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Special article

Dexketoprofen-mediated attenuation of ischemia-reperfusion injury in a rat model of lower limb ischemia

Modulación del daño por isquemia-reperfusión mediado por dexketoprofeno en un modelo de isquemia de extremidades inferiores en rata

Pablo Martínez-Rubio[a,b,1], Ignacio García-Alonso[a,c,1], Amador García Ruiz de Gordejuela[a,d], Daniel Alonso-Alconada[e], Sira Iturrizaga[f], Iñigo Cearra[c,g], Inmaculada Ruiz-Montesinos[a,d], Borja Herrero de la Parte[a,c,*]

[a] *Department of Surgery and Radiology and Physical Medicine, Faculty of Medicine and Nursing, University of the Basque Country UPV/EHU, 48940 Leioa, Spain*
[b] *Department of Medical Oncology, Hospital Universitario Clínico San Carlos, 28040 Madrid, Spain*
[c] *Biocruces Bizkaia Health Research Institute, 48903 Barakaldo, Spain*
[d] *Department of Gastrointestinal Surgery, Donostia University Hospital, Osakidetza Basque Health Service, 20014 Donostia, Spain*
[e] *Department of Cell Biology and Histology, Faculty of Medicine and Nursing, University of the Basque Country UPV/EHU, 48940 Leioa, Spain*
[f] *Department of Clinical Analyses, Galdakao-Usansolo Hospital, 48960 Galdakao, Spain*
[g] *Department of Orthopaedics, Basurto University Hospital, Osakidetza Basque Health Service, ES48013 Bilbao, Spain*

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ABSTRACT

Lower limb ischemia/reperfusion injury (IRI-LL) triggers a systemic inflammatory response after restoring blood flow to an ischemic limb. This study evaluated whether dexketoprofen (DEX) given before reperfusion mitigates IRI-LL. Fifty-six male WAG/RijHsd rats were assigned to one control, 3 vehicles, and 3 DEX-treated groups. After 3 h of hindlimb ischemia, reperfusion lasted 3 h, 24 h, or 14 days. Either DEX (1 mg/kg, po) or saline was administered 30 min before reperfusion. Biochemical markers, limb circumference, and motor performance (rotarod and treadmill) were assessed. DEX significantly reduced serum markers of muscle and hepatic injury but had no effect on renal parameters or edema. Functional recovery improved from day 5 onwards in treated animals. Prophylactic DEX administration effectively attenuated biochemical and functional damage secondary to IRI-LL, supporting its potential as a protective therapy against reperfusion injury.

Palabras clave:
Isquemia-reperfusión de extremidades inferiores
Dexketoprofeno, estudio en animales
Lesión por isquemia-reperfusión

RESUMEN

El síndrome de isquemia-reperfusión de las extremidades inferiores (IRI-LL) desencadena una respuesta inflamatoria sistémica tras la restitución del flujo sanguíneo en un miembro previamente isquémico. Este estudio evaluó si la administración de dexketoprofeno (DEX) antes de la reperfusión mitiga el daño por IRI-LL. Se utilizaron 56 ratas macho WAG/RijHsd distribuidas en un grupo control, tres grupos vehículo y tres tratados con DEX. Tras 3 h de isquemia en la extremidad posterior, la reperfusión se mantuvo durante 3 h, 24 h o 14 días. DEX (1 mg/kg, p.o.) o suero salino se administraron 30 min antes de la reperfusión. Se analizaron marcadores bioquímicos, perímetro del miembro y función motora (rotarod y treadmill). DEX redujo significativamente los marcadores séricos de daño muscular y hepático, sin modificar los parámetros renales ni el edema. Desde el día 5 se observó una mejor recuperación funcional. La administración profiláctica de DEX atenuó eficazmente el daño bioquímico y funcional secundario a la IRI-LL.

* Corresponding author.
E-mail address: borja.herrero@ehu.eus (B. Herrero de la Parte).
[1] These authors contributed equally to this manuscript.

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Introduction

Ischemia-reperfusion injury (IRI) refers to the tissue and systemic damage that occurs after blood flow restoration to previously ischemic tissues. Tissue reoxygenation promotes massive production of reactive oxygen species (ROS) and the activation of inflammatory responses, even exacerbating tissue injury more than ischemia itself.[1–3] Pneumatic tourniquets, commonly used in orthopedic surgery to create a bloodless surgical field, have been associated with several disadvantages, such as neuromuscular and thromboembolic injuries. In addition, their release may trigger lower limb ischemia-reperfusion injury (IRI-LL).[4–6] The failure of aerobic metabolic pathways during periods of ischemia results in the onset of anaerobic metabolism, culminating in the depletion of ATP and the accumulation of lactic acid.[7,8] Concurrently, inflammatory mediators such as IL-1, IL-6, TNF and thromboxane A2 are found to be released, activating the vascular endothelium and leukocytes.[5] Following reperfusion, the activation of neutrophils has been shown to increase the generation of ROS, as well as to enhance capillary permeability. Furthermore, it has been demonstrated that this process triggers intracellular signaling pathways, including NF-κB, MAPK and NRF2, which in turn mediate processes such as apoptosis and oxidative injury.[9]

