Original Article
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Effect of propofol anesthesia on skin flap survival in rat; comparison with ketamine

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Abstract:

Introduction:
This study aimed to evaluate non-anesthetic properties of propofol in skin flap survival in rats.

Materials and methods:
Thirty-two adult white female rats were randomly divided into two groups. Group 1 was anesthesized with ketamine (40 mg/kg) and group 2 was anesthesized with propofol (100 mg/kg) intraperitoneally. A full-thickness piece of skin on the back of rats (2.5 × 8 × 8 cm) was incised while the flap base was preserved. The flap was immediately returned and sutured using 5.0 separate sutures to the original location. Apparent healing was daily assessed by measuring the necrotic and healthy areas and the survival rate of the skin flaps was evaluated after photography. Histopathological evaluation was performed by a blinded pathologist on days 1, 4, 7 and 21 in three zones of the flap: up, down and middle. Serum interleukin 1 and 6 were measured before and 12, 24 and 168 hours after creating the flaps.

Results:
Flap survival rate on day 21, and IL-6 on day 0, 12 hour and day 1 were significantly higher in propofol group than in ketamine group. IL-1 was not significantly different. Histological signs of healing were more prominent in propofol group.

Conclusion:
It appears that propofol has a more significant effect on skin flap survival in comparison with ketamine, but further and more precise studies are required to confirm this finding.

Keywords: Anesthesia, Propofol, Skin, Flap, Rat

Introduction

Propofol has been used as an anesthetic drug after its introduction in the 1980s, not only in the operating rooms but also in other wards, due to its high potential. Besides its various properties associated with anesthesia, propofol has many other properties. Some of these properties include stimulating the production of structural nitric oxide, antioxidant, analgesic, anti-vomiting, inhibiting platelet aggregation, and inhibiting the increase of intracellular calcium, and each of them has been examined in various studies and has led to the increasing clinical use of propofol (1). Skin is a protective barrier against various environmental and external

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factors and protects the underlying layers against physical, chemical and microbial damage. Hence, it is subject to various injuries and damages. Hence, many studies have focused on the study of the mechanism of wound healing and various pharmaceutical and surgical interventions in order to improve and accelerate it or prevent its low pace. Various methods of wound healing from its oldest ones, like the use of natural gum and honey by ancient Egyptians or tea leaf extracts by the Chinese, to the modern use of disinfectants, are all in order to provide a favorable environment for accelerating the healing process of the damaged tissue. Wound healing is divided into three phases of inflammation, proliferation, and maturation, which are inter-dependent. All the wounds have these three stages regardless of their cause. In the inflammation phase, neutrophils become predominant within 24 hours after injury and then reduce after 3 days. Also, within 24 to 48 hours after injury, the macrophages become predominant and on the 5^{th} day, they comprise most of the cells in the wound. The proliferation phase begins at the end of the inflammation phase, which is the 3^{rd} day after the injury. Fibroblasts produce collagen and glycosaminoglycan. The rate of collagen synthesis increases continuously up to three weeks to reach a balance point, when collagen synthesis and degradation become equal. The maturation phase also begins three weeks after injury, in which there is a balance between collagen synthesis and degradation, but there is no net gain in collagen. This phase takes up to two years. Each healing phase prepares the wound for the next phase, and inhibition or suppression of each phase will have a negative effect on the subsequent phase(s) and the general process of healing. Considering the fact that the healing process depends on several factors, the type of anesthetic and its duration probably affect the postoperative healing process (2).

The healing of damaged skin due to injuries, tumor removal, infection and necrosis after ischemia is still a major challenge for plastic surgeons. Skin flaps are often used to cover the area for retaining function and beauty. Flap necrosis depends on the internal and external factors, and adequate blood flow is the most important internal factor whose impairment is the most common cause of necrosis failure. External factors are classified into systemic (blood pressure, vascular and arterial damage, and infection), environmental (temperature, pressure, and elasticity) and surgical technique, therefore, reperfusion is necessary to restore normal cell activity (3). The reperfusion injury refers to a cell damage caused when blood supply returns to the tissue after a period of ischemia. Various drugs have been studied to increase survival and reduce the effects of reperfusion injury in the experimental flap skin models (3).

