Autism Solutions Seminar

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The Protocol to diagnose Lyme disease non-invasively in ASD children


Abstract: Here we describe the Ruggiero-Klinghardt (RK) Protocol that is based on integration of Autonomic Response Testing (ART) with diagnostic ultrasonography and on application of therapeutic ultrasounds; the latter are used as a provocation tool and as an instrument to optimize drug uptake and utilization in specific areas of the body. This protocol consists of a precise sequence of diagnostic and therapeutic procedures with the ultimate goal of improving sensitivity and specificity of diagnosis at the same time evaluating and optimizing efficacy of treatments in chronic conditions including, but not limited to, persistent Lyme disease. The RK Protocol represents a paradigm shift in diagnostics and therapeutics: Thus, compartmentalized microbes, transformed cells, toxins and metabolites could be detected using a safe and non-invasive method. In addition, the RK Protocol allows optimization of efficacy of drugs and other therapeutic interventions. Although the RK Protocol was initially developed for persistent Lyme disease, it shows significant potential in conditions ranging from cancer to neurodegenerative diseases and autism. In oncology, the RK Protocol may serve to facilitate early diagnosis and to increase sensitivity of cancer cells to the killing effects of a variety of remedies ranging from conventional radio- and chemotherapy to more recent forms of immunotherapy. Thus, the 1st goal of the RK Protocol is diagnostic: That is, to make pathogens, toxins, transformed cells and cells infected by viruses that are inaccessible to conventional diagnostic and therapeutic tools, “visible” to the therapist who can detect them with laboratory methods and deal with them with appropriate interventions; and also to make them “visible” to the immune system that can fight them in a physiological manner. The 2nd goal is to optimize drug uptake and utilization in the organs and tissues studied and targeted with these procedures.

Aluminium in the brain of autistic children


Abstract

Autism spectrum disorder is a neurodevelopmental disorder of unknown aetiology. It is suggested to involve both genetic susceptibility and environmental factors including in the latter environmental toxins. Human exposure to the environmental toxin aluminium has been linked, if tentatively, to autism spectrum disorder. Herein we have used transversely heated graphite furnace atomic absorption spectrometry to measure, for the first time, the aluminium content of brain tissue from donors with a diagnosis of autism. We have also used an aluminium-selective fluor to identify aluminium in brain tissue using fluorescence microscopy. The aluminium content of brain tissue in autism was consistently high. The mean (standard deviation) aluminium content across all 5 individuals for each lobe were 3.82(5.42), 2.30(2.00), 2.79(4.05) and 3.82(5.17) μg/g dry wt. for the occipital, frontal, temporal and parietal lobes respectively. These are some of the highest values for aluminium in human brain tissue yet recorded and one has to question why, for example, the aluminium content
of the occipital lobe of a 15 year old boy would be 8.74 (11.59) μg/g dry wt.? Aluminium-selective fluorescence microscopy was used to identify aluminium in brain tissue in 10 donors. While aluminium was imaged associated with neurones it appeared to be present intracellularly in microglia-like cells and other inflammatory non-neuronal cells in the meninges, vasculature, grey and white matter. The pre-eminence of intracellular aluminium associated with non-neuronal cells was a standout observation in autism brain tissue and may offer clues as to both the origin of the brain aluminium as well as a putative role in autism spectrum disorder.


Abstract

Vaccines are being under investigation for the possible side effects they can cause. In order to supply new information, an electron-microscopy investigation method was applied to the study of vaccines, aimed at verifying the presence of solid contaminants by means of an Environmental Scanning Electron Microscope equipped with an X-ray microprobe. The results of this new investigation show the presence of micro- and nanosized particulate matter composed of inorganic elements in vaccines’ samples which is not declared among the components and whose unduly presence is, for the time being, inexplicable. A considerable part of those particulate contaminants have already been verified in other matrices and reported in literature as non biodegradable and non biocompatible. The evidence collected is suggestive of some hypotheses correlated to diseases that are mentioned and briefly discussed.

