Arthrex® IRAP II System

Arthrex Vet Systems
Arthrex Research and Development

Introduction

Two studies were performed comparing the newly designed Arthrex IRAP™ II to the IRAP syringe system. Study one examined the levels of two pro-inflammatory cytokines (IL-1β and TNF-α) and two anti-inflammatory cytokines (IL-1ra and IL-10) in human whole blood. Study two determined the levels of Fibroblast Growth Factor-b (FGF-b), Platelet Derived Growth Factor-AB (PDGF-AB), Transforming Growth Factor-β (TGF-β), and Vascular Endothelial Growth Factor (VEGF) using the IRAP syringe and Arthrex IRAP II device.

Study 1: Blood Incubation and ELISA

Blood was collected in four separate containers per donor (n=8): 2x 10 mL glass vacutainers, a 60 mL IRAP syringe (Orthogen, Düsseldorf, Germany), and a 50 mL Arthrex IRAP II device (Arthrex Vet Systems, Naples, FL). A vacutainer luer adapter connected to the butterfly cannula so the same needle/tubing could be used for all groups. Approximately 10 mL of blood was collected into each vacutainer, 50 mL of blood was collected into the large IRAP syringe, and then approximately 40 mL of blood was collected into the Arthrex IRAP II device. All groups were inverted ten times following collection and the IRAP syringe and Arthrex IRAP II device were placed into an incubator at 37ºC.

The blood samples were spun down in a Hermle Z300 centrifuge at 4000 rpm for 10 minutes. Baseline vacutainers were centrifuged approximately 15 minutes after it had been drawn, while the IRAP and IRAP II were centrifuged following a 24 hour incubation period at 37°C. After centrifugation, each donor's serum was removed from the container using a 5 mL polypropylene syringe and an 18 gauge needle. The serum was then filtered with 0.22 μm filters, aliquotted into 5 mL cryovials, and stored at approximately -80°C.

Serum samples were thawed to room temperature and then tested according to the Quantikine ELISA kit protocols (R&D Systems, Minneapolis, MN). The plate wells were washed using a Bio-Tek ELx50 microplate strip washer, and absorbance of each sample was determined using a Bio-Tek ELx808 absorbance microplate reader. Concentrations were calculated using KC Junior by converting absorbance to concentration based on a standard curve of optical density versus concentration. The mean values, range, and standard deviation of n=8 donors were calculated for TNF-α, IL-10, IL-1β, and IL-1ra. For statistical evaluation, one way repeated measure ANOVAs were used with post hoc multiple comparisons using the Holm-Sidak method. A significance level of 0.05 was used for all analyses. Figure 1, Figure 2, and Table 1 depict all cytokine mean levels.

TNF-α: IRAP II levels were not significantly different compared to baseline and IRAP ($p=0.186$, $P=0.712$ and $p=0.063$, $P=0.712$), respectively). Higher levels associated with IRAP were significantly different than baseline levels ($p=0.004$).

IL-10: Higher levels in IRAP II were significantly different than IRAP and baseline levels ($p<0.001$ and $p=0.015$, respectively). IRAP was not significantly different from baseline levels ($p=0.065$, $P=0.970$).

IL-1β: Higher levels in IRAP II were significantly different than baseline levels ($p=0.004$). IRAP II was not significantly different than IRAP ($p=0.077$, $P=0.730$), and IRAP was not significantly different from baseline levels ($p=0.138$, $P=0.730$).

IL-1ra: Higher levels in IRAP II were significantly different compared to both baseline levels ($p<0.001$) and IRAP levels ($p<0.001$). IRAP was not significantly different from baseline levels ($p=0.371$, $P=1.000$).

Figure 1:
Baseline, IRAP, and IRAP II levels of TNF-α, IL-10 and IL-1β (* denotes significant difference)

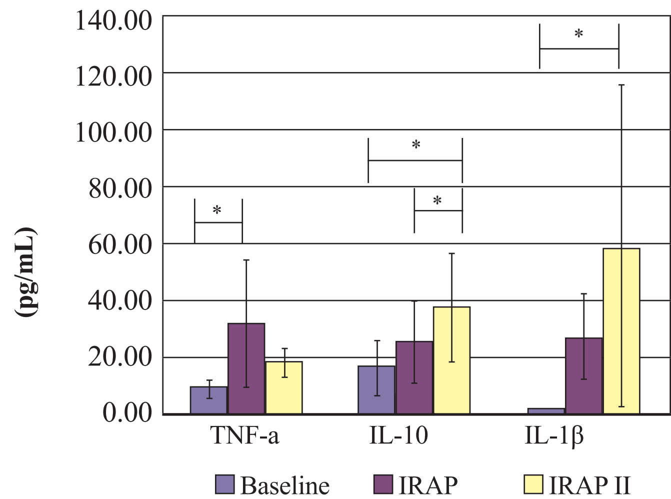


Figure 2:
Baseline, IRAP and IRAP II levels of IL-1ra
(* denotes significant difference)