Clinical evidence suggests that many aspects of anesthetic management such as increased oxygen therapy before, during and after surgery; prevention of hypothermia; provision of appropriate analgesia after surgery; and reduction in surgical stress significantly improve the outcome of surgical wound healing and reduce the duration of hospitalization (4). Most anesthetic drugs impair the immune response and thus lead to a temporary reduction in chemotaxis, phagocytosis, leukocyte bactericidal activity and T-Cell activity. Although these changes are

temporary, they may increase morbidity and mortality. The effects of surgery and anesthesia on the immune system are highly interrelated, but few studies have examined the effects of anesthesia on the immune system alone, making it difficult to differentiate the effects of anesthesia and surgery. (4). Lack of adequate information on the healthiness or toxicity of propofol is clear (5). Nowadays, plastic surgery, including free skin grafting, plays a major role in treating wounds and skin defects in humans and animals, especially horses and pets. One of the problems after plastic surgery is trying to increase the survival of skin flaps. Many studies have focused on the use of surgical and pharmaceutical approaches to improve the survival of flaps and skin grafts by reducing their defects, shortcomings, and side effects. Regarding the propofol properties obtained from different studies, the present study aimed to investigate the use of propofol as an anesthetic drug on the healing process and formation of flaps.

Materials and Methods

Thirty-two adult female rats were kept under similar nutritional and environmental conditions and randomly divided into two groups of sixteen. Group 1 was anesthetized with a single dose of ketamine (40 mg/kg). Group 2 was anesthetized with a single dose of propofol (100 mg/kg) intraperitoneally. The flap was created as described by McFarlane et al. (1965), as the most commonly used method (Sarifakioglu and Gokram, 2004; Dlimasilova et al., 2009). After marking the site on the skin of the animal's back, the hair was completely shaved in the area and the area was washed with a scrub solution, Betadine® (Povidone-iodine) and disinfectant alcohol. An area of 2.5 × 8 cm was marked in a rectangular shape using a ruler. Then under an aseptic condition, the two 8 cm and 2.5 cm sides were cut so that the base of the flap (in the posterior part of the flap) was maintained. The skin was completely dissected and the whole thickness was separated. The flap was then quickly returned to the original place and sutured with 0.3 nylon yarn with a single pattern so that it was consistent with the condition of the ischemia-reperfusion syndrome of the skin flap. All stages of the creation of a flap in all the rats were carried out by the same surgeon. Then, the rats were transferred to a room with clean and proper ventilation conditions in groups of four in separate cages. The daily healing was daily checked and photographed with a digital camera in completely similar conditions in terms of zoom power and spacing. The wound area was measured and recorded by the Digimizer software using the provided images on days 1, 4, 7 and 21. The histopathologic samples were evaluated by a pathologist blinded to the group of the samples. To this end, at the mentioned times, four mice were slaughtered according to ethical principles by cervical displacement. Three samples were taken from the upper, middle and lower flap areas of each rat. Subsequently, samples of each area were individually transferred to containers containing 10% formalin buffer. All stages including dehydration, clarification and molding were automatically performed by passing through distilled water, alcohol, glycols and paraffin. After molding, 5-micron sections were stained with hematoxylin-eosin. After preparation, the slides were studied and microscopically compared. Three 40x fields were examined in each slide. The collagen production and epithelialization, as well as the number of

inflammatory cells, fibroblasts, and blood vessels, were counted and their mean and standard deviation were calculated. Histological samples were examined and scored by a modified Ehrlich-Hunt numerical scale, in which fibroblast content, vascularity, collagen, and inflammatory cell infiltration were graded as 0 for absence, 1 for the occasional presence and light scattering, 2 for abundance, and 3 for the confluence of cells and fibers. Epithelial regeneration was scored as 0 for no epithelium, 1 for single-layer epithelium with partial closure, and 2 for multilayer epithelium with complete closure. Interleukin 1 (IL-1) and interleukin 6 (IL-1) levels were evaluated in blood samples before the creation of flap and at 12, 24 and 168 hours after the humane killing of animals and histopathologic sampling.

The independent t-test was used to compare the survival rate of the flaps and the IL-1 and IL-6 content between the two groups. The Mann-Whitney test was used to compare the histopathological evaluation data. Data were analyzed using the SPSS software at a significance level of $P<0.05$.

It is worth noting that the present research project was approved before implementation by the Research Ethics Committee of Shiraz University.