Results

The investigations verified the physical-chemical composition of the vaccines considered according to the inorganic component as declared by the Producer. In detail, we verified the presence of saline and Aluminium salts, but further presence of micro-, sub-micro- and nanosized, inorganic, foreign bodies (ranging from 100nm to about ten microns) was identified in all cases, whose presence was not declared in the leaflets delivered in the package.

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Keywords: Vaccine; Disease; Contamination; Protein corona; Biocompatibility; Toxicity; Nanoparticle; Immunogenicity; Foreign body; Environment; Industrial process; Quality control

Autism and Retroviruses
And a new approach to ASD

Whiteley, Paul, and K. Dodou. "We are all part virus—the role of human endogenous retroviruses." *Pharma J* 292 (2014): 244-5.

From the abstract: The constant bombardment of viral pathogens that the human race has fought off and developed immunity against down the ages has not been without consequences to our genome, as illustrated by the increasing interest in the role of endogenous retrovirus (ERVs) fragments and elements in health and disease.

Human endogenous retroviruses (HERVs) are the remnants of ancient retroviral infections, sometimes called fossil viruses, marked into our DNA via infection of germline cells (ie, the cells involved in reproduction). Passed down the generations, retroviral genes become part of the host genome and, gradually, generation after generation, pick up genetic mutations which eventually inactivate the virus, although still, in some cases, carrying the essential viral genes noted in exogenous retroviruses such as HIV. Around 4–8 per cent of the human genome is thought to comprise HERVs.


From the “discussion”: “To the best of our knowledge, this is the first evidence linking retrotransposon activity and ASD”


Several immune abnormalities have been noted in autistic subjects. These associations have been extended to the Major Histocompatibility Complex (MHC), a section of DNA remarkable for the number of encoded proteins with immunological functions. The strongest MHC association identified thus far is for the null allele of C4B in the class III region. The complex allelic composition of C4 as determined by immune-electrophoresis is discussed. Low levels of C4 resulting from the null allele may be important in disease pathogenesis especially since C4 has been identified in developing brain neurons. The DNA region just telomeric to C4 has several genes including tumor necrosis factor which encode proteins with immunological functions. These proteins may act in concert with C4 in disease contribution and the genes should be more closely examined……However, it appears that human endogenous retroviruses (HERV) and HERV fragments are involved.


**Summary:** Recent studies suggest that autism spectrum disorders (ASD) result from interactions between genetic and environmental factors, whose possible links could be represented by epigenetic mechanisms. Here, we investigated the transcriptional activity of three human endogenous retrovirus (HERV) families, in peripheral blood mononuclear cells (PBMCs) from Albanian ASD children, by quantitative real-time PCR. We aimed to confirm the different expression profile already found in Italian ASD children, and to highlight any social and family health condition emerging from information gathered through a questionnaire, to be included among environmental risk factors. The presence of increased HERV-H transcriptional activity in all autistic patients could be understood as a constant epigenetic imprinting of the disease, potentially useful for early diagnosis and for the development of effective novel therapeutic strategies.

**ADHD and retroviruses**


**Objectives.** Several lines of evidences suggest that human endogenous retroviruses (HERVs) are implicated in the development of many complex diseases with a multifactorial aetiology and a strong heritability, such as neurological and psychiatric diseases. Attention deficit hyperactivity Disorder (ADHD) is a neurodevelopmental disorder that results from a complex interaction of environmental, biological and genetic factors. Our aim was to analyse the expression levels of three HERV families (HERV-H, K and W) in patients with ADHD. **Methods.** The expression of retroviral mRNAs from the three HERV families was evaluated in peripheral blood mononuclear cells (PBMCs) from 30 patients with ADHD and 30 healthy controls by quantitative RT-PCR. **Results.** The expression levels of HERV-H are significantly higher in patients with ADHD compared to healthy controls, while there are no differences in the expression levels of HERV-K and W. **Conclusions.** Since the ADHD aetiology is due to a complex interaction of environmental, biological and genetic factors, HERVs may represent one link among these factors and clinical phenotype of ADHD. A future confirmation of HERV-H overexpression in a larger number of ADHD patients will make possible to identify it as a new parameter for this clinical condition, also contributing to deepen the study on the role of HERVs in the neurodevelopment diseases.

