RANTES/CCL5 Might Mediate Long-Term Renal Injury Resulting From Diabetic Ketoacidosis (DKA)

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Hypothesis

Acute kidney injury (AKI) occurs commonly during diabetic ketoacidosis (DKA) in children. We hypothesize that AKI during DKA is triggered by the hyperinflammatory response that occurs during DKA and involves pathophysiology that is similar multiple organ dysfunction syndrome (MODS) occurring during sepsis or trauma.

Background

Brain injury is the most serious and most extensively studied complication of pediatric DKA. Life-threatening brain injury occurs in 0.5-1% of pediatric DKA episodes (1), however, more subtle injury occurs frequently, causing cognitive deficits that are detectable after even a single DKA episode. Similarly, renal failure is an infrequent consequence of DKA, however, recent data demonstrate that less severe acute kidney injury (AKI) occurs commonly (2). These data suggest that organ injuries during DKA may not occur as isolated events but instead reflect a form of multiple organ dysfunction syndrome. Importantly, patterns of organ involvement in DKA largely mirror patterns of chronic organ dysfunction that are characteristic of T1D in adulthood. Data from other brain and kidney diseases suggests that inflammatory processes triggered during acute injury may persist chronically and contribute to long-term organ dysfunction. *Understanding the processes leading to organ dysfunction during DKA in childhood therefore may have far-reaching implications, extending beyond the acute time frame associated with DKA.*

Methods

To assess alterations in renal levels of inflammatory mediators by measuring the content of cytokines and chemokines in homogenized renal tissue specimens using multiplex ELISA. We assessed concentrations of various inflammatory mediators in the kidney during acute DKA (after 4 hrs insulin/saline treatment), and 28 days after DKA, using ELISA of renal tissue homogenates. Findings in rats exposed to DKA were compared to diabetic control rats without exposure to DKA (n=6 per group). Multiplex assays were used to measure cytokine and chemokine content in renal tissue homogenates.

The following proteins were evaluated:

GRO alpha KC/ CXCL1, RANTES /CCL5 and **MIP1 alpha/CCL3**: chemokines involved in neutrophil and macrophage chemotaxis and other inflammatory processes

TNFα, IFNγ, IL-6, IL-1ß , IL-10, IL-4, IL-2, IL-17 and IL12p70,: cytokines involved in the acute phase response, expression of adhesion factors and other aspects of the inflammatory response.

Homogenization. Transverse 2-3mm fresh frozen rat kidney block sections proximal to medial rat kidneys were sectioned and homogenized via the Invitogren homogenization protocol. Renal transverse specimen sections contained cortex, outer medulla, and inner medulla. (3)

Statistical analyses. Kidney samples from rats collected 28 days after a DKA episode were compared to samples from hyperglycemic rats of the same age and with diabetes of equal duration using Student's t-test.

Results

-> group = DKA 28 day

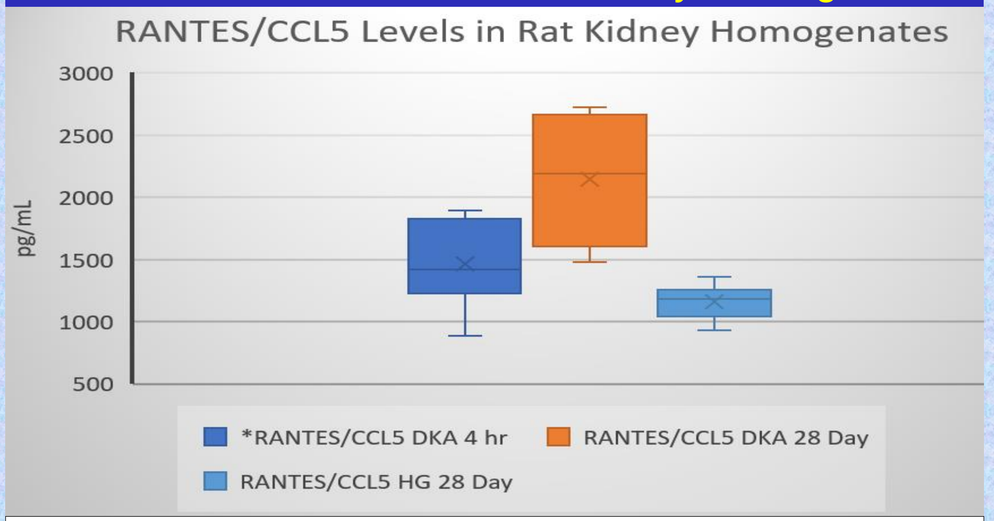
Variable	Obs	Mean	Std. Dev.	Min	Max
il10	6	3266.023	259.6114	2892.6	3651.46
il_1beta	6	739.4433	118.9844	611.62	945.74
il2	6	40.25333	7.107454	31.99	52.14
ifngamma	6	36.36333	4.506185	30.68	43.07
il12p70	6	112.2667	22.18489	80.14	140.47
groalphakc	6	2089.947	423.3015	1693.67	2888.76
rantes	6	2141.623	511.1475	1472.54	2719.2
tnfalpha	6	233.6317	52.00127	176.84	319.68
mip1alpha	6	67.28167	9.925377	58.4	82.37
il_17a	6	141.0817	21.01041	113.34	171.86

-> group = HG 28 day

Variable	Obs	Mean	Std. Dev.	Min	Max
il10	6	3137.192	441.3513	2574.66	3517.64
il_1beta	6	680.005	152.1891	501.51	838.14
il2	6	37.59333	6.530641	30.28	46
ifngamma	6	35.69833	6.046964	27.16	42.21
il12p70	6	112.6117	21.796	83.05	141.44
groalphakc	6	1962.595	337.0458	1538.41	2413.36
rantes	6	1155.383	144.9251	927.28	1356.88
tnfalpha	6	222.345	48.51877	160.61	271.4
mip1alpha	6	62.68333	10.74782	50.43	75.58
il_17a	6	132.255	26.94352	98.36	159.13