Results

There was a significant difference in terms of survival between the propofol (69.52 ± 3.82) and ketamine (47.13 ± 6.66) groups on the seventh day. On day 21, the survival rate of the flap in the propofol group (67.57 ± 9.52) was higher than that in the ketamine (61.18 ± 7.11) group, but the difference was not significant. The flap survival decreased significantly in the ketamine group on the 21st day ($P>0.001$). There was no significant difference in propofol group between these two days in terms of survival (Figure 1).

On day zero (before the start of the study) and 12 and 24 hours, and 7 days after the creation of the flaps, the activity of IL-1 was not significantly different between the propofol and ketamine groups. The IL-1 level at the above mentioned times was 4.5, 4.4, 5.5, and 3.5 pg/ml in the propofol group; and 4.8, 4.6, 5.8, and 3.6 pg/ml in the ketamine group, respectively (Figure 2).

On day zero and 12 and 24 hours after, the level of IL-6 activity was significantly different in the propofol and ketamine groups, such that it was significantly higher in the propofol group, however, on day 7 after the start of the study, this difference was not significant. The IL-6 levels in the propofol group at baseline, and 12 and 24 hours, and 7 days after were 4.8, 4, 3.5 and 4.1, pg/ml, respectively. These amounts in the ketamine group were 3.7, 3.4, 2.8 and 3.6 pg/ml, respectively (Figure 3).

Twenty-four hours after the creation of the flap, the collagen production level in the middle part of the flap in the propofol group was significantly higher than that of the ketamine group. Also on day 4, the collagen production level in the upper and lower parts of the flaps in the ketamine group was significantly higher than the propofol group. On day 7, the collagen production level in the lower part of the flaps in the propofol group was significantly higher than the ketamine group. On day 21, there was no significant difference between the two groups (Figure 4).

Twenty-four hours after, the epithelialization level in the middle part of

the flaps in the propofol group was significantly higher than that in the ketamine group. On day 4, this difference was significant in the lower part of the flap in the two propofol and ketamine groups, and it had a great increase in the ketamine group compared with the propofol group. On days 7 and 21, there was no significant difference between the two groups (Figure 5).

On day 21, fibroblast proliferation in the upper part of the flap in the propofol group was significantly higher than that in the ketamine group but no significant difference was observed on days 1, 4 and 7 (Figure 6).

On day 4, the inflammatory cell infiltration in the lower part of the flaps in the propofol group was significantly higher than the ketamine group. On day 7, the inflammatory cell infiltration level in the lower part of the flaps in the ketamine group was significantly higher than the propofol group. On day 21, the inflammatory cell infiltration level in the upper part of the flaps in the propofol group was significantly higher than the ketamine group. On day 24, there was no significant difference between the two groups (Figure 7).

On day 4, the vascularity level in the upper and lower parts of the flaps in the propofol group was significantly higher than that in the ketamine group. On day 7, the vascularity level in the upper and middle parts of the flaps in the propofol group was significantly higher than that in the ketamine group. On day 21, the vascularity level in the upper and middle parts of the flaps in the propofol group was also significantly higher than that in the ketamine group. On day 24, there was no significant difference between the two groups (Figure 8).