**Key words::** Human endogenous retrovirus, HERV-H, ADHD, neurodevelopmental diseases, peripheral blood mononuclear cells

**Schizophrenia and Retroviruses**

Abstract

Both genetic and environmental factors appear to contribute to the causation of schizophrenia. Evidence indicating that fetal development is disrupted in schizophrenia and the finding of an excess of winter births among schizophrenic patients have led to continued speculation that an intrauterine viral infection may cause developmental lesions, genetic mutations, or persistent infections that lead to schizophrenia. Certain unique characteristics of the retroviruses render them plausible as candidate “schizo-viruses” and the involvement of an endogenous retrovirus would be compatible with some of the puzzling epidemiological findings in schizophrenia. Reverse transcriptase (RT) is a retrovirally encoded enzyme essential for retroviral integration into host DNA. While attempts to detect retroviral infections by measuring RT activity in the peripheral lymphocytes and serum of schizophrenic patients have been unsuccessful, such negative findings may simply mean that the virus is not active in peripheral lymphocytes. A more sensitive and comprehensive approach to detect a retrovirus is to search the genomes of schizophrenic patients directly for the presence of retroviral DNA sequences encoding RT and one possible approach is described.


Retroelements, such as Human Endogenous Retroviruses (HERVs), have been implicated in many complex diseases, including neurological and neuropsychiatric disorders. Previously, we demonstrated a distinctive expression profile of specific HERV families in peripheral blood mononuclear cells from Autistic Spectrum Disorders (ASD) patients, suggesting their involvement in ASD. Here we used two distinct ASD mouse models: inbred BTBR T+tf/J mice and CD-1 outbred mice prenatally exposed to valproic acid. Whole embryos, blood and brain samples from the offspring were collected at different ages and the expression of several ERV families (ETnI, ETnII-α, ETnII-β, ETnII-γ, MusD and IAP), proinflammatory cytokines (IL-1β, IL-6 and TNF-α) and Toll-like receptors (TLR3 and TLR4) was assessed. In the two distinct mouse models analysed, the transcriptional activity of the ERV families was significant higher in comparison with corresponding controls, in whole embryos, blood and brain samples. Also the expression levels of the proinflammatory cytokines and TLRs were significantly higher than controls. Current results are in agreement with our previous findings in ASD children, supporting the hypothesis that ERVs may serve as biomarkers of atypical brain development. Moreover, the changes in ERVs and proinflammatory cytokines expression could be related with the autistic-like traits acquisition in the two mouse models.

Retroviruses in vaccines


**Background:** Safety considerations require that biological products for human use are free from any agent that might pose a potential health hazard. One method to detect the presence of retroviral particles is the reverse transcriptase (RT) assay. This assay is capable of detecting all infectious retrovirus particles, irrespective of genome or protein composition. Recently, a family of ultrasensitive RT tests, named product-enhanced reverse transcriptase (PERT) assays, has been designed with a detection limit that is $10^6 - 10^7$ times lower than that of conventional RT tests.

**Objectives:** To investigate with the PERT assay whether RT activity is detectable in live attenuated virus vaccines and to characterize eventual RT activities.

**Study design:** A total of 12 different monovalent and one trivalent virus vaccines containing live attenuated viruses were tested for RT activity with the PERT assay and a conventional RT test. RT activities were investigated with respect to their susceptibility to RT inhibitors, association with physical particles, and their possible origin.

