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ORIGINAL ARTICLE

Anesthesia may alter mRNA expression of certain wound healing-associated genes in dermal wound environment of the rats

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Abstract

Some anesthetics including ketamine/xylazine and thiopental have been shown to alter the expression of genes related with inflammatory cytokines and chemokines in previous studies unassociated with wound healing, arising the question of whether commonly used anesthetics in wound healing models could interfere with the transcriptional responses of the genes associated with skin wound healing. The gene expression profile in wound biopsies of rats who received widely used anesthetics doses of intraperitoneal ketamine/xylazine (50 mg/kg and 10 mg/kg) or thiopental (50 mg/kg) in comparison with control rats was analyzed by monitoring the expression of genes effective on various phases of wound healing. The expression levels of 84 genes were determined on 3rd, 7th and 14th days of post-wounding using a qPCR array system. Of the genes either up or downregulated fivefolds or more, three (*Egf, Col5a1* and *Cxcl3*) and two (*Tgfa* and *Il2*) genes were found to be the most responsive ones to ketamine/xylazine or thiopental anesthesia respectively in a period of 14 days after correction for multiple testing. However, up to 22 and 24 genes for ketamine/xylazine and thiopental were found to be differentially expressed in the same period without correction for multiple-comparisons testing ($p < 0.05$). In conclusion, our data suggest that ketamine/xylazine and thiopental may alter the transcriptional responses of some genes associated with wound healing in rats. We strongly suggest to consider the possible alteration effect of these anesthetics on gene expression in animal models of dermal wound healing.

Keywords Ketamine/xylazine · Thiopental · Anesthetics · Wound healing · Gene expression

Introduction

Presently available evidence indicates that approximately 100 genes, which also include a group of genes involved in the inflammatory responses are highly regulated at the transcriptional, translational and post-translational levels during different phases of wound healing [1, 2]. Some of the key findings for these genes were obtained from studies focusing on the transcriptional responses of the genes associated with skin wound healing on experimental animals. PCR array, covering major genes for the wound healing response is commercially available and it is not only used in understanding the molecular mechanisms involved in wound healing, but also in understanding how various therapeutic agents and medicinal plants used for skin wounds treatment affect mRNA expression during healing [3–6].

In wound healing models, the animals, especially rats as a preferred model organism, undergo general anesthetics at least 3–4 times during wounding and biopsy processes. In these models, 3rd or 4th, 7th and 10th or 14th days covering the different phases of wound healing (hemostasis, inflammation, proliferation, and remodeling) are considered to be the appropriate time-points to evaluate the wound healing [7]. Ketamine and thiopental sodium are two prominent anesthetics used in these studies and can be administered

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by intraperitoneal or intravenous injection. Ketamine is often used in combination with xylazine to anesthetize animals. Data from human and animal studies irrelevant to the wound healing showed that both anesthetic agents have possible immunomodulatory and anti-inflammatory effects. Recent molecular studies mainly focusing but not limited to ketamine show that these anesthetics can modulate the transcriptional expression, the production, and activation of several molecules, which have also a role in molecular events of wound healing, such as *NF-κB, IL-6, TNF-α, Collagen, CD18*, and *CD62L* [8–13]. A recent in vitro study on macrophage functions have shown that ketamine exerts suppressive effects by altering the expression of TNF-α, IL-1β, and IL-6 mRNAs [14], important components of wound healing. It has been shown that thiopental inhibits global protein synthesis by repressing Eukaryotic Elongation Factor 2 (EF-2) [15]. In the proliferation phase of wound healing, global protein synthesis is in its most intense term and repressing this synthesis may affect wound healing. Considering that there are many genes related with wound healing, it seems likely that use of the aforementioned anesthetics either individually or together may alter the of gene expression, therefore, interfering with the scientific goals of cutaneous wound experiments. A limited number of studies have investigated the possible effects of widely used anesthetics on wound healing [16–18]. However, little is known about the effects of anesthetics on genes related to wound healing in animal models. In the present study, we aimed to evaluate the effects of ketamine/xylazine (K/X) and thiopental on genes related to wound healing by mRNA expression profiling with a specific panel. Our results will help for a better evaluation of the past, current and future experimental animal studies focusing on genes related to wound healing and may give a pre-warning to the researchers who use or aim to use these widely used anesthetics whether there is an interfering effect of the agents on the expression of the genes related to wound healing or not.