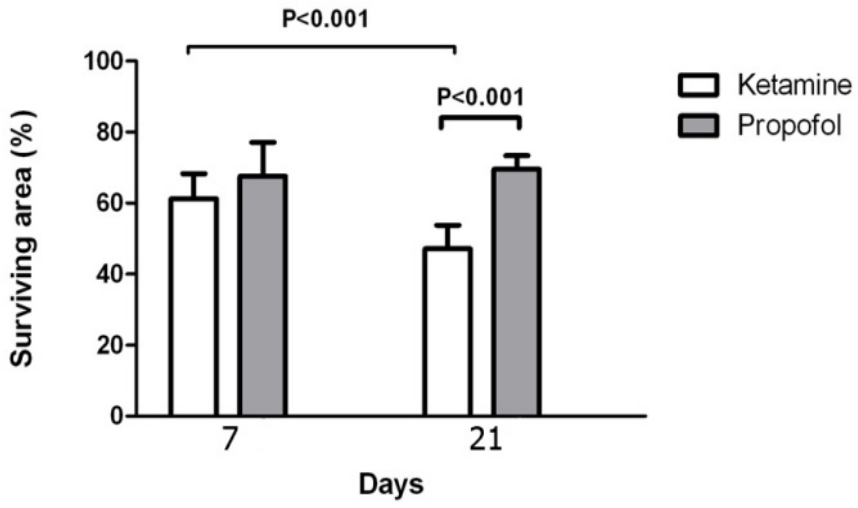

Figure 1: The flap survival in propofol and ketamine groups at different times

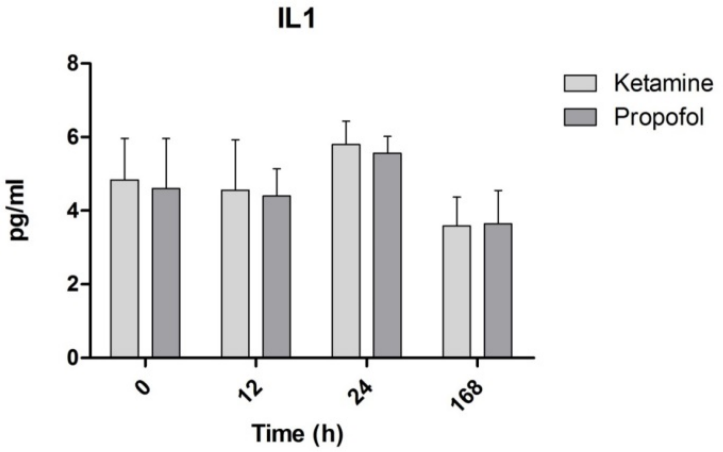

Figure 2: The IL-1 activity in propofol and ketamine groups at different times

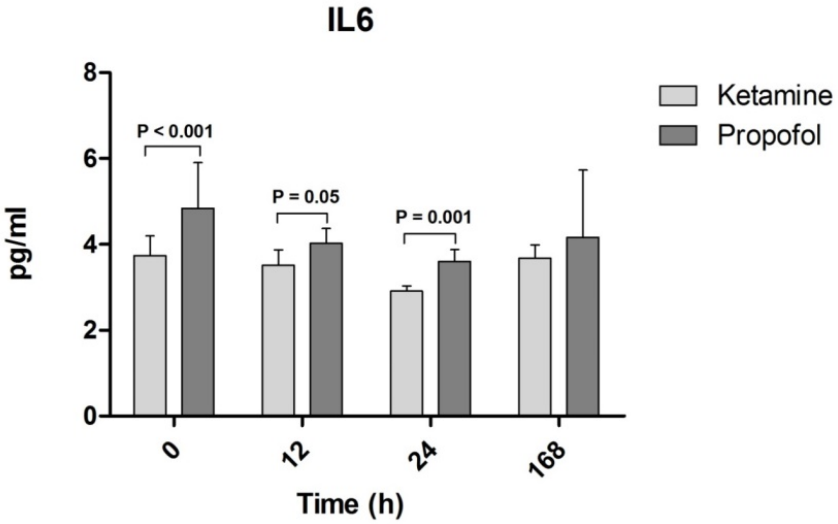

Figure 3: The IL-6 activity in propofol and ketamine groups at different times

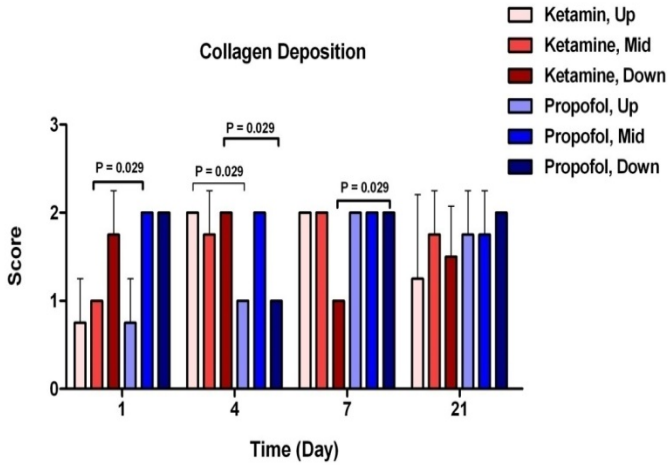

Figure 4: The collagen production levels in the propofol and ketamine groups at different times and in the upper, middle and lower parts of the flap