From a clinical perspective, IRI is characterized by the presence of edema, relative hypovolemia, hemoconcentration, and the release of enzymes such as creatine kinase (CK), lactate dehydrogenase (LDH), or myoglobin.[6,7] It is important to note that vital organs like the lungs, liver and kidneys are especially susceptible to systemic inflammatory and oxidative injury, which can lead to multi-organ failure and coagulopathies.[10,11] Furthermore, prostaglandins and thromboxanes, synthesized by the cyclooxygenase (COX) enzyme, which is also activated by cytokines, contribute to endothelial dysfunction and oxidative stress.[12,13]

Non-pharmacological strategies have demonstrated partial efficacy in reducing IRI-related damage, including regional hypothermia[2,14] and pre- or post-conditioning (pre-CI, post-CI)[15–17] Regarding pharmacological treatment, there is currently no established protocol for IRI management. Nonetheless, various experimental studies have reported promising results with antioxidant drugs, such as vitamins C and E,[18,19] or agents such as allopurinol, curcumin, or folic acid.[8,20–23] In clinical settings, hypertonic mannitol has shown benefits in acute IRI-LL,[24] while other drugs, like statins, have reduced edema and neutrophilic infiltration in models of muscle damage.[25] Some anesthetics, such as propofol or ketamine, have also demonstrated protective effects, likely due to their antioxidant properties.[26–29]

Non-steroidal anti-inflammatory drugs (NSAIDs) are distinguished by their ability to inhibit COX, thereby decreasing the production of pro-inflammatory prostanoids. In addition, evidence has emerged that inhibition of both COX-1 and COX-2 may offer protective effects in various *in vivo* models of renal and mesenteric IRI.[30,31] Dexketoprofen (DEX), a non-selective NSAID with anti-inflammatory, analgesic and antipyretic properties, has been shown to offer better tolerability than its racemic isomer, ketoprofen, and greater analgesic potency compared to drugs such as paracetamol.[32–34] Despite its routine clinical use in the treatment of postoperative pain and acute inflammatory conditions, its effect in the context of IRI remains poorly understood. In a murine model of renal IRS, a significant reduction in oxidative stress (as measured by decreased MDA levels) was observed, although results regarding renal function were mixed, possibly due to vasoconstrictor effects.[35] These findings underscore the necessity for additional research in alternative experimental models of IRI, such as IRI-LL, to elucidate the function of this pharmaceutical agent.

The aim of this study is to evaluate the effect of dexketoprofen on damage induced by IRI in an experimental model of lower limb ischemia in rats.

Material and methods

Fifty-six male WAG/RijHsd rats (4 months old) were used in the study. The animals were maintained under controlled conditions of temperature, humidity and light/dark cycles, with unrestricted access to food and water. The animals were randomly assigned to three groups: a) control (n = 8), rats in the control group were not subjected to any experimental procedure; b) vehicle (n = 24), this group underwent 3 h of right hind limb ischemia followed by reperfusion, and received the equivalent volume of saline; c) DEX (n = 24), the animals were subjected to the same experimental procedure as the vehicle group but received dexketoprofen treatment.

Ischemia was induced using a previously validated custom-made device.[1,8,20] Briefly, under anesthesia (diazepam 15 mg/kg + ketamine 80 mg/kg ip), the animals were placed in a supine position and exsanguination was performed from distal to proximal using a rubber band. A mechanical tourniquet was applied using a nylon loop connected to a dynamometer, which exerted a constant tension of 1 kg. Following the release of the tourniquet, flumazenil (1 mg/kg ip) and fentanyl (0.1 mg/kg sc) were administered to facilitate recovery.

An oral suspension of dexketoprofen (1 mg/kg, Enantyum® 25 mg, Laboratorios Menarini) or saline was administered via flexible 18G orogastric tube 30 min before the end of the ischemia period.

The animals in the vehicle and DEX groups were randomized and sacrificed after 3 h, 24 h or 14 days of reperfusion. Blood samples were collected through the aorta artery at each of these times for serum analysis. The following serum parameters were evaluated: a) renal injury: creatinine, urea; b) muscle injury: CK and LDH; c) cytolysis enzymes: aspartate (AST) and alanine aminotransferase (ALT), and alkaline phosphatase (ALP); and d) electrolytes: Na^+, K^+, Cl^-. All analyses were performed using a Cobas 8000 c702 analyzer (Roche Diagnostics). Additionally, the perimeter of both lower limbs was measured after 24 h, and the gastrocnemius muscle was removed, weighed, and preserved in 4% paraformaldehyde from both legs.