Materials and methods

Animals, experimental protocol and wound creation

Twenty-five healthy male Wistar albino rats (250–300 g weighing, 6 months old) were used in this study. Animal studies were performed in accordance with the guidelines of the Istanbul University Experimental Animal Studies Local Research Ethics Committee. The rats were grouped as follows: group I (5 rats/group)— anesthetized with ketamine (50 mg/kg, intraperitoneally) and xylazine (10 mg/kg, intraperitoneally) on 0, 3rd, 7th, and 14th days and group II (5 rats/group)— anesthetized with thiopental sodium on 0, 3rd, 7th, and 14th days (50 mg/kg, intraperitoneally) and group III (15 rats/group)— control group, administrated with tramadol (4 mg/kg, subcutaneously) at 0 day prior to wounding.

A total 15 rats for the control group were decapitated prior to taking granulation tissues on the 3rd day, (5 rats), on the 7th day (5 rats), and on the 14th day (5 rats).The dorsal hair was shaved and the skin was sterilized with 70% alcohol. Three full thickness dorsal skin wounds with the size of 1 cm in diameter were carried out. The wounds were cleansed with normal saline solution (0.9% w/v) on days 0, 3, and 7. Granulation tissues were surgically removed on 3rd, 7th and 14th days (tissues are taken from different wounds in different days) after wounding and stored at − 80 °C for molecular genetic analysis (Fig. 1). The study protocol was approved by the Animal Research Ethics Committee of Istanbul University, Istanbul, Turkey (December 5, 2014, No: 116).

Calculation of wound contraction percentage

Following wound creation wound boundaries were traced on a planimetric transparent paper on days of biopsy with a fine tip permanent marker. Wound healing was followed-up by calculating the squares in the traced area. Percentage of wound contraction (wound closing) has been calculated using the Wilson's formula shown below and contraction was expressed when the size of the wound was reduced compared to the original size.

$$\% \text{ wound contraction} = \frac{0 \text{ day wound area} - \text{wound area on the day biopsy taken}}{0 \text{ day wound area}} \times 100.$$

Total RNA extraction and cDNA synthesis

Total RNA was extracted with the High Pure RNA Tissue Kit (Roche, Mannheim, Germany) from snap-frozen tissue samples following tissue homogenization. Extracted RNA samples were stored at − 80 °C until analysis. NanoDrop 2000c UV–Vis Spectrophotometer (Wilmington, USA) was used for determination of RNA quantity and purity by absorbance measurement at 260 nm and 280 nm. 200 ng of total RNA was used for cDNA synthesis with Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions.

PCR array and real time polymerase chain reaction

All amplification reactions were performed in a 96-well ready to use Rat Wound Healing RT^2 Profiler PCR Array (Qiagen, Hilden, Germany) containing primers and probes for 84 tested genes of interest related to wound healing and two sets of five housekeeping genes *Actb* (actin beta), *B2m* (beta-2 microglobulin), *Hprt1* (hypoxanthine phosphoribosyl

