

Premedication with Clonidine Does Not Attenuate Suppression of Certain Lymphocyte Subsets After Surgery

John E. Ellis, MD*, Steven Pedlow, BS†, and Jujhar Bains, BA*

Departments of *Anesthesia and Critical Care and †Statistics, The University of Chicago, Chicago, Illinois

Sixty-four patients undergoing elective major surgery were randomly assigned into a double-blinded, placebo-controlled, clinical trial to test the hypothesis that premedication with clonidine would attenuate postoperative reductions in circulating lymphocytes. The treatment group ($n = 28$) received a clonidine skin patch (0.3 mg/d) and a 0.6-mg oral loading dose 60–90 min before surgery. The control group ($n = 36$) received placebo patches and pills. Absolute blood levels of the following lymphocyte subsets were measured before induction of a standardized general anesthetic (baseline) and the morning after surgery: CD2, CD3, CD4, CD8, CD20, CD56, and the CD4:CD8 ratio. Significant decreases in lymphocyte subsets CD2, CD3, and CD4 were found in both groups; CD56 was significantly decreased only in the placebo group. However, the extent of lymphocyte depletion from baseline to Postoperative Day 1 between the clonidine and placebo groups was not different. Plasma concentrations of

epinephrine, norepinephrine, and cortisol were measured from blood samples drawn at 8:00 AM on Postoperative Day 1. Plasma norepinephrine levels were significantly lower among patients who received clonidine. However, no significant differences were found in plasma epinephrine or cortisol levels between the clonidine and placebo groups. With a clinical dose, clonidine did not prevent postoperative lymphocyte depletion. α_2 -Agonists may not suppress adrenocortical stress responses sufficiently to prevent postoperative immune suppression. **Implications:** Lymphocyte (white blood cell) counts often decrease after major surgery. We hypothesized that clonidine would reduce hormonal stress and blunt reductions in lymphocytes after major surgery. In a randomized trial, we found no differences from placebo in cortisol levels or lymphocyte changes. Lymphocyte levels did not predict infectious complications.

(Anesth Analg 1998;87:1426–30)

Immunosuppression may complicate the recovery of patients after major surgery, when infectious complications are common and their progression may be fatal. Immune suppression also may be associated with tumor metastasis after cancer surgery (1). Postoperative perturbations of the immune system may be caused by a generalized endocrine stress response (2), transfusion (3), hypothermia (4,5), or endotoxemia (6). Lymphopenia is also common in patients admitted to intensive care units (ICUs) (7). Surgery performed under general anesthesia is associated with a marked decrease in the numbers of both B- and T-lymphocyte subpopulations (8). Decrements in circulating lymphocyte populations and function, as well as the normally increased endocrine stress

response associated with major surgery, can be prevented with epidural anesthetic techniques (9).

Maze and Tranquilli (10) focused on the use of α_2 -agonists as anesthetic adjuvants. These compounds produce sedation and analgesia and reduce the postoperative adrenergic response to surgery performed under general anesthesia (11). However, the effects of α_2 -agonist-mediated sympatholysis on the immune status of surgical patients is unknown. We therefore designed this trial to test the hypothesis that premedication with clinical doses of the α_2 -agonist clonidine would attenuate postoperative lymphopenia.

Methods

After obtaining institutional review board approval and individual written, informed consent, 64 patients aged 69 ± 9.4 yr (mean \pm SD) undergoing major noncardiac surgery were randomly allocated in a double-blinded trial to receive a placebo ($n = 36$) or clonidine ($n = 28$) before surgery. Operations performed included 45 vascular, 5 orthopedic, and 14 other (Table 1). These patients were part of a larger

Supported by Grant 5 K14 HL03163 from the National Heart Lung and Blood Institute.

Presented in part at the 1995 annual meeting of the International Anesthesia Research Society, Honolulu, HI.

Accepted for publication September 23, 1998.

Address correspondence and reprint requests to Dr. Ellis, Anesthesia and Critical Care, MC 4028, 5841 South Maryland Ave., Chicago, IL 60637. Address e-mail to j-ellis@uchicago.edu.