Functional performance over the 14 days following ischemia was assessed using the rotarod and treadmill tests. Evaluations were conducted on days −1, +1, +3, +5, +7, +10, and +14 relative to the ischemia day (day 0). For the rotarod test, the rats were placed in a rotating cylinder (model LE8305), which accelerated progressively from 15 to 25 rpm. The time of fall was recorded. For the treadmill test (model LE8710RTS), the rats underwent 3 consecutive 10-minute sessions of forced walking at 25 rpm, with mild electrical stimulation (0.1 mA) that was applied if they stopped and touched the back of the treadmill. The early endpoints of the test were set at either 20 cumulative seconds or 3 continuous seconds of stimulation. Data were recorded using SeDaCom® software v2.0.03 (all from Harvard Apparatus).

Data were analyzed using GraphPad Prism®. After verifying normality, results were expressed as mean and standard deviation. For comparisons involving 3 or more groups, one-way ANOVA was used followed by Tukey's post hoc test. For functional analyses (rotarod and treadmill), two-way repeated-measures ANOVA was performed, assessing the effects of treatment and time. A P-value $< .05$ was considered statistically significant.

Results

Morphological assessment of the limb after 24 h showed no significant differences between the DEX and vehicle groups in the perimeter of the gastrocnemius muscle of the ischemic limb (63 ± 7.8 vs 73 ± 10 mm, respectively; $P > .05$), nor were any differences observed in the fresh weight of the gastrocnemius muscle.

Functional evaluation revealed marked functional deterioration following the induction of IRI-LL. On days +1 and +3, the treadmill test revealed that the animals in both the vehicle and DEX groups

travelled significantly shorter distances than at baseline (353.5 ± 35.2 m). No significant differences were observed between groups at these early time points. However, by day 5, statistically significant differences were found. The DEX group demonstrated a much faster functional recovery, covering twice the distance of the vehicle group (358.1 ± 45 vs 180.1 ± 49.3 m; $P < .0001$). This difference remained consistent on days 7 and 10, with the DEX group covering around 100 m more than the control group ($P < .01$). On day 14, both groups reached the baseline level of distance covered (Table 1, Fig. 1).

The distance-to-stimulus ratio (the distance travelled, in meters, divided by the number of electric stimuli received during the treadmill test) showed that higher electrical stimulation was needed on days 1 and 3. From day 5 onward, the ratio increased, being significantly higher in the DEX group from day 10 (94.3 ± 68.3 vs 36.1 ± 53.5; $P < .01$), and reaching a maximum difference on day 14 (Table 2, Fig. 2). Analysis of variance revealed that dexketoprofen treatment explained 6.32% of the total variance in distance travelled ($P < .0001$), while the time factor (post-ischemia evolution) accounted for 71.3%, and the treatment-time interaction accounted for 4.6%.

The rotarod test also showed clear improvement in the DEX group. From day 1, the DEX group exhibited greater resistance, with times twice as long as those of the vehicle group, although statistical significance was only reached on day 5 (96.1 ± 36.6 vs 50 ± 32.5 s; $P < .05$), with the DEX group maintaining twice the performance time (Table 3, Fig. 3). This improvement persisted through days 7, 10, and 14, with significantly higher times in the DEX group, reaching a maximum on day 14 (181.7 ± 33.6 vs 135.6 ± 29.8 s; $P < .01$).

Table 1
Distance covered in the treadmill test. Data represent the mean and standard deviation of the distance (in meters) covered during the 30-minute test by animals in the untreated (vehicle) and dexketoprofen-treated (DEX) groups. *P*-values indicate the statistical significance between both groups for each of the 7 time points (ns: $P > .05$; $^{**}P < .01$; $^{****}P < .0001$).

Day	Vehicle	Dex	*P* value
−1	353.5 ± 35.2 m		
1	13.1 ± 24 m	29.9 ± 18.2 m	Ns
3	130.7 ± 52.1 m	197.8 ± 137.1 m	Ns
5	180.1 ± 49.3 m	358.1 ± 45.9 m	****
7	260.5 ± 89.8 m	371.2 ± 48.4 m	**
10	273.5 ± 91.6 m	372.7 ± 63.4 m	**
14	354.7 ± 39.8 m	376.8 ± 47.2 m	Ns

