glutamine were measured before and after the surgical intervention in patients pre-medicated or not with betametasone. The bar graphs show the % of glutumine increase or reduction between the two groups. Each data point is the mean ± S.E.M calculated from the two separate group. Asterisks denote values significantly different (p ≤ 0.05).

Age (years) (mean ± SD)	Group B 34 ± 12	Group P 34 ± 11
ASA I	18	16
ASA II	5	7
Multiparous	8	10
Nulliparous	15	13
Weight (Kg) (mean ± SD)	63 ± 15	65 ± 14

Table 1: Characteristics of the two groups studied.

Patient:	Time of surgery (min)	Gln conc (sample A)	Gln conc (sample B)	Gln variation	Gln variation (%)	Creatinine (mg/dL)
1	5	2.3	1.69	reduced	26	0.6
2	8	2.04	1.61	reduced	21	0.54
3	11	2.1	1.65	reduced	21.4	0.45
4	6	1.98	1.53	reduced	22.7	0.78
5	12	2.56	2.01	reduced	21.5	0.69
6	10	1.99	1.24	reduced	37.6	0.64
7	11	2.02	1.53	reduced	23.8	0.74
8	11	2.6	2.01	reduced	22.6	0.82
9	8	2.45	1.33	reduced	45.7	0.72
10	9	2.68	1.08	reduced	59.7	0.7
11	9	2.79	1.74	reduced	43.7	0.7
12	7	2.34	1.57	reduced	32.9	0.64
13	8	2.88	2.07	reduced	28	0.67
14	11	2.53	1.77	reduced	30	0.54
15	13	2.64	2.02	reduced	23.4	0.56
16	14	2.22	1.63	reduced	22.2	0.73
17	15	2.08	1.55	reduced	23.2	0.7
18	9	2.2	1.56	reduced	29	0.6
19	7	2.3	2	reduced	13	0.6
20	9	2.04	1.71	reduced	16	0.6
21	5	2.1	1.8	reduced	14	0.69
22	8	2.31	2.69	increased	16.4	0.5
23	7	2.01	2.4	increased	19.4	0.6

Table 2: Plasmatic levels of glutamine measured in patients belonging to the placebo group.

Interestingly, when the two blood samples were compared, we found that in 34 patients out of 46, the gynaecological surgery caused a significant decrease of plasma levels of glutamine (Figure 1, Tables 2 and 3). In particular, 18 patients out of 23 belonging to the placebo group showed a reduction of Gln >20%; while in 3 patients the decrease of Gln was less strong. In the remaining 2 patients belonging to this group, the Gln level was increased.

Patient:	Time of surgery (min)	Gln conc (sample A)	Gln conc (sample B)	Gln variation	Gln variation (%)	Creatinine (mg/dL)
1	5	2.6	3.2	increased	23	0.53
2	8	2.01	1.61	increased	29	0.52
3	8	2.1	2.64	increased	26.2	0.6
4	9	2.67	3.43	increased	31.7	0.6
5	12	2.4	3.01	increased	25.4	0.84
6	11	1.9	2.34	increased	23.1	0.72
7	10	2.11	3.53	increased	67.2	0.7
8	11	2.26	3.01	increased	33.2	0.81
9	10	2.5	3.03	increased	21.2	0.73
10	5	2.67	3.28	increased	22.3	0.69
11	9	3.01	2.6	reduced	15.7	0.6
12	6	2.03	1.77	reduced	12.8	0.6
13	8	1.93	1.67	reduced	13.4	0.6
14	9	2.03	1.81	reduced	10.8	0.64
15	8	2.4	2.02	reduced	15.8	0.7
16	5	2.02	1.65	reduced	18.3	0.85
17	6	2.18	1.75	reduced	19.7	0.64
18	8	3.02	2.56	reduced	15.2	0.7
19	9	2.4	1.82	reduced	24.1	0.72
20	12	3.03	2.31	reduced	24	0.63
21	15	2.2	1.63	reduced	25.6	0.61
22	6	2.32	1.59	reduced	31.4	0.7
23	13	2.23	1.4	reduced	59.2	0.72

Table 3: Plasmatic levels of glutamine measured in patients pre-medicated with bethametasone.