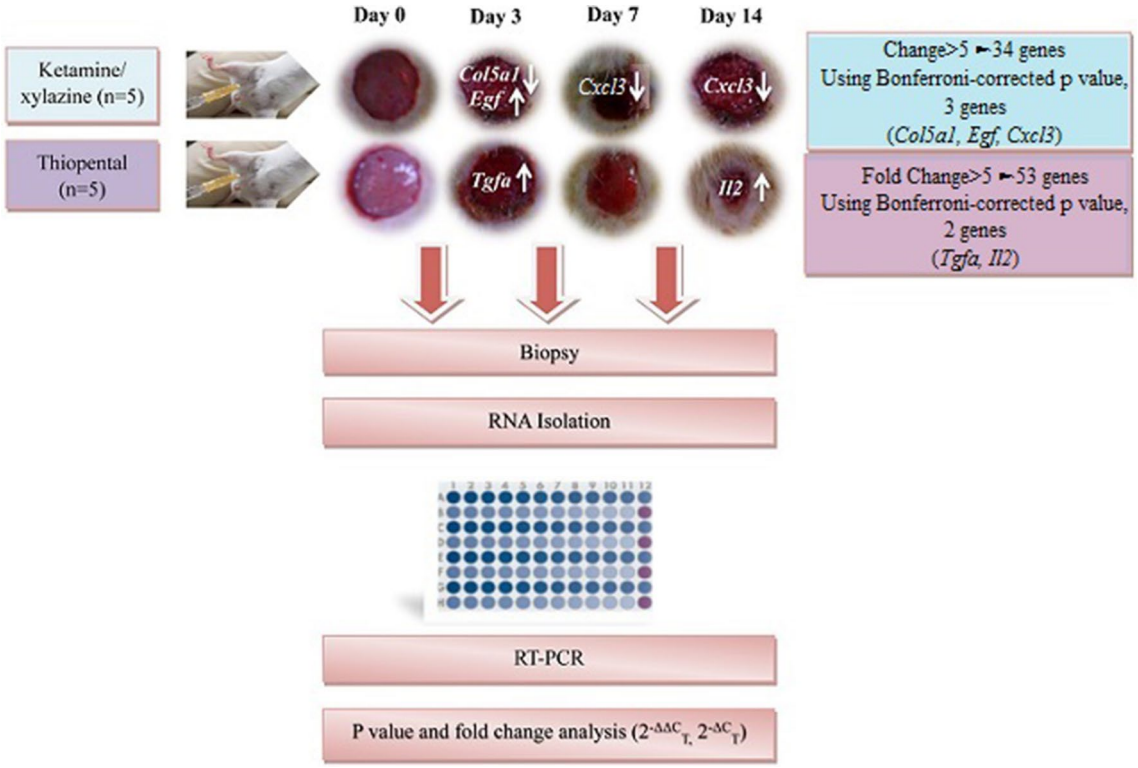

Fig. 1 Flow diagram showing the highlights and the study design

transferase 1), *Ldha* (lactate dehydrogenase A), *Rplp1* (ribosomal protein large P1) and blank wells for negative control. Genes of interest can be found on the manufacturer's web page (http://www.sabiosciences.com/rt_pcr_product/HTML/PARN-121Z.html) regarding the Rat Wound Healing RT^2 Profiler PCR Array. The cDNA was diluted with distilled water at 1:1 ratio, and added into the RT^2 qPCR Master Mixes (Qiagen, Hilden, Germany) according to the manufacturer's instructions and loaded into each well of a 96-well PCR array plate. PCR was performed on a BioRad Cfx Connect (Hercules, CA, USA). PCR conditions were as follows: initial denaturation at 95 °C for 10 min, and then 40 cycles of denaturation at 95 °C for 15 s, annealing at 58 °C for 20 s and extension at 72 °C for 20 s. All reactions were performed three times and the mean values were used.

Data and statistical analyses

The expression levels in wounds from K/X and thiopental sodium anesthetized rats relative to controls were analyzed using the $2^{-\Delta\Delta Ct}$ method [19]. ΔCt indicated the difference in expression levels with the Ct value of the related gene and mean of five housekeeping genes (ΔCt = Ctx gene – Ct housekeeping), and ΔΔCt indicated the difference in the ΔCt value between treatment and control groups (ΔΔCt = ΔCt treatment – ΔCt control). Student's t-test was used to determine p values from the replicate $2^{-\Delta Ct}$ values for each gene in the control and treatment groups. Kruskal–Wallis test was used for comparison of percentage contraction. The statistical significance was set at $p < 0.05$ and a mean difference equal to or greater than fivefold change in expression levels.

A Bonferroni correction was also applied to the p-values [ECM & cell adhesion molecules, $p < 0.0012$ (0.05/42); inflammatory cytokines & chemokines, $p < 0.0038$ (0.05/13); growth factors, $p < 0.0029$ (0.05/17) and signal transduction, $p < 0.0042$ (0.05/12)] to compensate the multiple testing error, considering the gene numbers in each category as associated the 84 genes represented in the wound healing PCR array panel.

Results

PCR array analysis of the genes related to wound healing and anesthetics

In order to assess the effects of anesthetic doses of K/X or thiopental sodium on genes related to wound healing, we performed an expression analysis of 84 genes crucial for the wound healing response in the full-thickness excisional

wound tissues of rats at 3, 7, and 14 days post wounding by using RT-PCR array analysis.