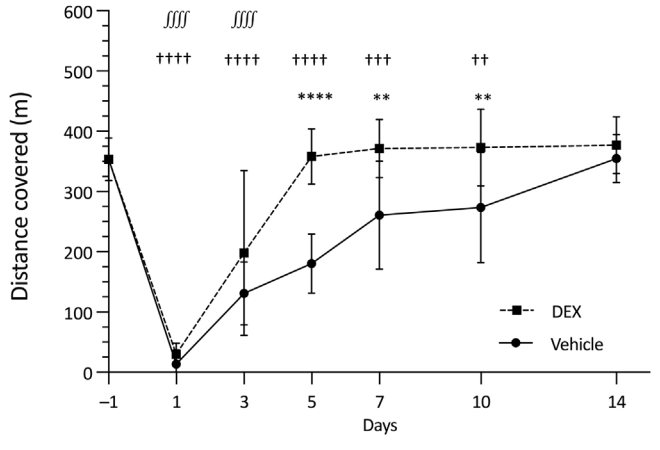

Fig. 1. Total distance travelled (in meters, m) in the treadmill test. The vehicle group is shown as circles with a solid line; the dexketoprofen-treated group (DEX) as squares with a dashed line. Asterisks indicate statistically significant differences between DEX and vehicle ($^{**}P < .01$; $^{****}P < .0001$). The † symbol denotes significance versus baseline within the vehicle group ($^{\dagger\dagger\dagger\dagger}P < .0001$; $^{\dagger\dagger\dagger}P < .001$; $^{\dagger\dagger}P < .01$). The $^{\int}$ symbol indicates significance versus baseline within the DEX group ($^{\int\int\int\int}P < .0001$).

Table 2
Ratio of distance per stimulus in the Treadmill test. Data represent the mean and standard deviation of the meter/stimulus ratio in animals from the untreated (vehicle) and dexketoprofen-treated (DEX) groups. *P* values indicate the statistical significance between the 2 groups on each of the 7 test days (ns: $P > .05$; $^{**}P < .01$; $^{***}P < .001$).

Day	Vehicle	Dex	*P* value
−1	4.6 ± 5.4		
1	0.9 ± 0.5	1.2 ± 1.1	Ns
3	7.4 ± 4.5	8.1 ± 9.9	Ns
5	21.3 ± 25.4	27.4 ± 10.7	Ns
7	30.4 ± 38.8	57.5 ± 61.8	Ns
10	36.1 ± 53.5	94.3 ± 68.3	**
14	78 ± 73.6	180.5 ± 32.4	***

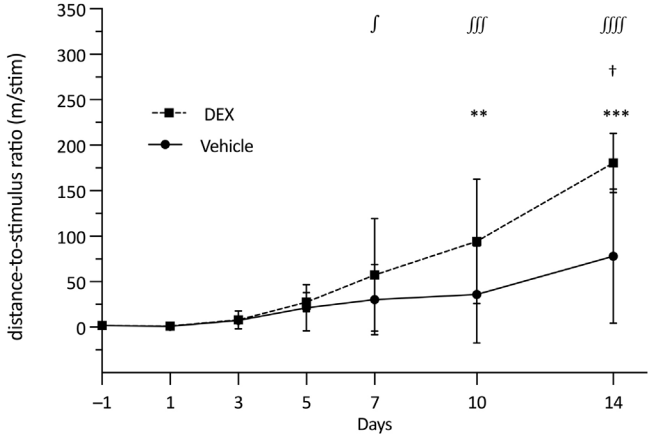

Fig. 2. Ratio between distance travelled and number of electrical stimuli received in the treadmill test (distance-to-stimulus ratio, m/stim). Vehicle: circles with solid line; DEX: squares with dashed line. Asterisks indicate statistically significant differences between DEX and vehicle ($^{**}P < .01$; $^{***}P < .001$). †: significant difference from baseline in the vehicle group ($^{\dagger}P < .05$). $^{\int}$: significant difference from baseline in the DEX group ($^{\int}P < .05$; $^{\int\int\int}P < .001$; $^{\int\int\int\int}P < .0001$).

Table 3
Time on the rod in the Rotarod test. Data represent the mean time in seconds (s) and standard deviation for animals in the untreated (vehicle) and dexketoprofen-treated (DEX) groups. *P* values indicate the statistical significance between the 2 groups on each of the 7 test days (ns: $P > .05$; $^{*}P < .05$; $^{**}P < .01$).

Day	Vehicle	Dex	*P* value
−1	120.6 ± 50.7 s		
1	11.8 ± 6.4 s	24.8 ± 20.3 s	Ns
3	24.7 ± 16.2 s	37.4 ± 27.7 s	Ns
5	50.0 ± 32.5 s	96.1 ± 36.6 s	*
7	96.6 ± 31.3 s	144.7 ± 56.7 s	*
10	118.9 ± 36.9 s	162.7 ± 44.9 s	*
14	135.6 ± 29.8 s	181.7 ± 33.6 s	**

Biochemical markers were assessed 3 h, 24 h, and 14 days after reperfusion to evaluate renal, muscle, and cellular injury as well as electrolyte balance (Table 4, Figs. 4–6). Serum levels of urea and creatinine significantly increased after 3 h of reperfusion in both vehicle and DEX groups, doubling the values observed in control animals. This elevation persisted 24 h later. No significant effects—either protective or detrimental—were attributable to dexketoprofen treatment for these renal markers at any time point. By day 14, levels of both parameters returned to near-baseline values, indicating renal recovery (Fig. 4).