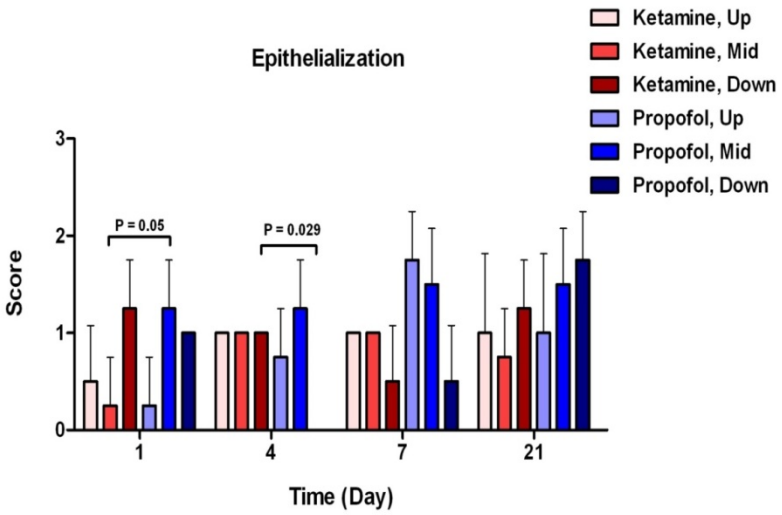

Figure 5: The epithelialization levels in the propofol and ketamine groups at different times and in the upper, middle and lower parts of the flap

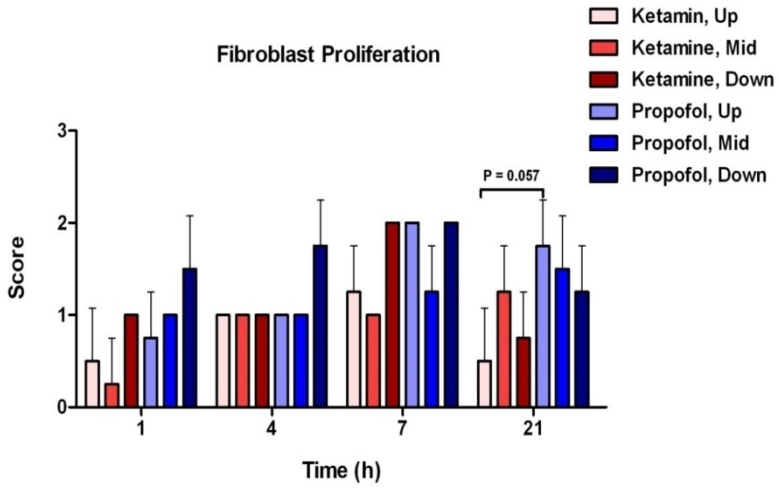

Figure 6: The fibroblast proliferation levels in the propofol and ketamine groups at different times and in the upper, middle and lower parts of the flap

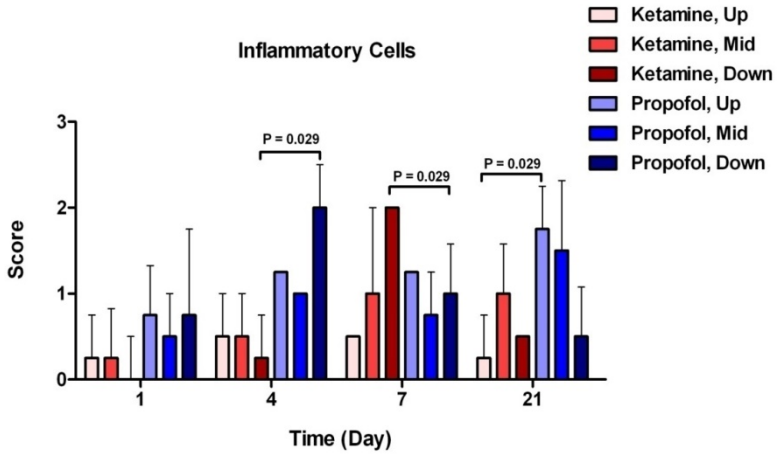

Figure 7: The inflammatory cell infiltration level in the propofol and ketamine groups at different times and in the upper, middle and lower parts of the flaps

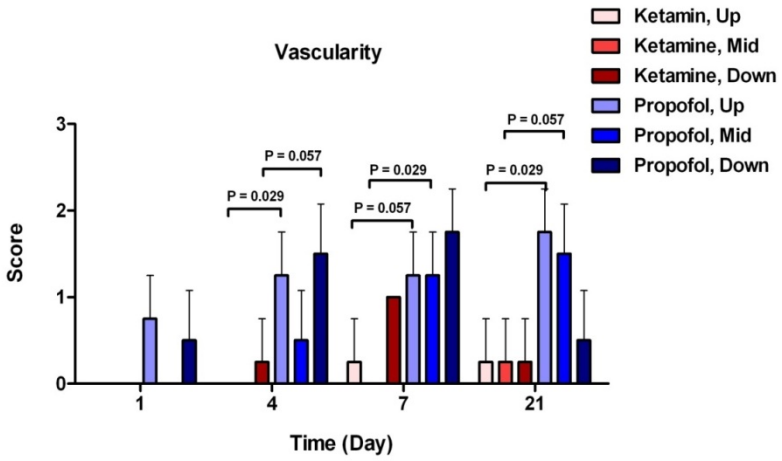

Figure 8: The vascularity levels in the propofol and ketamine groups at different times and in the upper, middle and lower parts of the flap