K/X anesthesia

As shown by the Volcano plot (Fig. 2a–c) and by the Heat map (Fig. 3) 34 out of 84 genes during the first 14 days of healing were differentially expressed (≥ fivefold change, either up-or down-regulated) in the wounds of the rats exposed to K/X anesthesia in comparison to the control wounds exposed to tramadol. Of 84 genes tested, 18 genes were found to be significantly different between the K/X anesthesia and control group at $p < 0.05$ level. In addition, we also set a conservative significance threshold that was based on a Bonferroni-corrected p value of 0.0012–0.0042, considering the gene numbers in each subset represented in the wound healing PCR array panel. In this case, totally three genes exceeded these conservative significance thresholds in K/X anesthesia group on days 3, 7, and 14 (Table 1).

At day 3 after wounding, fold regulation analysis (≥ fivefold change) showed that there were a total of 23 genes showing differential expression with 11 genes up-regulated and 12 genes down-regulated, compared to control group (Fig. 2a–c). Among these genes, 13 genes were found to be significantly different at $p < 0.05$ level (Table 1). On the other hand, considering a stringent p value cut-off of 0.0012 for the subset of ECM & cell adhesion genes, up-regulation of one gene (*Col5a1*) passed this filter of $p < 0.0012$. In addition to this subset, only down-regulated *Egf* gene among the growth factor genes exceeded the associated significance threshold of $p < 0.0029$.

On day 7 post-wounding compared to the control wounds, there were six differentially expressed mRNAs in K/X group according to the fivefold change criterion with three genes up- and three genes down-regulated (Fig. 2a–c). Statistical analyses showed a significant difference at $p < 0.05$ level for the two genes (*Cxcl3* and *Fgf10*). Using Bonferroni-corrected p value, we found that only *Cxcl3* gene from the subset of inflammatory cytokines & chemokines genes among these genes passed the associated Bonferroni cut-off (Table 1).

At 14 day old wounds from the rats which received third injections of K/X before the start of skin biopsy, among 12 genes displaying a fivefold difference, only three down-regulated genes (*Cxcl1, Cxcl3*, and *Tnf*) remained significant at $p < 0.05$ level (Table 1; Fig. 2a–c). Furthermore, only *Cxcl3* gene exceeded the significance threshold of $p < 0.0038$ for the subset of inflammatory cytokines & chemokines genes.