Serum CK and LDH, classical markers of muscle damage, showed significant increases in the vehicle group after ischemia (Fig. 5). In the

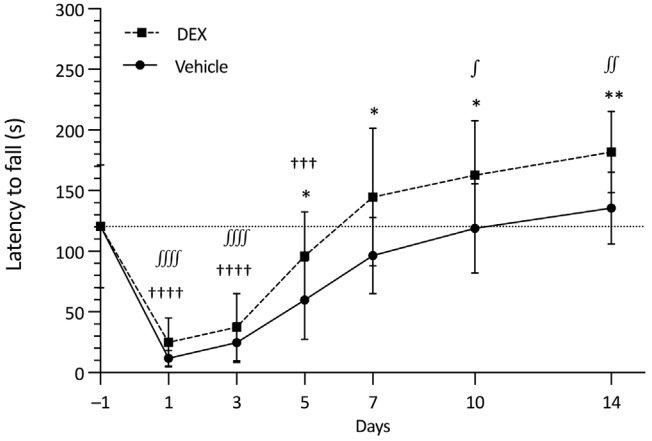

Fig. 3. Time to fall (in seconds) in the rotarod test. Baseline: dotted line; vehicle group: circles with solid line; DEX group: squares with dashed line. Asterisks indicate statistically significant differences between DEX and vehicle (*$P < .05$; **$P < .01$). †: significant difference from baseline in the vehicle group (†††$P < .001$; ††††$P < .0001$). ∫: significant difference from baseline in the DEX group (∫$P < .05$; ∫∫$P < .01$; ∫∫∫∫$P < .0001$).

DEX group, CK levels were consistently lower throughout the study, with significant reductions at 24 h ($P < .001$) and on day 14 ($P < 0.05$). LDH values also decreased with treatment, reaching significance at the same time points, although levels did not return to control values.

Finally, AST and ALT followed a similar pattern, with significant increases after IRI-LL that were markedly attenuated in the DEX group (Figs. 6A and 6 B). After 3 h of reperfusion, AST levels were lower in the DEX group compared to vehicle (671 ± 122 IU/L vs 1129 ± 137 IU/L; $P < .0001$). Similarly, ALT levels were reduced in the DEX group (69 ± 14 IU/L vs 92 ± 8.7 IU/L; $P < .01$). By day 14, both enzymes had returned to near-baseline levels in both groups, suggesting resolution of cytolytic injury. Conversely, ALP declined significantly across all groups over time, reaching a 30% reduction from baseline at day 14, with no differences attributable to dexketoprofen (Fig. 6C). Serum sodium, potassium, and chloride remained stable throughout the study and did not differ significantly between groups (Figs. 6D–F).

Discussion

Improved living conditions and healthcare access have markedly increased life expectancy in Western countries.[36] In Spain, average life expectancy in 2020 was around 85.2 years for women and 79.6 years for men,[37] which is expected to exceed 87.7 and 83.2 years, respectively, by 2035.[36] This increase in longevity is accompanied by a higher prevalence of chronic diseases, mental health conditions, and physical dependence, limiting the number of years lived in optimal health.[37] Therefore, maintaining healthy life expectancy is crucial, as it accounts for approximately 95.7% of total life expectancy.[38] The ageing population and improved survival after acute illnesses have led to an increased demand for surgical interventions. Successful surgical outcomes in this population not

Table 4
Biochemical markers measured in serum samples from the control group and from vehicle- and dexketoprofen-treated (DEX) groups. The table shows the mean and standard deviation values of serum samples collected 3 h, 24 h, and 14 days after the ischemic period. Results are expressed in international units per liter (IU/L) for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and cholinesterase; in milligrams per deciliter (mg/dL) for glucose, cholesterol, and total bilirubin (TBil); and in grams per deciliter (g/dL) for total protein and albumin. Data are presented as mean ± standard deviation.

	Control	Vehicle 3 h	DEX 3 h	Vehicle 24 h	DEX 24 h	Vehicle 14 D	DEX 14 D
Urea (mg/dL)	29 ± 3.1	65 ± 8.9	66 ± 7.7	53 ± 15	51 ± 16	36 ± 2.7	34 ± 2.9
Creatinine (mg/dL)	0.36 ± 0.03	0.78 ± 0.26	0.72 ± 0.16	0.86 ± 0.21	0.73 ± 0.05	0.46 ± 0.04	0.38 ± 0.03
CK (UI/L)	86 ± 4.7	7694 ± 828	6739 ± 1702	5193 ± 798	2536 ± 842	574 ± 189	296 ± 96
LDH (UI/L)	53 ± 11	1570 ± 249	1342 ± 218	1246 ± 184	950 ± 117	897 ± 202	488 ± 44
AST (UI/L)	52 ± 1.2	1129 ± 137	671 ± 122	467 ± 93	378 ± 64	82 ± 13	56 ± 7.1
ALT (UI/L)	41 ± 1.4	92 ± 8.7	69 ± 14	67 ± 12	64 ± 12	39 ± 4.2	31 ± 4.4
ALP (UI/L)	124 ± 3.4	101 ± 10	98 ± 11	99 ± 12	100 ± 6.5	87 ± 9.3	86 ± 5.8
Na^+ (mEq/L)	143 ± 5.0	144 ± 2.8	144 ± 5.1	143 ± 3.8	143 ± 3.3	143 ± 2.6	145 ± 3.8
K^+ (mEq/L)	3.9 ± 0.22	4.1 ± 0.25	4.2 ± 0.24	4.0 ± 0.28	4.1 ± 0.39	4.1 ± 0.27	4.0 ± 0.31
Cl^- (mEq/L)	104 ± 2.5	102 ± 2.3	104 ± 6.4	107 ± 6.1	105 ± 3.0	106 ± 1.8	104 ± 1.9