Table 1. Patient Demographics

	Placebo	Clonidine	P value
<i>n</i>	36	28	
Age (yr)	74 (63.00, 76.75)	69.5 (63.25, 72.75)	0.18
Weight (kg)	74 (63.50, 83.50)	78 (66.00, 87.00)	0.64
Women	19 (52.8)	8 (28.6)	0.052
Black	16 (44.4)	12 (42.9)	0.90
Vascular/orthopedic/other operations	26/2/8	19/3/6	0.75
Patients with cancer	6 (16.7)	1 (3.6)	0.10
Patients taking β -adrenergic blockers	7 (19.4)	3 (10.7)	0.34
Patients taking NSAIDs	7 (19.4)	7 (25.0)	0.59
Patients taking steroids	0	2 (7.3)	0.10

Values are median (interquartile range) or *n* (%).
NSAIDs = nonsteroidal antiinflammatory drugs.

study examining cardiac outcomes in patients with coronary artery disease or at least two of the following risk factors: age >70 yr, previous vascular surgery, hypertension, abnormal electrocardiogram, diabetes, or cigarette smoking. Exclusion criteria included the following: the presence of bundle branch block, plasma creatinine level >3.0 mg/dL, planned carotid or thoracic surgery, electrocardiogram with PR interval >240 ms, heart rate <50 bpm, long-term clonidine treatment, or diabetes with symptomatic hypoglycemia.

Patients in the treatment group received a clonidine skin patch (0.3 mg/d) and a clonidine 0.6-mg oral loading dose 60–90 min before surgery. The control group received placebo patches and pills. The clonidine skin patch was removed 72 h after application, unless hypotension (systolic blood pressure <90 mm Hg unresponsive to fluid challenge) or a major complication (myocardial infarction, cardiac arrest) ensued; in such cases, the patch, whether active or placebo, was immediately removed.

Patients received morphine premedication (0.1 mg/kg IM) and any chronic cardiac and antihypertensive medications 60 min before surgery. Midazolam 1–2 mg IV was administered to facilitate arterial line placement. Balanced general anesthesia was induced with sufentanil (0.5 μ g/kg), thiamylal (50-mg increments to a maximum of 5.0 mg/kg), and vecuronium (0.1 mg/kg). After endotracheal intubation, anesthesia was maintained with enflurane (\leq 2.0% inspired) in 50% N₂O. All patients received postoperative care in an ICU setting until at least 8:00 AM on Postoperative Day 1. Patients were assessed daily while in the hospital for infections or other complications.

To determine lymphocyte levels, citrated blood samples were collected from an indwelling arterial line 10 min before the induction of anesthesia (baseline) and between 7:00 AM and 9:00 AM on Postoperative Day 1. Absolute levels of the following lymphocyte subsets were measured using fluorescence-activated flow cytometry (12): total B cells (CD20), total T cells (CD2), mature T cells (CD3), helper cells

(CD4), suppressor/cytotoxic cells (CD8), natural killer cells (CD56), and the CD4:CD8 ratio.

To determine plasma catecholamine levels, three separate heparinized blood samples were collected from an indwelling arterial line at 30-min intervals between 7:00 AM and 9:00 AM on Postoperative Day 1. Plasma norepinephrine and epinephrine levels were measured using high-performance liquid chromatography by solid-state extraction (13). The detection limit was 20 pg/mL for norepinephrine and 25 pg/mL for epinephrine. The average value of the measured norepinephrine and epinephrine concentrations from three blood samples was used for final statistical analysis. Plasma cortisol levels were determined by a radioimmunoassay technique from the final blood sample (14). Blood samples were obtained at a relatively constant time of day, although surgery may disrupt normal circadian hormonal rhythms (15).

All continuous data were analyzed using the Wilcoxon rank-sum test of medians. Categorical data were analyzed using Fisher's exact test or the χ^2 test of independence. $P < 0.05$ was considered significant.

Results

There were no significant differences in the medians of lymphocyte subsets compared at baseline and on Postoperative Day 1 between the placebo and clonidine groups (Table 2). There was a significant decrease from baseline in CD2, CD3, and CD4 lymphocyte subsets in both the placebo and clonidine groups. Within the placebo group, there was also a significant decrease in the number of CD56 lymphocytes from baseline to Postoperative Day 1. However, compared across the two groups, no significant difference was found in the extent of postoperative lymphocyte reduction (Table 3). Plasma norepinephrine levels were significantly lower among patients who received clonidine. However, plasma epinephrine and cortisol levels were not significantly different between the placebo and clonidine groups (Table 4).