Discussion

The in vitro effects of Thiopental, Methohexital, Propofol, Etomidate, and Ketamine have been evaluated on the chemotaxis of eosinophilic leukocytes. Researchers have concluded that thiopental and etomidate have deteriorating effects on the wound healing process by inhibition and degradation of chemotaxis (6), while propofol and ketamine did not have a significant effect on chemotaxis, which is consistent with the results of the present study. Antiproteases, including aprotinin, a broad-spectrum antiprotease in the skin flap model of 32 rats, were evaluated before and after the creation of intravenous flap and in comparison with saline as control. Researchers concluded that administering aprotinin prior to ischemia can significantly improve the survival of skin flaps by reducing neutrophils in the skin flap (7). A large influx of neutrophils to a skin flap has been shown in numerous studies, and many studies have been undertaken to control or reduce the effects of free radicals and damage caused by neutrophils. Sympathetic inhibitors, vasodilators, prostaglandin inhibitors, glucocorticoids, anticoagulants, and free radical scavengers are in this category (8). Certain compounds include minoxidil, superoxidase dismutase, catalase, allopurinol, vitamins A, E and C, dexamethasone, cyclosporine, methy-lprednisolone, azathioprine, heparin, deferoxamine, arginine, vascular endothelial growth factor, lidocaine and prilocaine, carnitine and aspirin have also been used in several studies (3). In a damaged tissue, neutrophils produce necrotizing agents, such as free oxygen radicals (9), which are the main cause of reperfusion injury in tissues and organs (10). Consequently, compounds that inhibit the formation of these substances, such as allopurinol and those that eliminate their effects, such as superoxide dismutase, mannitol, and dimethyl sulfoxide have been shown to increase the survival of skin flaps in various studies. IL-1, IL-6, TNF and interferons are the main cytokines that are secreted in response to surgical injuries. It has been shown that sedative and anesthetic drugs, having an effect on the immune system, can inhibit the activity of granulocytes and

multinucleated cells and their phagocytosis. An increase in the percentage of T-helper lymphocytes was observed following anesthesia with propofol. Also, decreased activity of phagocytosis in alveolar macrophages was observed in anesthesia with propofol or sevoflurane. It appears that abnormal perfusion of small vessels after the process of reperfusion injury is associated with the infiltration of inflammatory cells and the release of inflammatory mediators. The activated neutrophils that attach to the endothelium of the small vessels, secrete the myeloperoxidase enzymes, causing free radicals and tissue damage, vascular damage and vasospasm, hemostasis, and thrombosis and cellular autolysis and the extracellular matrix proteins. With the onset of lipid peroxidation by free radicals, the cellular function will be affected and reperfusion-associated necrosis will occur (11, 12). This damage has a complex process, and increasing the tissue resistance to deal with this phenomenon is very necessary (3). As stated and since neutrophils are the main cells responsible for reperfusion injury, reducing or inhibiting the pharmacologic response of neutrophil to ischemia will reduce reperfusion injury (13). Although the use of ketamine is preferred in the induction of anesthesia in infectious patients, cautious use of propofol due to decreased immobilization of leukocytes or thiopental has also not been ruled out (4).

It has been shown that the secretion of cytokines and their imbalance, which was attributed to surgical stress alone, is also affected by anesthetics. The most important consequence of these cytokines is increased neutrophil aggregation (14). In general, intravenous anesthetic induction drugs decrease the activity of neutrophils, and opiates increase it (14). Recent studies have shown the role of cytokines in wound healing and increasing the pain around the wound. It has been determined that the skin around the incision and biopsy contains elevated amounts of several cytokines, including IL-1β, IL-6 and TNFα (15). Reduction in and inhibition of IL-1β secretion by human peripheral mononuclear cells were reported in the presence of anesthetic gases of sevoflurane, isoflurane, and enflurane in comparison to air (16). Therefore, it appears anesthetics affect secretion of cytokines by peripheral mononuclear cells.