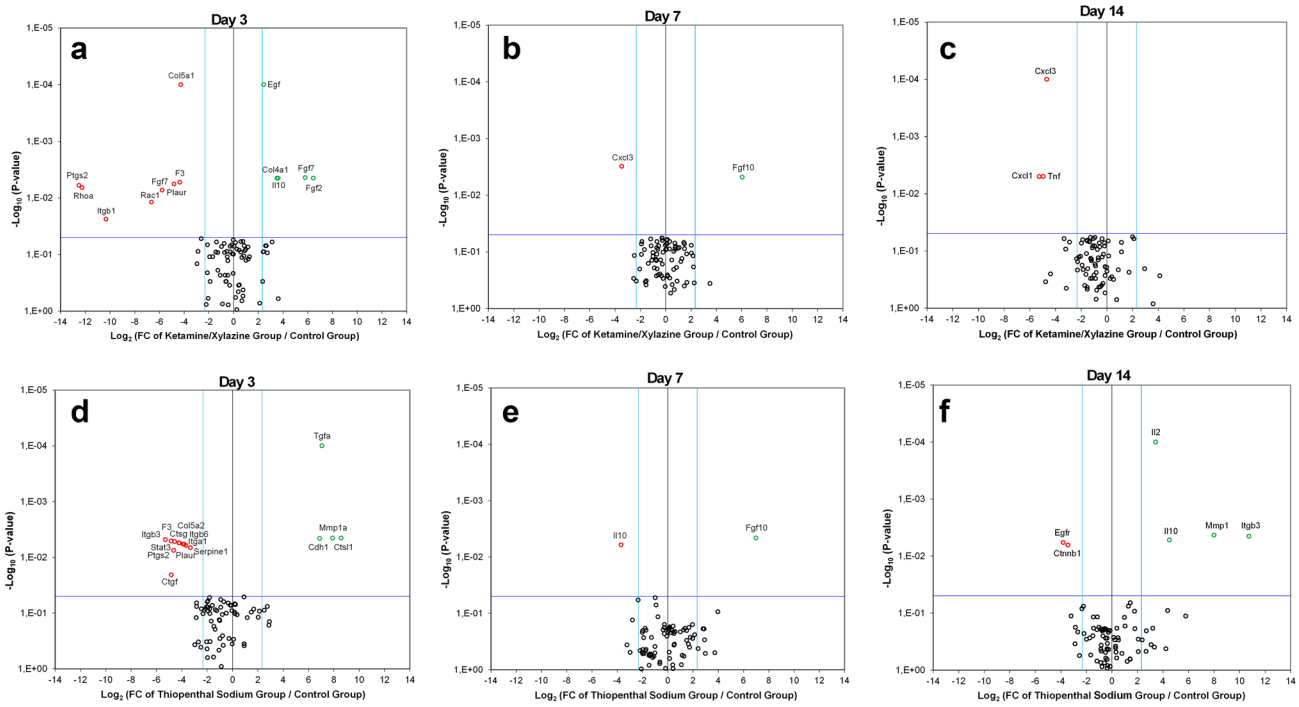

Fig. 2 Volcano plots representing real-time RT-PCR analysis at each time point. The 84 genes in the skin of rat associated with wound healing were analyzed at day 3 (**a**, **d**), day 7 (**b**, **e**) and day 14 (**c**, **f**) post-wounding. The vertically central line indicates a fold-change in gene expression of 1. Two blue vertical lines either side of the central line indicate a threshold of fivefold-changes in gene expression. The dark blue horizontal line indicates a p-value of 0.05. The green and red circles represent significant down and up regulation, respectively. (Color figure online)

Thiopental anesthesia

As a consequence of the exposure to thiopental anesthesia at doses commonly, 53 (out of 84) genes during a period of 14 days exhibited a greater than fivefold change in expression, either increasing or decreasing, as shown by the Volcano Plot representation and by the Heat map (Figs. 2d–f, 3). Comparison of the $2^{-\Delta Ct}$ values obtained from measurement of the control and thiopental anesthesia groups showed differential expression of several mRNAs in various stages of the healing process. Although 23 genes showed significant difference at $p < 0.05$ levels, only two genes (*Tgfa* and *Il2*) remained statistically significant after multiple testing correction (Table 2).

At day 3 after wounding, the fold regulation analysis showed that there were 12 upregulated and 27 downregulated genes, compared to the control group. Among all genes that displayed at least a fivefold difference, 11 down-regulated and four up-regulated genes remained significant at $p < 0.05$ level. However, only one mRNA (*TgFa*) from growth factors genes passed the significance threshold of $p < 0.0029$.

In the granulation tissues of the rats which received a second intraperitoneal injection of thiopental before skin biopsy at day 7 post-wounding, the results of the fold changes showed that there were 13 differentially expressed mRNAs in thiopental group with eight up-regulated and 5 down-regulated. The statistical significance of fold changes for all genes in both anesthesia groups did not achieve the Bonferroni cut-off.

On day 14 post-wounding compared to the control wounds, there were 21 differentially expressed mRNAs in thiopental anesthesia group according to the fivefold change criterion, with 13 genes up- and 8 genes down-regulated. However, the mRNA level for only one gene (*Il2*), encoding a type of cytokine signalling molecule was decreased significantly according to Bonferroni criterion.

Effects of anesthetics on wound closure

Wound contraction percentage is used to assess wound healing. There was no significant difference in total wound area between the K/X or thiopental sodium-treated wounds with respect to the control wounds through the 3rd, 7th and the 14th days (Table 3).

Discussion

The current study investigated whether K/X or thiopental anesthesia could interfere with transcriptional responses of the genes associated with skin wound healing. We aimed to assess the effects of these anesthetics on expression levels of 84 gene transcripts in wound biopsies obtained from the rats on 3rd, 7th, and 14th days, which are characteristic time points of the different phases of wound repair, by using quantitative RT-PCR array with primers specific for genes encoding extracellular matrix components, signal transduction proteins, cellular adhesion molecules, cytoskeleton proteins as well as inflammatory cytokines and chemokines.