In contrast, 10 patients out of 23 pre-medicated with betamethasone an increase of glutamine concentration >20% was observed. Whilst, in 8 patients we observed a decrease of Gln by about <20%. In the remaining 5 patients, the reduction of Gln was larger than >20%.

Interestingly, 10 patients belonging to the placebo group with decreased levels of Gln, declaimed post-operative pain (VAS >30) within the first hour after surgery (Table 4). In this case, 1 gm Paracetamol (i.v.) was administered. In contrast, for those patients pre-medicated with betamethasone showing increased levels of Gln, the use of analgesic drugs was not necessary (VAS <30, Table 4).

The degree of satisfaction was better in Group B (group B: 5 ± 0.9 vs. group P: 3.6 ± 0.8). The results are summarized in Table 4.

However, upon an accurate clinical observation (4-6 h) and once reached a complete clinical recovery, the patients were discharged.

VAS	Group B Scores			Group P Scores		
	0	<30	>30	0	<30	>30
Pain during surgery	23	-	-	23	-	-
Pain 1 h after surgery	20	3	-	11	2	10

Pain on discharge	22	1	-	17	6 -	-
Degree of satisfaction		5 ± 0.9			3.6 ± 0.8	
Glutammine deplection <20%		8 pt			3 pt	
Glutammine deplection >20%		5 pt			18 pt	
Glutammine increased		10 pt			2 pt	

Table 4: Pain scores, degree of satisfaction and glutamine levels.

Discussion

In the present study we found that in patients that underwent minor gynaecological surgery, the plasma levels of glutamine were significantly decreased, and that the pre-medication with betamethasone prevents the deplection of glutammine, improves postoperative pain and determines a better outcome of patients.

These findings seem to be in agreement with other studies performed by Carli and Emery [37]. In these studies, the authors found that the surgical procedures very often cause a robust decrease of glutamine or other free aminoacid. Moreover, they suggested that this effect was not related to the type of anesthesia carried out (although they have taken analysis of patients anesthetized with halothane or with an epidural block).

In contrast in a subsequent study Lattermann and colleagues [38] observed a significant reduction of muscular and protein catabolism with a decrease of plasmatic levels of glutamine larger than the group anesthetized with isoflurane that in the other one with subarachnoid anesthesia.

In this regard we previously found that the plasma Gln concentration after surgery was lower than pre surgery values and that in major surgery the decrease of Gln was higher than in minor surgery [39]. In this current study we discovered that minor gynaecological surgery also caused a significant decrease of plasma Gln in the majority of patients (32 patients out of 46). However, pre-medication with 4 mg betamethasone (i.v.) significantly prevented the observed decrease of Gln levels in the plasma levels after surgery. Importantly, we found significant differences in the post-operative pain's score between the two groups of patients. In fact in 10 out of 23 patients belonging to the placebo group showed a VAS score >30, therefore paracetamol was administered to reduce the post-operative pain. In contrast, patients pre-medicated with betamethasone, did not require the use of analgesic drugs. A possible mechanism of glutamine on postoperative pain would depend of a connection between plasmatic decrease of glutamine and the increase of inflammatory cytokines released during surgery. As a consequence, we could think that premedication with Betametasone protected better the patient from surgical stress and from the following post-operative pain [21].

Moreover we found a correlation with the duration of the surgery and with parity, in fact in all 12 cases in which we observed a glutamine increase, the surgery lasted a short time, between 7 and 10 min. May be that a surgery lasting less, in a parous patient, and therefore less invasive, cannot cause a depletion of glutamine levels.

However, this study gives rise many questions.

Could the postoperative pain be reduced or prevented by direct glutamine administration? As also previously suggested [40].

The administration of analgesic drugs in pre-emptive can reduce body reaction to surgical stress and therefore glutamine depletion too, in postoperative?

In considering of result that shows an association between depletion of glutamine and pain in postoperative time, can the Gln be accepted in future as prognostic marker of postoperative pain?

Is there a difference in the Gln levels profile between men and women during surgeries? This also based on the evidence that in rat the sexual hormones differently affect visceral pain-related behavioral responses [41].

In conclusion, we suggest that it is important to increase the studies on the Gln profile in surgery and that the premedication with betamethasone can be an useful therapeutic opportunity to increase the plasma level of Gln during the minor surgeries, to reduce the post-operative pain and to ameliorate the satisfaction of the patients.

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