The role of intramuscular injection of nonsteroidal anti-inflammatory drug in development and severity of deep soft tissue infection in mice

Zeynep Ture [1] Hayati Demiraslan [1], Olgun Kontas [2], Emine Alp [1], Mehmet Doganay [1]
[1]Erciyes University; Infectious Diseases and Clinical Microbiology, Kayseri, Turkey
[2] Erciyes University; Pathology, Kayseri, Turkey

Background: Soft tissue infection (STI) is a serious problem after im injection. Recent our clinical observation showed that NSAID injection increased the severity of STI and lead to severe sepsis. Our hypothesis is that NSAID injection may have a role on the severity of local infection and spreading of STI. For this reason, STI model in mouse with Group A streptococcus subtype M3(GASM3) was carried out.

Material/methods: Balb-c female mice were used in experimentation. Inoculum dose was estimated as 10^6 cfu /0.1 ml and injected into gluteal muscle. Diclofenac sodium (DS) was choiced and the dose was 20 mg/kg. In-vitro effect of DS on GAS was determined by time-kill studies. The study groups and design are seen in Table 1.

Table 1. Study design and work scheme

Times	- 48th hour	-24th hour	Zero time	24th hour	48th hour	96th hour
Group 1	DS	DS	DS+GASM3	S	S	Assess
Group 2	S	S	GASM3+DS	DS	DS	Assess
Group 3	S	S	DS	DS	DS	Assess
Group 4	S	S	GASM3	S	S	Assess

DS; diclofenac sodium, S; serum sale, GASM3; Group A streptococcus subtype

Two times, at 48 and 24 hours before bacterial inoculation, DS was injected in group 1, and serum physiologic was injected for the others. After GAS inoculation, mouse was observed for 96 hours and than sacrificed. Blood samples were taken for estimate the level of TNF-alpha and interleukin-6. Cytokine studies were performed by using Culture was performed from heart and lung. Injected right leg muscle was extracted.

Quantitative culture and histopathological examination were performed. Sepsis was defined if bacterial growth was seen at least in two organs. For the comparison of histopathological findings, an inflammation score was used as from 0 to 4 (Table 2).

Table 2: Inflammation grading score in the muscle tissue

Score	Histopathological findings
0	Normal skeletal muscle tissue
1	Skeletal muscle tissue with mild edema and sparse inflammatory cells
2	Skeletal muscle tissue including marked inflammatory cell and sparse septal infiltration
3	Intensive infiltration of inflammatory cells
4	Intensive infiltration of inflammatory cells, multinucleated giant cell formation and skeletal muscle tissue including necrosis

Cytokine Analysis: The plasma samples stored at -20 ° C were studied IL-6 and TNF alpha according to the kit procedure by using Enzyme-linked immunosorbent assay (ELISA) method (Boster, Mouse TNF alpha PicoKine ™ ELISA Kit EK0527, and Mouse IL-6 PicoKine ™ ELISA Kit EK0411) in Histology Lab, Erciyes University. The microplates were read at 450nm by using ELISA reader (Tecan, Sunrise™)

Results: In time-kill study, high concentration (40 mg/L) of DS inhibited the bacterial growth until 24h. However, lower concentration (0.4 mg/L) of DS did not affect (Figure 1).

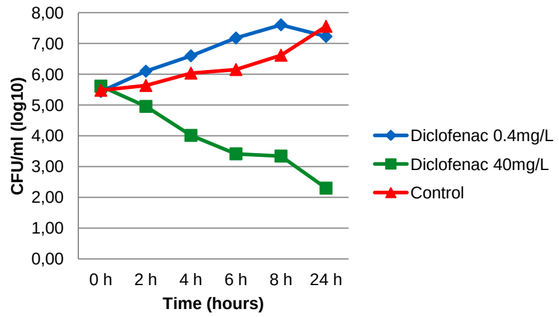

Figure 1: The time-kill effect in the different concentrations of diclofenac on *S. pyogenes*

Sepsis was observed only in groups 1 and 2 that were including DS injection. In regard to histologic examination, the highest inflammation in the muscle tissue was observed in the group 2. Groups 1 and 2 had a higher inflammation score than group 3 and 4 but not significant (Table 3). Group 4 had statistically significant higher bacterial load than other groups (p= 0.001).

Table 3. Results of the study

Groups	Sepsis rate, (%)	Bacterial load, cfu/g, (mean±SD)	Inflammation score, (mean±SD)
Group 1	36	1.28±1.80	3.60±0.89
Group 2	10	1.40±1.21	3.80±0.42
Group 3	0	0	3.50±0.83
Group 4	0	*5.56±1.53	3.30±1.05

* Statisticallly significant

The mean level of TNF-alpha in the group 1, 2 and 3 was higher than those in group 4. But only, group 1 and 3 had higher level of IL-6 than group 3. But differences were not significant between the all groups (Figure 2).

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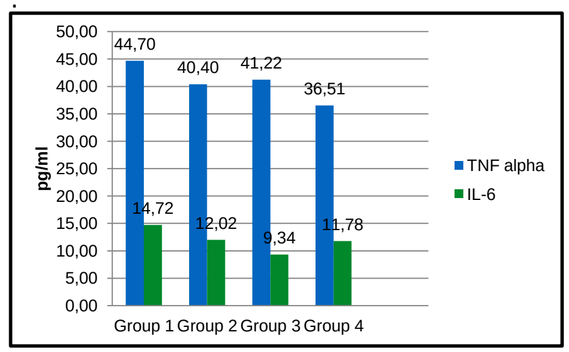

Figure 2. TNF alpha and IL-6 level in serum of study groups

Conclusion: Intramuscular injection of DS may cause the higher level inflammation and necrosis in the muscle tissue. DS injection before/after the inoculation of bacteria leads to reduce bacterial load in the muscle tissue at 96th hours. However, the sepsis rate increases in those groups. Before inoculation of the bacteria, DS injection for two days leads higher cytokine levels.

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