PREMATURITY

Abstracts 9 – 17

Moderators: Catalin Buhimschi, MD; Edward McCabe, MD, Medical Director, March of Dimes

9 The effect of simvastatin on infection induced inflammatory response of human fetal membranes

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OBJECTIVE: The inflammatory response to infections is a major factor associated with spontaneous preterm birth (PTB) and perinatal morbidity. Using an in vitro fetal membrane organ explant system we tested if simvastatin (sim), a lipophilic statin with strong anti-inflammatory properties, regulates the inflammatory response to lipopolysaccharide (LPS).

STUDY DESIGN: Normal term human fetal membrane (n = 11) explants were divided into 6 treatment groups: control, LPS (100ng/ml), sim (125ng/ml), sim given 6 hours prior to LPS (S-L), sim given 6 hours post LPS (L-S), and sim and LPS given simultaneously (L+S) and incubated for 24 hours. Multiplex ELISA for IL-1β, IL-1 receptor antagonist (IL-1ra), IL-6, soluble IL-6 receptor (sIL-6R), IL-10, TNF-α and soluble TNF receptors (sTNF R1 and R2) was performed in tissue culture supernatants. Differences between individual treatments and untreated controls were evaluated by comparison of least squares means estimates. Mechanistic properties of sim in (L+S) group were documented by Western blot analysis of membranes for NF-κB.

RESULTS: LPS stimulation increased cytokines compared to controls ($p = 0.01$), confirming membrane immune reactivity. Regulation of cytokine production by sim in LPS-stimulated membranes was specific and limited to pro-inflammatory cytokines. S-L reduced IL-1ra ($p = 0.0005$), IL-6 ($p = 0.02$) and TNF-α ($p = 0.02$); L-S reduced IL-1β ($p = 0.02$) and TNF-α ($p = 0.04$) while LS only reduced IL-1ra ($p = 0.03$). Compared to controls, LPS increased the TNF-α/sTNF R1 or R2 molar ratio favoring increased tissue bioavailability of TNF-α ($p = 0.0001$) while it was restored by L-S ($p = 0.01$), S-L ($p = 0.01$) and L+S ($p = 0.05$) compared to LPS (Table). Sim blocked the LPS induced phosphorylation of NF-κB/p65 subunit but did not inhibit IκB-α proteolysis.

CONCLUSION: Simvastatin treatment down-regulated LPS induced inflammatory response and restored homeostasis between pro and antiinflammatory agents, by controlling NF-κB activation. Simvastatin may reduce the fetal inflammatory response in PTB.

Cytokines and their soluble receptor concentrations (pg/ml) in various study groups

	Control	LPS	Sim	L-S	S-L	L+S
IL-1β	2.72 ± 1.79	9.15 ± 1.86 [a]	2.88 ± 1.83	5.84 ± 1.83 [b]	4.41 ± 1.87	6.53 ± 1.91
IL-1ra	16 ± 10.98	105.81 ± 11.54 [a]	21.44 ± 10.98	70.60 ± 11.55	44.70 ± 11 [b]	63.88 ± 11.54 [b]
IL-6	2812.98 ± 426.1	4321.23 ± 426.1 [a]	2985.45 ± 444.36	3898.24 ± 426.1	3181.63 ± 409.68 [b]	3780.98 ± 409.68
sIL-6R	50.68 ± 9.33	39.29 ± 9.60	46.83 ± 8.94	31.09 ± 8.94	31.73 ± 9.35	36.83 ± 9.35
IL-6/sIL-6R	295.53 ± 182.08	444.12 ± 189.14 [a]	277.68 ± 176.15	578.09 ± 171.51	388.94 ± 176.3	453.56 ± 176.3
IL-10	192.18 ± 149.44	687.52 ± 139.35 [a]	117.42 ± 139.35	383.33 ± 144.15	354.48 ± 149.65	667.75 ± 149.28
TNF-α	23.51 ± 33.89	276.23 ± 37.81 [a]	30.31 ± 33.88	125.05 ± 40.35 [b]	139.57 ± 37.79 [b]	144.79 ± 40.31
sTNF R1	404.58 ± 47.52	461.01 ± 47.52	445.82 ± 49.02	482.64 ± 47.52	502.74 ± 47.52	493.05 ± 47.52
sTNF R2	599.01 ± 191.87	1100.83 ± 198.1 [a]	656.89 ± 192.02	1289.77 ± 192.02	1196.72 ± 186.86	1503.53 ± 186.86 [b]
TNF-α / sTNF R1	0.20 ± 0.50	2.79 ± 0.56 [a]	0.25 ± 0.53	0.75 ± 0.60 [b]	0.95 ± 0.56 [b]	0.91 ± 0.60 [b]
TNF-α / sTNF R2	0.21 ± 0.48	2.40 ± 0.50 [a]	0.49 ± 0.48	0.42 ± 0.54 [b]	0.50 ± 0.50 [b]	0.38 ± 0.54 [b]

Data expressed as least square means (LSM) ± SE.

LSM, least square means concentration in picogram/milliliter; *SE*, standard error.

[a] When LPS was significantly different from control ($p<0.05$); b: When either L-S, S-L or L+S was significantly different from LPS ($p<0.05$); Sim alone was not significantly different from control for any analyte tested.

10 Is thrombin activation predictive of preterm delivery?

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OBJECTIVE: It has been suggested that increased thrombin generation (measured by thrombin-antithrombin complexes, TAT) may share in the pathogenic pathways to preterm delivery (PTD). We aimed to determine the relation between TAT complexes in amniotic fluid early in pregnancy and subsequent PTD, and to evaluate TAT complexes as predictor of PTD.

STUDY DESIGN: Prospective cohort of 680 singleton pregnancies undergoing 2nd trimester amniocentesis. Participants were followed to delivery with standardized evaluation of outcome. Primary outcome: PTD. Amniotic fluid TAT levels were determined by ELISA. Continuous and categorical (quartiles) levels were compared between women who delivered preterm and those who did not. Binary logistic regression was used to control for confounders. ROC analysis was applied to determine a discriminatory cutoff level for TAT complexes.

RESULTS: TAT concentration was significantly higher in women who subsequently delivered preterm (median 98.9 mcg/l, IQR 49.6-181.4) than in those who did not (median 66.2 mcg/l, IQR 39.5-122.2; $P<.001$). This difference persisted when 55 spontaneous PTD's (median 87.6 mcg/l, IQR 40.3-177.5) and 34 indicated PTD's (median