Table 2. Baseline Lymphocyte Counts and Change on Postoperative Day 1

Cell type	Baseline level (cells/mm ³)	Change in median cell count (%)	<i>P</i> value ^a	<i>P</i> value ^b
CD2				
Clonidine	1269.0 (927.0, 1722.0)	-24.1	0.36	0.032
Placebo	1385.0 (1069.0, 1838.0)	-29.6		0.006
CD3				
Clonidine	1212.0 (882.0, 1646.0)	-39.5	0.57	0.011
Placebo	1230.0 (973.0, 1658.0)	-26.7		0.014
CD4				
Clonidine	736.0 (533.7, 972.5)	-27.3	0.37	0.004
Placebo	838.0 (630.0, 1049.0)	-29.6		0.005
CD8				
Clonidine	345.5 (251.5, 584.7)	-10.7	0.30	0.240
Placebo	426.0 (316.0, 590.0)	-29.3		0.053
CD20				
Clonidine	236.5 (113.3, 387.2)	8.5	0.46	0.840
Placebo	188 (117.0, 302.0)	30.0		0.260
CD56				
Clonidine	149.5 (78.7, 206.8)	-29.3	0.40	0.280
Placebo	176.0 (93.0, 251.0)	-51.1		0.007

Values are median (interquartile range) or %.
^a Clonidine versus placebo group at baseline.
^b Change from baseline to Postoperative Day 1.

Table 3. Median Change in Lymphocyte Levels from Baseline to Postoperative Day 1

Cell type	Placebo (cells/mm ³)	Clonidine (cells/mm ³)	<i>P</i> value
CD2	-240 (-641.8, -65.3)	-270 (-840, -15)	0.85
CD3	-154 (-634.5, -16.5)	-233 (-828, -92)	0.28
CD4	-145 (-432.0, 26.2)	-149 (-557, -8)	0.35
CD8	-66 (-172.3, -4.2)	-78 (-203.5, 30.8)	0.94
CD20	12 (-27.3, 121.7)	-1.5 (-57.2, 76.2)	0.45
CD56	-48.5 (-121.3, -7.8)	-44.5 (-102.3, 35)	0.42

Values are median (interquartile range).

Table 4. Catecholamine and Cortisol Levels on Postoperative Day 1

	Placebo	Clonidine	<i>P</i> value
Epinephrine (pg/mL)	72.7 (41.4, 140.8)	62.1 (32.7, 87.8)	0.14
Norepinephrine (pg/mL)	657.7 (416.5, 1074.0)	300.3 (202, 530)	0.0004
Cortisol (μg/dL)	16.00 (12.40, 18.80)	17.55 (15.25, 23.92)	0.17

Values are median (interquartile range).

Infectious complications occurred in seven patients and included three urinary tract infections, four cases of pneumonia, two wound infections, two cases of cellulitis, and one case of parotiditis. Infections were not significantly different between the clonidine (5 of 28, 17.9%) and placebo (2 of 36, 5.6%) groups (*P* = 0.118). Additional factors that were not predictive of infection on univariate analysis included cancer status, type of operation, and use of nonsteroidal antiinflammatory drugs. However, gender had an association with infection in this cohort; all seven patients with infections were male (*P* = 0.017). Neither postoperative lymphocyte depletion nor median epinephrine, norepinephrine, or cortisol levels were different between patients with and without infections after surgery (Table 5).

Discussion

With the emergence of acquired immunodeficiency syndrome, aggressive medical and surgical therapy of malignancies, and a growing elderly population, increasing numbers of patients with preexisting immunodeficiency present for major surgery. Effective methods to facilitate recovery of postoperative immune status in such high-risk patients should be identified. In the present series, we were unable to show that perioperative clonidine therapy could reduce postoperative lymphocyte depletion.