Another study on rabbits showed that propofol increases hepatic blood flow, and, especially in biliary-hepatic surgery, where there is a risk of ischemic and reperfusion injury, the amount of hepatic oxygen creates the balance (17). A study by Delacros et al. on rats showed that propofol not only inhibits lipid peroxidation but also enhances the cellular antioxidant defense system. Propofol, therefore, appears to prepare cells to cope with oxidative influx by increasing reduced glutathione levels. This mechanism (glutathione system) is the most important defense mechanism against cell damage and can be used in patients with ischemia (18). Following the creation of a skin flap, ischemic-dependent tissue damage results due to disruption of oxygenation. Reperfusion, along with tissue damage and small vessel obstruction will even lead to tissue necrosis. The mechanism of this phenomenon is still not well known and how drug interventions affect the ischemic tissue is the subject of many investigations. Three main mechanisms of platelet aggregation, vascular spasm, and autolysis or neutrophilic cell destruction have been

reported for ischemic injury (13). Vascular thrombosis followed by surgical injury is another major cause (19). Improvement of oxygenation was reported following administration of a high dose propofol in rabbits with acute pulmonary injury (20). In this study, IP administration of Dimethyl sulfoxide (1.5 mg/kg) during and after the onset of reperfusion ischemia, significantly improved the survival of a 4×9 cm skin flap in rats. Free radical scavenging and vascular dilation are reported as the causes of this effect (10). Another study examined the effect of 1, 2 and 8 hours of anesthesia with propofol or sevoflurane on wound healing in 32 rats. Examining the amount of blood flow around the wound and the size of the wound on day 0 (before surgery) and 3 and 7 days after surgery, it was observed that 8-hour administration of sevoflurane reduced the healing process by reducing blood flow and increasing the size of the wound. While IV administration of propofol through the tail vein (10 mg/kg/hour) on day 7, increased wound blood flow in 8-hour administration and the size of the wound did not have a significant difference (21). In the present study, an increased vascularity in days 4, 7 and 21 after creating the flap was observed in the propofol group in comparison with the ketamine group, which is consistent with the increase in blood supply in the above mentioned studies.

The role of propofol in decreasing the activity of neutrophils and subsequent reduction of myeloperoxidase activity in rats anesthetized with propofol was reported to increase the flap survival (11). The 48-hour continuous administration of propofol versus midazolam in a study suggested that propofol stimulates the production of pre-inflammatory cytokine production (IL-1b, TNF-a, IL-6, IL-8) IL-1β, IL-6, and TNF-α, while midazolam inhibits them (22). IL-1 induces chemokine production by fibroblasts, keratinocytes, and macrophages that are present in the wounds (22). The role of IL-1 in wound biology have been investigated by Hu et al. The administration of Interleukin-1 receptor antagonist (IL-1RA) in rats reduced pain response and reduced the production of many inflammatory mediators in the wound. It was found that IL-1 plays an important role in regulating the production of inflammatory mediators in the wound and by stimulating the production of chemokines and cytokines plays an important role in the biology of the wound (23). Though, its short-term inhibition has a positive effect on wound healing. Free radicals change the vascular endothelium by inhibiting nitric oxide synthase activity, which ultimately leads to the inhibition of factors affecting vasodilation and prevents platelet aggregation (18). Nitric oxide dilates the blood vessels and regulates blood flow through small vessels of the skin (24). In vitro studies have shown that propofol enhances the production of nitric oxide by leukocytes, stimulation of endothelial cells in its synthesis and the inhibition of platelet aggregation (18). The examination of the effect of propofol on nitric oxide production route by leucocytes in vitro and in vivo suggested that propofol increases the production of nitric oxide in both conditions. Plasma concentration of IL-1β, IL-6, and TNFα decreased, too. None of these changes were observed in the group anesthetized with sevoflurane. It is reported that propofol creates such effects by affecting the inflammatory mediators (25).

Conclusion

According to the results, administration of a single dose of propofol for short-term sedation and short-term anesthesia does not have adverse effects on skin flap compared to ketamine and can help flap survival. Although the positive effects of improved flap survival by propofol were observed, further studies are needed to confirm its effectiveness.

Acknowledgments

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Conflict of interest

The authors declare no conflicts of interest.

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