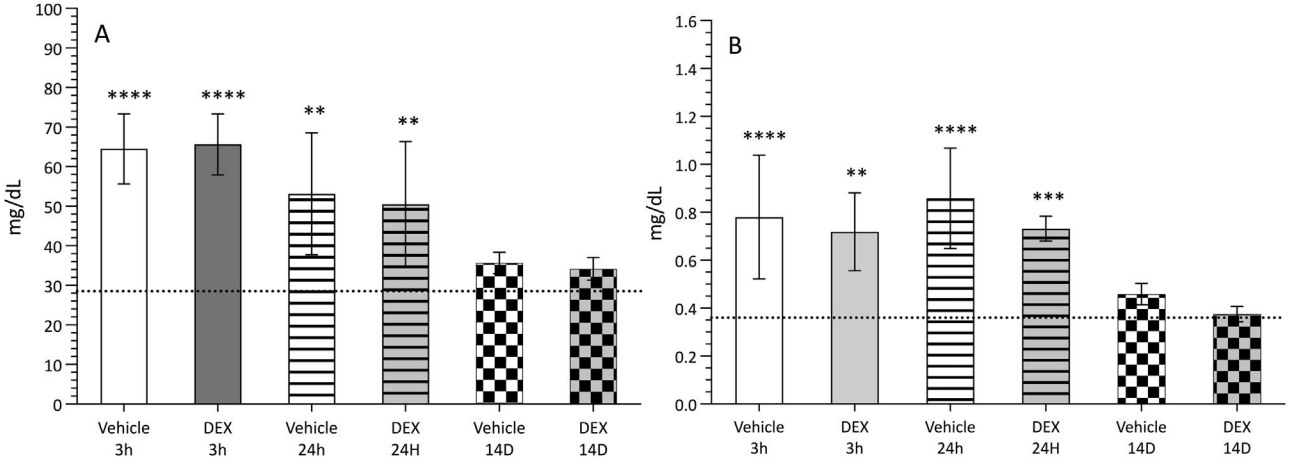

Fig. 4. Serum levels of urea (A) and creatinine (B) expressed in mg/dL. Measurements were taken at 3 h (solid bars), 24 h (striped bars), and 14 days (checkered bars) post-ischemia in control animals (dotted line), vehicle-treated (white bars), and DEX-treated rats (grey bars). Asterisks indicate differences versus control (*$P < .01$; **$P < .001$; ***$P < .0001$); hashtags indicate differences between vehicle and DEX groups ($^{\#\#}P < .01$; $^{\#\#\#}P < .001$).