Using a fold change of ≥ 5 either up- or down-regulation and p-value significance level of 0.05, we observed significant changes in the expression of a number of wound healing associated genes by the wounds of the rats exposed to K/X anesthesia at doses commonly. Similarly, rat anesthetized with thiopental demonstrated differential gene expression for several of tested genes. Furthermore, the results of the present study clearly show that these anesthetics exerted a different expression for some genes. The present research work demonstrated that the most prominent alterations for differently expressed genes in wound microenvironment due to the administration of K/X or thiopental occurred at the early phase of wound healing. The first three days after injury include the most complicated processes of healing with many influencing factors, involving the participation of multiple cell types and signal transduction pathways. In addition, it is worth noting that most of the differentially expressed genes by both anesthetic agents were down-regulated.

Among differentially expressed genes by K/X anesthesia during a period of 14 days, the most remarkable changes were a 1276–5791-fold decrease in *Itgb1, Rhoa* and *Ptgs2* genes related to cellular adhesion, signal transduction and conversion of arachidonic acid to prostaglandin H2, respectively, an important step in inflammatory process, and a 21–87-fold increase in three growth factor genes (*Fgf2, Fgf7* and *Hbegf*) on the 3rd day.

The genes influenced by thiopental anesthesia were also associated with those involved in numerous biological functions or pathways as well as inflammatory cytokines and chemokines-related genes. Of the genes which showed a remarkable up regulatory pattern, Itgb3 was the highest up-regulated transcript with 1710 fold change that codes a cellular adhesion molecule. The transcript from *Mmp1* gene which codes a remodeling enzyme as other examples exhibited a 240–257-fold increase on days 3 and 14.

Generally, many researchers approve twofold cut off to define differentially expressed genes as it does suffice their purpose. On the other hand, most of the researchers use the double filtering procedure (fivefold cut-off together with $p < 0.05$ level) to reduce the number of false positives. In addition to this, there is an alternative approach the using fivefold for cutoff with the Bonferroni corrected significance level to compensate the multiple testing error. Although some authors have mentioned that this last approach have some disadvantages such as type two errors with in the


a
Extracellular Matrix & Cell Adhesion Genes
K3 K7 K14 T3 T7 T14
Acta2 (A-Sma)
Actc1
CDH1 (E-caderin)
Col1a1
Col1a2
Col3a1
Col4a1
Col4a3
Col5a1
Col5a2
Col5a3
Col14a1
Ctsg
Ctsk
Ctsl1
F13a1
F3 (tissue factor)
Fga (fibrinogen)
Itga1
Itga2
Itga3
Itga4
Itga5
Itga6
Itgav
Itgb1
Itgb3
Itgb5
Itgb6
Mmp1
Mmp2
Mmp7
Mmp9
Plat (tpa)
Plau (upa)
Plaur (upar)
Plg
Rac1
Rhoa
Serpine1 (Pai-1)
Tagln
Timp1
Vtn
-5 0 5
b
Growth Factors
K3 K7 K14 T3 T7 T14
Angpt1
Csf2 (Gm-Csf)
Csf3 (Gcsf)
Ctgf
Egf
Fgf2
Fgf7
Fgf10
Hbegf (Dtr)
Hgf
Igf1
Mif
Pdgfa
Tgfa
Tgfb1
Tnf
Vegfa
c
Signal Transduction
K3 K7 K14 T3 T7 T14
Ctnnb1
Egfr
Il6st (Gp130)
Mapk1 (Erk2)
Mapk3 (Erk1)
Pten
Ptgs2
Stat3
Tgfbr3
Wisp1
Wnt5a
d
Inflammatory Cytokines & Chemokines
K3 K7 K14 T3 T7 T14
Ccl12
Ccl7 (Mcp-3)
Cd40lg (Tnfsf5)
Cxcl1
Cxcl11 (I-tac/Ip-9)
Cxcl3
Cxcl5
Ifng
Il10
Il1b
Il2
Il4
Il6

◂**Fig. 3** Heat maps showing the expression rates of different gene groups associated with **a** extracellular matrix and cell adhesion, **b** growth factors, **c** signal transduction and **d** inflammatory cytokines and chemokines on 3rd, 7th and 14th days of post wounding. Color red shows negative expression and color green shows positive expression. The darker the color, the higher the fold change is. Only differential expression of five or more fold in either direction is shown. *K* ketamine/xylazine, *T* thiopental. (Color figure online)

rejection of some "true positive" responses, it may reduce the likelihood of false positives and may infer big if anything about the biology [20]. Therefore, results were also evaluated with a Bonferroni-corrected p value of 0.0012–0.0042, considering the gene numbers in each functional groups as associated the 84 genes represented in the wound healing PCR array panel.