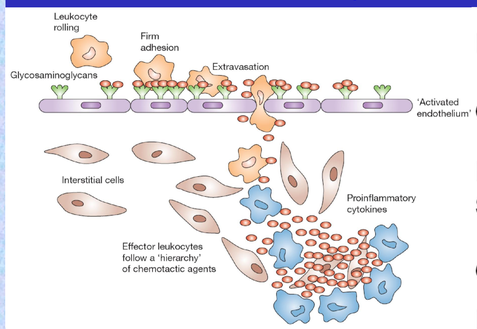
CHEMOKINE	P-VALUE
IL-10	0.55
IL-1b	0.47
IL-2	0.51
IFN Gamma	0.83
IL-12p70	0.98
RANTES/CCL5	**0.001**
TNF Alpha	0.71
MIP 1 Alpha	0.46
IL-17	0.54

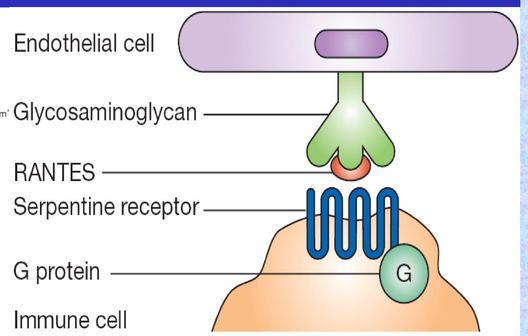
RANTES/CCL5 Levels in Rat Kidney Homogenates



*NOTE: Comparisons of rats with acute DKA (after 4 hours saline/insulin treatment) with normal and hyperglycemic rats of the same age showed consistently low levels of all cytokines and chemokines measured. These levels were reduced by a uniform amount for all cytokines/chemokines suggesting a measurement error, likely due to dilution related to large volumes of saline in the peritoneum and circulating through the kidneys. Additional experiments are underway to investigate this issue.

Inflammation, RANTES/CCL5 and T-Cells





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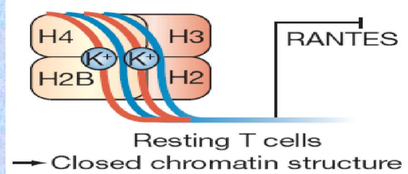

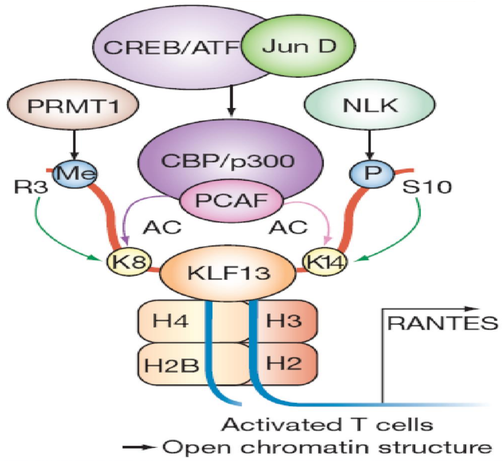

Discussion

Studying cytokines in kidneys of rats after exposure to DKA is novel. A literature review was performed before commencing our experiments to acquire background knowledge and to assist with protocol development. Methods previously used to study brains of rats with DKA were employed to generate homogenized samples to conduct multiplex cytokine and chemokine assays. Our results showed that most cytokine and chemokine levels were similar in rats with and without exposure to DKA, however, RANTES (Regulated on Activation, Normal T-Cell Expressed and Secreted)/CCL5 levels were markedly elevated in rats exposed to DKA. These findings suggest links between DKA and organ inflammation that may contribute to diabetic kidney disease and suggest that RANTES/CCL5 may be an important mediatory of ongoing organ inflammation/injury.

RANTES/CCL5 is known to be directly associated with renal diseases (4) but our findings are influential in demonstrating the impact of a single DKA episode on renal inflammatory pathways. Our findings suggest that DKA triggers a substantial increase in RANTES/CCL5 expression far above that attributable to diabetes alone.

RANTES/CCL5 findings in rats with acute DKA after 4 hours of treatment with intraperitoneal (IP) saline and SC insulin were also included in the figure. Our DKA brain research has documented increased chemokines in the brain during acute DKA treatment. Our current data suggest that RANTES/CCL5 is also increased during acute DKA in the kidneys, however, treatment with large volumes of IP saline may have diluted chemokines concentrations, resulting in lower than expected chemokine levels. To investigate this question, a new n=6 cohort of DKA rats will be studied, with kidney specimens collected prior to administration of DKA treatment.

Conclusion

- A single DKA episode in rats with diabetes results in elevated renal RANTES/CCL5 levels 28 days after the DKA episode. Differences between diabetic rats with and without DKA exposure were substantial with no overlap between the ranges of RANTES/CCL5 levels observed.
- Increased levels of RANTES/CCL5 and resultant T-cell infiltration might play a role in multi-organ dysfunction observed during and after DKA in children.
- RANTES/CCL5 levels in kidneys of rats with acute DKA were lower than expected which could be due to a dilution artifact from DKA treatment with intraperitoneal normal saline boluses.

Further Research

- Harvest kidneys from n=6 rats with acute, untreated DKA followed by homogenization and multiplex cytokine assay protocol to r/o dilution artifact.
- Immunohistochemical staining of rat kidney sections to identify inflammatory cells and renal location of inflammation.

References

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