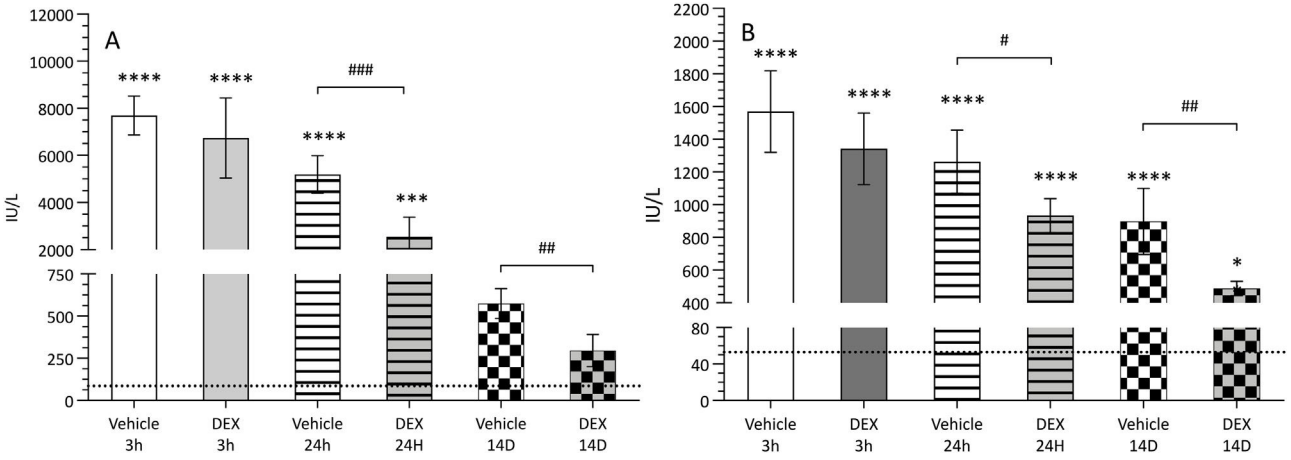

Fig. 5. Serum levels of creatine kinase (CK, A) and lactate dehydrogenase (LDH, B) expressed in IU/L. Values from control (dotted line), vehicle (white bars), and DEX groups (grey bars) at 3 h, 24 h, and 14 days after ischemia. Asterisks indicate differences versus control (**$P < .01$; ***$P < .001$; ****$P < .0001$); hashtags show differences between treatment groups ($^{\#}P < .05$; $^{\#\#}P < .01$; $^{\#\#\#}P < .001$).

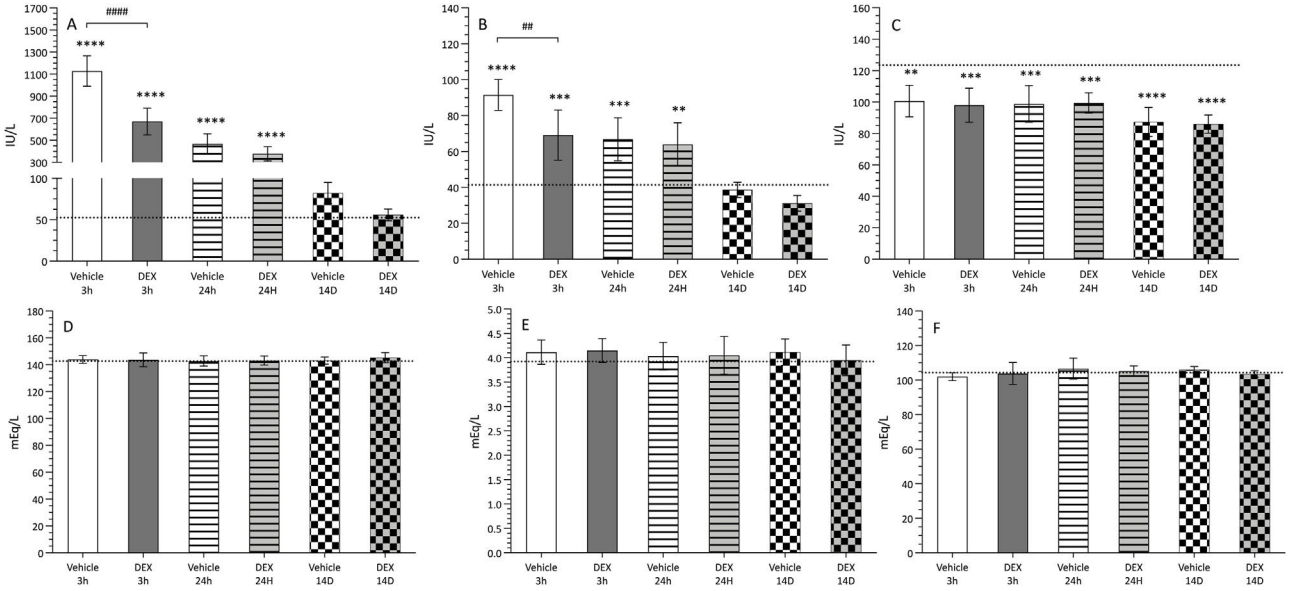

Fig. 6. Serum levels of AST (A), ALT (B), ALP (C), sodium (Na^+, D), potassium (K^+, E), and chloride (Cl^-, F). Enzymes are expressed in IU/L; electrolytes in mEq/L. Data were obtained at 3 h (solid bars), 24 h (striped bars), and 14 days (checkered bars) from control (dotted line), vehicle (white bars), and DEX (grey bars) groups. Asterisks indicate differences versus control (**$P < .01$; ***$P < .001$; ****$P < .0001$); hashtags indicate intergroup differences ($^{\#\#}P < .01$; $^{\#\#\#\#}P < .0001$).

only depend on proper anatomical reduction of fractures or avoidance of complications, but also on restoring functional and social status. In this context, the use of tourniquets has become common practice to minimize bleeding and enhance surgical field visibility. However, their use carries significant risks, including nerve, vascular, and muscle damage, as well as postoperative complications such as stiffness and inflammation.[39]