Even when we used the most stringent criteria (intersection of $p < 0.0012–0.0042$ and > fivefold change), it was observed significant changes in the expression of three genes (*Egf, Col5a1*, and *Cxcl3*) during a period of 14 days in the wounds of the rats exposed to K/X anesthesia. In similar conditions, rat anesthetized with thiopental demonstrated differential gene expression for *Tgfa and Il2*.

Sufficient evidence from previous studies of several independent groups with different experimental designs those are not associated with wound healing demonstrated that administration of anesthetics could affect expression levels of genes related with inflammatory cytokines and chemokines, including *Tgfa, Il1b* and *Il2* [10, 13, 14]. However; there is lack of sufficient evidence for *EGF* (Epidermal Growth Factor), gene encoding EGF, an important factor in early events of the mitogenic cascade, and the collagen, type V, alpha 1 (*Col5a1*) gene, encoding the α1 chain of type V collagen, an important regulator of the assembly of heterotypic fibers.

Our present results may suggest that effects of K/X or thiopental on modulation of gene expression at transcriptional level was not just limited to inflammatory cytokines/chemokines, and neurotransmitter receptors/regulation-related genes, an effect previously documented in studies those are not associated with wound healing. In addition, to our knowledge this is also the first evidence showing that the administration of K/X or thiopental as an anesthetic in skin wound healing experiments may alter expression of wound healing-associated genes in the wound environment.

There are a number of evidences showing that many medications have the capacity of affecting wound healing by modulating gene transcription and interfering clot formation or platelet function or inflammatory responses and cell proliferation (see review) [21]. Considering the

Table 1 Differentially expressed genes on days 3, 7, and 14 in the wounds obtained from rats with ketamine/xylazine anesthesia, compared to wounds obtained from rats without anesthesia

	Ketamine/xylazine								
	Day 3			Day 7			Day 14		
	Gene symbol	Fold change	p value	Gene symbol	Fold change	p value	Gene symbol	Fold change	p value
Extracellular matrix (ECM) and cell adhesion molecules									
Extracellular matrix (ECM) structural constituents	*Col5a1*	−19.27	**<0.0001**[a]						
Extracellular matrix (ECM) remodeling enzymes	*Ctsl*	11.4	0.0045						
	F3	−20.4	0.0053						
	Plaur	−28.2	0.0057						
Cell adhesion molecules	*Itgav*	−54.8	0.0073						
	Itgb1	−1276.9	0.0238						
Cytoskeleton regulators	*Rac1*	−99.9	0.0119						
	Rhoa	−4864.1	0.0066						
Inflammatory cytokines and chemokines	*Il10*	12.3	0.0045	*Cxcl3*	−11.08	**0.0031**[b]	*Cxcl1*	−38.59	0.0050
							Cxcl3	−25.67	**<0.0001**[b]
Growth factors	*Egf*	5.4	**<0.0001**[c]	*Fgf10*	66.3	0.0048	*Tnf*	−30.91	0.0050
	Fgf2	87.3	0.0045						
	Fgf7	55.7	0.0044						
Signal transduction									
Other signal transduction genes	*Ptgs2*	−5791.8	0.0060						

The $2^{-\Delta Ct}$ values obtained from triplicate measurement of the control and ketamine /xylazine anesthesia groups are statistically significant (Student's *t* test) at [a]$p < 0.0011$, [b]$p < 0.0038$, and [c]$p < 0.0029$ levels. The fold change values (given as $2^{-\Delta\Delta Ct}$) are mean of triplicate measurement for each gene

Table 2 Differentially expressed genes on days 3, 7, and 14 in the wounds obtained from rats with thiopental sodium anesthesia, compared to wounds obtained from rats without anesthesia