The exact mechanisms by which anesthesia, operative trauma, and other interventions impair the immune system are complex and poorly defined. Although volatile anesthetics (16) and endogenous

Table 5. Relationship of Stress Hormones and Percentage Change in Median Lymphocyte Levels on Postoperative Day 1 to Infectious Complications

	Infection	No infection	P value
Epinephrine (pg/mL)	69.0	66.7	0.88
Norepinephrine (pg/mL)	465.5	534.0	0.66
Cortisol (μ g/dL)	17.2	16.2	0.76
CD2	-23.3%	-23.9%	0.70
CD3	-37.7%	-22.3%	0.51
CD4	-37.7%	-22.4%	0.59
CD8	-35.7%	-21.9%	0.40
CD20	11.5%	9.3%	0.89
CD56	-9%	-34.8%	0.19

cytokines (17) also suppress immune function, increasing levels of surgical stress also affect the degree of immune suppression in patients after major surgery (18). Regional anesthetic techniques seem to block both adrenergic and cortisol responses, as well as lymphocyte depletion after major surgery (8). The literature suggests that the cortisol stress response may be the dominant inhibitory mechanism responsible for mediating postoperative lymphopenia. The link between hyperadrenergic states and immune suppression may be more tenuous. However, when an etomidate-based anesthetic is used for cardiac surgery, lymphopenia may result even when cortisol elaboration is delayed (19). Increased plasma catecholamine levels do follow surgical trauma; their association with decreased perioperative immune status is unclear. The experimental infusion of inotropic medication in humans can have varying effects, increasing CD3, CD4, and CD8 after an epinephrine infusion but decreasing them after the administration of dobutamine and dexmedetomidine (20). Other experimental studies in humans suggest that CD3 and CD4 T cells decrease significantly 5–60 min after an injection of adrenaline (21). We believe that the relationship between surgical stress and immune function is complex and not fully understood.

There are several limitations to our study. First, we measured circulating T-cell populations, but not their functional activity. In animal studies, lymphopenia after surgery may represent a redistribution of lymphocytes from peripheral blood to lymphatic tissues, rather than a true absence of cells (22,23). Second, we only measured stress hormones on the first postoperative day and therefore cannot comment on the relative time course of stress hormone elaboration and lymphopenia. Third, we used a fixed dose of oral and transdermal clonidine; administering larger doses of clonidine or the more specific α_2 -agonist dexmedetomidine parenterally may provide superior sympatholysis. Indeed, using our regimen, we were able to

demonstrate reductions only in norepinephrine, but not in epinephrine or cortisol. However, other studies by our group (11) have shown a significant decrease in both epinephrine and norepinephrine levels using this same regimen of clonidine. The large statistical variation in catecholamine levels in humans often requires relatively large numbers of patients to show significant differences.

Power analysis for this study is illustrated by the CD3 population. The point estimate for the difference in median postoperative decrease between the placebo and clonidine groups is 100 cells/mm³. Power calculations indicate that if the actual difference had been 100 cells/mm³, the power with the number of patients enrolled in this trial would be 40%; to reach 80% power, we estimate that 70 patients would have had to be enrolled in each group. As designed, the study (64 patients) had 80% power to detect a difference of 167 cells/mm³.

Two gender differences are to be noted in this study. The study was double-blinded, with group assignment determined from random number tables after patient enrollment. Despite this, women seemed more likely to be assigned to the placebo group. Similarly, we cannot explain why all of the patients with infectious complications were men; this may deserve further study. We did not find any difference in T-lymphocyte populations in those patients with and without infectious complications (Table 5); similar findings have been found in ICU patients.

It is not surprising that clonidine therapy did not result in differences in postoperative cortisol levels. Other studies have shown that clonidine may increase cortisol secretion in humans under normal conditions but may decrease it or produce no changes in humans under stressful conditions (24–26). Because increased cortisol levels are associated with immune suppression in other studies (2), clonidine may not be expected to suppress cortisol-related responses. The stress reduction provided by regional anesthetic techniques, however, consistently reduces the adrenocortical and adrenergic responses to surgery.

In our study, a clinical dose of clonidine decreased norepinephrine levels but did not significantly reduce cortisol levels or postoperative lymphocyte depletion. Further investigations that include analysis of both lymphocyte function and the time course of changes in immune status are required to fully assess any effects of α_2 -agonists on postoperative immunosuppression.

We acknowledge the important contributions of Dr. Scott Laff to data collection for this study. In addition, we acknowledge Ms. Sally Kozlik for her editorial assistance.

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