How to translate neuro-cognitive and behavioural outcome data in animals exposed to paracetamol to the human perinatal setting?

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Background: Epidemiological observations link perinatal paracetamol (acetaminophen) exposure to impaired neuro-cognition and behavior, but animal models may assist to better understand mechanisms.

Methods: To provide an overview on preclinical data and mechanisms explored, we conducted a structured literature search on animal models and neuro-cognition and behavioural outcome following perinatal paracetamol exposure.

Results: This search resulted in 20 papers ***[rat*** (n=9), ***zebrafish larvae*** (n=6), ***mice*** (n=5)], published between 2009-2020.
Eight discussed pregnancy/fetal paracetamol exposure, 6 juvenile, 6 studies combined pregnancy and juvenile exposure.
Most papers (n=16) described ***paracetamol exposure without indication***, so rather toxicology models, except for an induced fever and repetitive needle pricking (rat), brain injury (mice), and a zebrafish nociception model.

Reported outcomes related to ***biochemistry*** (mono-amines, amino-acids, protein expression), ***anatomy*** (teratogen, morphology, nuclear size) or ***behaviour*** (spatial memory, motor, social behaviour and exploration, sexual behaviour).
On mechanisms, the cumulative data support an interesting 'cannabinoid' hypothesis to link paracetamol to neuro-cognitive and behavioural outcome. Besides limited species diversity, there is relevant within-species paracetamol dosing variability (dose, duration) with undocumented exposure.