We aimed to replicate these conditions using a standardized IRI-LL model as described by Cearra et al.,[6] which closely resembles human anatomical and biochemical responses. Unlike other models using simple devices like elastic bands to induce ischemia,[26,40,41] our system allows for precise pressure control, avoiding excessive mechanical injury, as previously demonstrated by Gersoff[42] and Saunders in surgical patients.[43] In our experiment, the applied tourniquet pressure was 1 kg, which is sufficient to exceed the mean arterial pressure in rats and ensure homogeneous ischemia, while minimizing variability and preventing pressure-induced injury. This strict control is essential to accurately interpret the effects of prophylactic treatments such as dexketoprofen. Functional recovery is likely the most clinically relevant endpoint, as tourniquet-induced functional impairment negatively impacts postoperative outcomes. Various techniques have been used to assess muscle injury after IRI-LL, including electromyography and functional tests.[25,44] In our study, motor ability and endurance were evaluated using treadmill and rotarod tests—validated tools in models of cerebral and myocardial ischemia[45–49] as well as locomotor disorders.[50–53]

Compared to previous studies showing early functional improvement (by day 3) with folic acid treatment,[8,20] dexketoprofen demonstrated significant recovery starting on day 5 and surpassing baseline performance by day 7. This indicates a more effective functional recovery than with other treatments, albeit with a slightly delayed onset. The improved distance/stimulation ratio in the DEX group also suggests enhanced exercise capacity and greater motor efficiency, requiring fewer stimuli to cover longer distances. Similarly, rotarod performance, which measures coordination and endurance,[51,53] confirmed better neuromuscular recovery in dexketoprofen-treated animals. Some methodological limitations must be acknowledged, particularly regarding preconditioning with function-

al devices. The high variability observed in the distance/stimulation ratio on days -1 and +1 may reflect insufficient familiarization with the treadmill—an issue previously described by Castro.[54] Future studies should include standardized training protocols before ischemia to improve reproducibility and test sensitivity.

Regarding renal injury markers (urea and creatinine), our study found no evidence of dexketoprofen-mediated protection within the first 3 h after reperfusion, in contrast to the findings of Cearra,[8] who reported a significant reduction with folic acid. Nonetheless, by day 14, both markers were normalized in all groups, suggesting that acute renal injury may be reversible over time even without pharmacologic intervention. These findings confirm the presence of acute kidney damage associated with the ischemia–reperfusion process.[55]

Previous evidence from a renal IRI model[35] suggests that dexketoprofen reduced systemic oxidative stress but worsened renal injury, indicating potential nephrotoxicity. This aligns with known adverse effects of other non-selective NSAIDs like diclofenac, which increase urea levels.[56] Simultaneous COX-1 and COX-2 inhibition—part of the mechanism of these drugs—may adversely affect renal hemodynamics, as COX-1 is essential for glomerular blood flow regulation and COX-2 for renal excretion.[57,58] This potential nephrotoxicity must be considered when evaluating dexketoprofen as a prophylactic agent in IRI-LL. Selective COX-2 inhibitors such as etoricoxib have shown benefits in animal models of renal I/R injury, likely due to COX-1 preservation and reduced gastrointestinal and renal side effects.[59] However, prolonged treatment with these agents may induce adverse cardiovascular effects.[8,60] Our model involved a single prophylactic dose, which is unlikely to induce such effects, but this should be confirmed in future studies.

Regarding liver injury markers, dexketoprofen showed a significant protective effect only during the first 3 h post-reperfusion, reducing AST and ALT by 40% and 25%, respectively. This pattern resembles the results observed with folic acid[8] and other antioxidant agents, such as melatonin or hydrogen sulfide, which have also been shown to attenuate early hepatic injury in IRI models.[61–63] These findings underscore the importance of the early reperfusion phase as a key therapeutic window for hepatoprotection, regardless of the agent used. In terms of muscle injury, our results indicate a clearly protective effect of dexketoprofen. Serum levels of CK and LDH were significantly reduced at 24 h and 14 days post-ischemia. These markers are widely used and clinically relevant for assessing muscle injury, although they are less specific than oxidative stress indicators like MDA.[26–29] Other studies have shown faster recovery with antioxidant agents like folic acid,[8,20] possibly due to differences in pharmacokinetics and mechanisms of action.

Tissue edema, assessed by the perimeter of the ischemic limb, showed no statistically significant differences between groups. These findings suggest that, in the short term, dexketoprofen did not prevent inflammation or detectable muscle volume loss at this time point. However, a trend toward reduced swelling in the DEX group may reflect a mild anti-inflammatory effect, as also reported for folic acid and simvastatin in similar models.[8,20,64]

Conclusion

Dexketoprofen improved functional recovery and reduced muscle and liver damage in a rat model of IRI-LL. However, its lack of renal protection and potential nephrotoxicity mean it should be used with caution in clinical practice.

Informed consent statement

Not applicable.

Institutional review board statement

All animal experiments were conducted in accordance with EU Directive 2010/63/EU for animal experiments and were approved by the institutional Review Board (M20/2022/013).

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Data availability

Data is contained within the article.

Declaration of competing interest

The authors declare no conflict of interest.

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