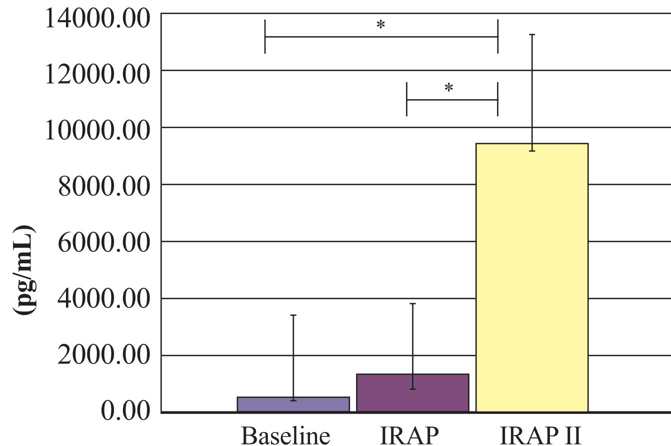
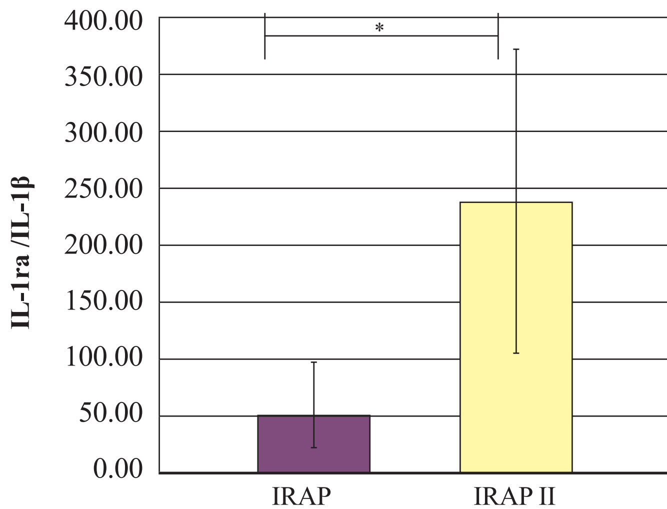


Table 1:
Cytokine production of the IRAP™ II and IRAP Systems

(pg/mL)	TNF alpha	IL-10	IL-1beta	IL-1ra
Baseline	9.5 ± 2.9	16.9 ± 9.5	1.8 ± 0.3	355 ± 326
IRAP	32.3 ± 22.7	25.7 ± 14.3	27.5 ± 15.2	1233 ± 973
IRAP II	18.8 ± 4.9	37.8 ± 19.0	58.8 ± 56.7	9688 ± 3382

IL-1ra /IL-1β ratios are illustrated in Figure 3. A t-test (α=0.05) was performed and the higher ratio produced by Arthrex® IRAP II is significantly different than IRAP (p=0.002).

Figure 3:
IRAP and IRAP II IL-1ra /IL-1β ratios
(* denotes significant difference)



Study 2: Blood Incubation and ELISA

Identical methods were used for serum isolation and ELISA analysis for FGF-b, PDGF-AB, TGF-β, and VEGF (n=24). Table 2 illustrates the growth factor levels and Table 3 depicts the increased levels of growth factors compared to baseline levels.

Table 2:
Growth Factor production of the IRAP II and IRAP Systems

(pg/mL)	FGF-b	PDGF-AB	TGF-β	VEGF
Baseline	1.4 ± 1.2	123.3 ± 97.0	7.0 ± 4.7	16.3 ± 10.8
IRAP II	11.5 ± 4.6	680.3 ± 398.5	30.1 ± 11.4	41.1 ± 8.0
IRAP	11.9 ± 4.6	839.2 ± 421.5	29.9 ± 10.4	46.8 ± 9.4

Table 3:
Increase in concentration of growth factors produced by the IRAP II and IRAP systems over baseline

	IRAP II	IRAP
FGF Increase	8.4x	8.1x
PDGF-AB Increase	4.3x	4.3x
TGF-β Increase	2.9x	2.5x
VEGF Increase	6.8x	5.5x

FGF-b: IRAP II levels were not significantly different compared to IRAP (p=0.631, P=1.000). Higher levels associated with IRAP II and IRAP were significantly different than baseline levels (p<0.001 for both).

PDGF-AB: IRAP II levels were not significantly different compared to IRAP (p=0.955, P=1.000). Higher levels associated with IRAP II and IRAP were significantly different than baseline levels (p<0.001 for both).

TGF-β: Higher levels associated with IRAP II were significantly different compared to IRAP and baseline (p=0.028 and p<0.001, respectively). Higher IRAP levels were significantly different than baseline levels (p<0.001).

VEGF: Higher levels associated with IRAP II were significantly different compared to IRAP and baseline (p=0.036 and p<0.001, respectively). Higher IRAP levels were significantly different than baseline levels (p<0.001).

Conclusion

In vitro incubation of human whole blood at body temperature for 24 hours using the IRAP II system induces production of anti- and pro-inflammatory cytokines. IL-10, IL-1β and TNF-α levels in the serum of the Arthrex IRAP II system were not different compared to the current IRAP. However, the Arthrex IRAP II produces higher levels of IL-1ra when compared to the IRAP. The higher levels of IL-1ra associated with the IRAP II also led to a higher ratio of IL-1ra /IL-1β. Compared to baseline measures, the IRAP II also induced significantly higher levels of FGF-b, PDGF, TGF-β, and VEGF. Since the serum produced by IRAP II contains a concentrated combination of biologically relevant autologous GFs, it can be an attractive option in treatment for tissue injury.

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