Thiopental sodium									
	Day 3			Day 7				Day 14	
	Gene symbol	Fold change	p Value	Gene symbol	Fold change	p Value	Gene symbol	Fold change	p Value
Extracellular Matrix (ECM) and cell adhesion molecules									
Extracellular matrix (ECM) structural constituents	*Col5a2*	−18.3	0.0055						
Extracellular matrix (ECM) remodelling enzymes	*Ctsg*	−18.6	0.0055				Mmp1	257.3	0.0043
	Serpine	−10.0	0.0067						
	Ctsl	383.8	0.0045						
	Mmp1	239.9	0.0045						
	F3	−23.7	0.0052						
	Plaur	−14.7	0.0058						
Cell adhesion molecules	*Cdh1*	118.2	0.0046				Itgb3	1710.5	0.0045
	Itga1	−14.1	0.0058						
	Itgb3	−39.3	0.0048						
	Itgb6	−12.8	0.0061						
Inflammatory cytokines and chemokines				*Il10*	−13.0	0.0061	*Il2*	10.8	**0.0001**[a]
							Il10	22.7	0.0052
Growth factors	*Ctgf*	−28.4	0.0206	*Fgf10*	126.7	0.0046			
	Tgfa	134.0	< **0.0001**[b]						
Signal transduction									
TGFβ signaling	*Stat3*	−28.5	0.0051						
WNT signaling							*Ctnnb1*	−10.8	0.0064
Cell surface receptors							*Egfr*	−14.0	0.0058
Other signal transduction genes	*Ptgs2*	−24.9	0.0075						

The $2^{-\Delta Ct}$ values obtained from triplicate measurement of the control and thiopental groups are statistically significant (Student's *t* test) at [a]$p < 0.0038$, and [b]$p < 0.0029$ levels. The fold change values (given as $2^{-\Delta\Delta Ct}$) are mean of triplicate measurement for each gene

Table 3 Effect of ketamine/xylazine and thiopental anesthesia exposure on wound contraction

Group	Percentage wound contraction		
	Day 4	Day 7	Day 14
Control	15.08 ± 1.28	68.97 ± 5.81	83.90 ± 7.89
Ketamine/xylazine	16.07 ± 2.64	62.02 ± 9.74	80.39 ± 9.09
Thiopental	15.28 ± 2.61	61.47 ± 11.26	82.28 ± 8.79

Percentage wound contraction is calculated regarding the wound area created on specified days on each animal. Values are given as mean ± SD

known effects of both anesthetics on gene expression and the results observed, it may be speculated that the different transcription profiles expressed in wound microenvironment in response to K/X or thiopental anesthesia can interfere with one or more phases through all precisely highly programmed phases of the wound, causing improper or impaired healing. Our results show that the process of wound healing followed by wound contraction is not affected by administration of K/X or thiopental which can be concluded as these agents have no effect on wound healing at least in macro scale. However; it is not possible to clearly explain what causes changes in the gene expression levels of various genes in wound microenvironment due to administration of K/X or thiopental and whether these changes cause impaired wound healing at molecular levels with our experimental design or not and the literature published until now is far away from shedding the light on this question. Therefore, additional experiments are necessary to establish the precise mechanism underlying this phenomenon.

The present study necessarily has several limitations. Firstly, we focused on the expression profile of 84 key genes central to the wound healing response. However; other gene(s), along with the genes investigated in this study, may be differentially expressed in the wounds of the rats exposed

to K/X or thiopental anesthesia or other anesthetics taking into consideration that wound healing is a complex process. One clear limitation of this study is the use of topical tramadol, which is an effective analgesic widely used for the relief of acute and chronic pain. Due to ethical and obligatory reasons, the animals were desensitized with tramadol as it has been reported that tramadol does not interfere with collagen synthesis and wound healing [18]. Since there is no extensive data in literature focusing on gene expression related to topical tramadol application per se on the skin, we can not predict whether topical tramadol administration has an effect on the observed results. Unless a new method is developed in order to prevent animals to suffer, medications such as tramadol must be used which may create an additional consideration point while calculating the levels of expression. Another limitation of this study is that we did not evaluate the effects of administration of ketamine alone or xylazine per se on transcriptional responses of the genes associated with skin wound healing, and the individual effects of these anesthetics remain to be seen. This may be a promising subject for future research.

It appears that K/X or thiopental anesthesia may influence the transcriptional responses of some genes associated with wound healing at the wound site of rats and some other genes might be involved in wound healing in addition to inflammatory cytokine and chemokine genes previously mentioned in the literature. This suggests that the use of these anesthetics in animal models of wound healing might be an interference factor for evaluating the changes at the transcriptional levels of wound-expressed genes.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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