study reference	animal	fetal, or pregnancy	postnatal, juvenile	intervention	outcomes	relevant findings
Blecharz-Klin 2015a [26]	rat	+	+	placebo, 5 or 15 mg/kg/day paracetamol, from pregnancy to lactation until day 60 (3 x 9 animals), only male rats.	spinal cord mono-amines, amino-acids and blood chemistry (renal, liver, muscle safety) on day 60.	3-methoxy-4-hydroxyphenylglycol (MHPG) and dopamine higher in controls, aspartate was higher in cases, reflecting modulation of dopaminergic and noradrenergic systems.
Blecharz-Klin 2015b [27]	rat	+	+	placebo, 5 or 15 mg/kg/day paracetamol, from pregnancy to lactation until day 60 (3 x 10 animals), only male rats.	biochemical alterations in medulla oblongata + safety assessment on day 60.	noradrenaline and dopamine, homovanillic acid, taurine, alanine higher, 5-hydroxy tryptamine (5-HT) lower in controls, reflecting modulation of serotonergic, dopaminergic and noradrenergic systems. Body weight on day 60 was different.
Blecharz-Klin 2016 [28]	rat	+	+	placebo, 5 or 15 mg/kg/day paracetamol, from pregnancy to lactation until day 60 (3 x 10 animals), only male rats.	cerebellar levels of mono-amines and amino-acids.	MHPG higher in cases, and 5-hydroxy-indole-acetic acid (5-HIAA) higher in the 5 mg cases, reflecting modulation of serotonergic and noradrenergic systems.
Blecharz-Klin 2017 [29]	rat	+	+	placebo, 5 or 15 mg/kg/day paracetamol, from pregnancy to lactation until day 60, decapitation (3 x 10 animals), only male rats.	neurotransmitters (prefrontal cortex, hippocampus, striatum), spatial memory and motor performance.	dopamine higher in cases (striatum), 3-methoxytyramine (3-MT) higher (prefrontal); 5-HT higher and 5-HIAA lower (prefrontal) in 15 mg cases, reflecting modulation of serotonergic and dopaminergic systems. *staircase*: no differences; *hole board*: motor activity lower in 5 mg cases; *water maze*: platform crossing higher in 5 mg cases; spatial reversal: elevated latency in 5 mg cases; reversal probe: swim distance and speed higher in 5 mg cases, related to difference in amino acids levels.
Blecharz-Klin 2018 [30]	rat	+	+	placebo, 5 or 15 mg/kg/day paracetamol, from pregnancy to lactation until day 60 (3 x 10 animals), only male rats.	Brain-derived neurotrophic factor (BDNF, prefrontal cortex, hippocampus, striatum), social behaviour and exploration.	BDNF 2-fold lower (striatum) in exposed cases. *Social interaction*: higher frequency of social interaction and sniffing, but lower pinning in controls. *Novel objection recognition*: higher in 15 mg cases.
Blecharz-Klin 2019 [31]	rat	+	+	placebo, 5 or 15 mg/kg/day paracetamol, from pregnancy to lactation until day 60 (3 x 10 animals), only male rats.	biochemical alterations in hypothalamus on day 60.	MHPG and glutamic acid higher in controls, and dopamine higher in 5 mg cases, reflecting modulation of dopaminergic and noradrenergic systems.
David 2009 [32]	zebrafish	+, larvae	-	developing eggs (30), exposed to different paracetamol (0,1,5,10,50,100 mg/l) levels for 7 consecutive days.	early development, hatching, organogenesis, larval growth and morphology, tail and tail-fin, pigmentation, behaviour and survival.	dose-related lower survival and affected embryo development; if successful, also delayed hatching; lower body mass; lack of pigmentation; malformations more present.
Ellis 2018 [33]	zebrafish	+, larvae	-	nociception model (acetic acid recovery), post exposure normalization of activity (150 min).	Does paracetamol, ibuprofen or tramadol affect post exposure normalization.	paracetamol has no early, but a late effect (reduced hyperactivity after acetic acid exposure)
Escapa 2019 [34]	zebrafish	+, larvae	-	environmental toxicity assessment, zebrafish embryo assay (paracetamol in effluents)	toxic effects (e.g. developmental delay, length, pigmentation, hatching)	Paracetamol displays concentration-related negative effects in the zebrafish embryo model.
Hay-Schmidt 2017 [35]	mice	+	-	maternal paracetamol (50, or 150 mg/kg/day) or aniline (30, or 90 mg/kg/day), compared to placebo, gavage) from day 7 until delivery (5x10 pregnant animals).	week 8 pups. Sexually dimorphic nucleus (SDN, preoptic area) anatomy; behavioural (urinary, aggression, sexual) testing (150 mg)	paracetamol (150 mg) and aniline both decreased (50%) SDN cell number. Less masculine territorial marking, less territorial display, reduced mating.
Klein 2020 [36]	rat	+	-	maternal paracetamol (350 mg/kg/day), from day 6 until delivery (gavage, 40/47 litters included).	offspring behavioural testing (nest seeking, behavioural stereotypy, 3 chamber sociability, open-field, elevated plus-maze, hot plate); neurochemical (prefrontal cortex, hippocampus)	in paracetamol group, *nest seeking*: impaired; *stereotypy*: augmented apomorphine-induced; *elevated plus-maze*: decreased rostral grooming. neurochemical assessment (glutathione, lipid hydroperoxide, BDNF): no differences.
Leroux 2010 [37]	mouse	-	+	excitotoxic brain injury, ibotenate cortical injection. Aspirin, indomethacin, paracetamol (10, 100 µg) before/after injection.	lesion size on day 10 (pathology).	post-treatment with paracetamol protected for white matter lesions, pre-treatment not.
Nogueira 2019 [38]	zebrafish	+, larvae	-	effects of environmental realistic exposure to paracetamol (0.005-3.125 mg/l)	embryonic development, locomotor activity, biochemical, epigenetics.	in paracetamol exposed: embryotoxicity (pigmentation, deformities); higher locomotor activity; increased cholinesterase, glutathione peroxidase, lipid peroxidation; methylation differs.
Philippot 2017 [39]	mice	-	+	mice, male/female, exposed to paracetamol (2 x 30 mg/kg) or saline (subcutaneous) on postnatal day 3,10 or 19.	spontaneous behaviour (locomotion, rearing, total activity) in a new environment at adult equivalent age (2 months).	adverse effects on behaviour and cognition on day 3 and 10, but not 19 in both male and female.
Philippot 2018 [40]	mice	-	+	male mice, paracetamol (2 x 30 mg/kg) +/- cannabinoid receptor type 1 agonist (WIN 55 212-2) on day 10. *short-term*: 24h after initial exposure; *long-term*: adult mice.	*short-term*: transcript levels (cerebral cortex, hippocampus). *long-term*: spontaneous behaviour (locomotion, rearing, total activity) in adult male mice (new environment).	in paracetamol + WIN co-exposed mice: *short-term*: fatty acid amide hydroxylase (cortex), synaptophysin and tropomyosine receptor kinase B (hippo) lower. *long-term*: lack of habituation
Reuter 2016 [41]	zebrafish	+, larvae	-	zebrafish, exposed to sub-liver toxic levels of paracetamol (up to 1000 (acute), 100 (chronic) mg/l).	locomotor activity levels, wild *vs* latrophilin-3 (Attention Deficit Hyperactivity Disorder (ADHD) model).	no ADHD trait in larvae exposed to paracetamol.
Saeedan 2018 [42]	albino rat	-	+	fever induced in pups (vaccines (MMR, DPT), or LPS) +/- paracetamol (subcutaneous, 50 mg/kg).	growth; behaviour (swimming, olfactory, geotaxis, nociception, locomotor activity); pro- and anti-inflammatory markers.	paracetamol (+/-) exposed: weight gain differs; decreased locomotor activity; negative geotaxis; increases thermal nociception time; swimming behaviour, nest seeking, olfactory discrimination and exploration affected. Correlation between behaviour and pro- or anti-inflammatory markers.
van den Hoogen 2016 [43]	rat	-	+	repetitive needle pricking model (day 0-7) +/- paracetamol (30 to 20 mg/kg, subcutaneous) vs sham handling, n = 3 x 14 pups.	short- (mechanical hypersensitivity, von Frey) and long-term effects (postoperative hypersensitivity, ipsilateral paw incision 8th week).	no effects on short-term mechanical sensitivity, nor on baseline (week 3-8). reduced duration of mechanical hypersensitivity after incision at adult equivalent age after neonatal exposure.
Viberg 2014 [44]	mice	-	+	10 days old mice, one or two paracetamol doses (30 mg/kg, subcutaneous) vs normal saline. Only data in male mice collected.	short- (BDNF, frontal, parietal, hippocampus, day 11) and long-term behavioural effects (spontaneous behaviour, radial arm maze, hot plate, elevated plus maze) at 2 months.	BDNF higher (frontal, parietal) in paracetamol cases. altered locomotor behaviour in novel home cage, impaired spatial learning, but not thermal nociception or anxiety related behaviour.
Xia 2017 [45]	zebrafish	+, larvae	-	ibuprofen, diclofenac or paracetamol (5-500 µg/l) effects on the zebrafish embryo.	short-term effects of ibuprofen, diclofenac or paracetamol on hatch, motor behaviour and RNA extraction.	paracetamol hatch: no effects; behaviour: no effects; RNA neuron related genes no effects.

Conclusions: toxicology models should shift to clinical indications, as non-exposure is the obvious safest setting in the absence of an indication. Besides pain and fever and related to the, this should include perinatal brain injury, as there is animal experimental evidence that cannabinoids are neuroprotective in newborn brain injury or asphyxia, further supported by evidence in non-perinatal models of paracetamol-related neuroprotective effects.

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