**Results:** One trivalent and five different monovalent vaccines contained RT activity when tested with the PERT assay, but were negative in a conventional RT assay. All lots tested of these vaccines showed RT activity. The activity in all vaccines was sensitive to AZT-triphosphate and ddTTP and at least part of it was associated with particles. Mg$^{2+}$-dependent RT activity banded at a density of 1.14 g/ml. All positive vaccines were produced using chicken cells.

**Conclusions:** The data indicate the systematic presence of partially particle-associated retroviral reverse transcriptase in attenuated live virus vaccines that are produced in chicken-derived cells. The identification and further characterization of these particles, as well as the elucidation of possible interactions with the human organism are imperative goals despite the fact that these vaccines have been safely used for many years.

### The treatment of Retroviral Activity

#### 1. Retroviruses and Baicalin (Scullcap root/Scutalaria)

Abstract

Baicalin (BA), (formulated as 7-D-glucuronic acid-5,6-dihydroxy-flavone), was purified from the plant Scutellaria Baicalensis Georgi. It has been used as a traditional Chinese herbal medicine. The inhibitory effect of BA against human immunodeficiency virus (HIV-1) infection and replication has been studied in vitro. The compound inhibits HIV-1 infection and replication as measured by: (1) a quantitative focal syncytium formation on CEM-ss monolayer cells; and (2) HIV-1 specific core antigen p24 expression and retroviral reverse transcriptase (RT) activity in the HIV-1-infected H9 cells. We have further demonstrated that the enzymatic activity of purified recombinant HIV-1/RT was inhibited by BA. In addition to lymphoid cell lines, the anti-HIV-1 activity of BA was also observed in cultures of primary human peripheral blood mononuclear cells infected with HIV-1 in vitro. Neither cytotoxic nor cytostatic effects on the indicator cells were found under the assay condition. This data suggests that BA may serve as a useful drug for the treatment and prevention of HIV infections.

2. Retroviruses and ST John’s Wort


Studies of the mechanisms of action of the anti-retroviral agents hypericin and pseudohypericin

G Lavie, F Valentine, B Levin, Y Mazur, G Gallo, D Lavie, D Weiner and D Meruelo
PNAS 1989 August, 86 (15) 5963-5967.

Abstract

Administration of the aromatic polycyclic dione compounds hypericin or pseudohypericin to experimental animals provides protection from disease induced by retroviruses that give rise to acute, as well as slowly progressive, diseases. For example, survival from Friend virus-induced leukemia is significantly prolonged by both compounds, with hypericin showing the greater potency. Viremia induced by LP-BM5 murine immunodeficiency virus is markedly suppressed after infrequent dosage of either substance. These compounds affect the retroviral infection and replication cycle at least at two different points: (i) Assembly or processing of intact virions from infected cells was shown to be affected by hypericin. Electron microscopy of hypericin-treated, virus-producing cells revealed the production of particles containing immature or abnormally assembled cores, suggesting the compounds may interfere with processing of gag-encoded precursor polyproteins. The released virions
contain no detectable activity of reverse transcriptase. (ii) Hypericin and pseudohypericin also directly inactivate mature and properly assembled retroviruses as determined by assays for reverse transcriptase and infectivity. Accumulating data from our laboratories suggest that these compounds inhibit retroviruses by unconventional mechanisms and that the potential therapeutic value of hypericin and pseudohypericin should be explored in diseases such as AIDS.

**Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin.**

D Meruelo et al., Proc Natl Acad Sci U S A

3. **Green Tea and retroviruses**


Tea polyphenols (i.e., green tea catechins and black tea theaflavins) are strong inhibitors of human immunodeficiency virus (HIV)-reverse transcriptase. The galloyl moiety is important for their inhibitory effect because it is essential in catechins for inhibition and enhances the inhibitory potency of theaflavins. Tea polyphenols had considerable inhibitory activity against cellular DNA and RNA olymerases but were less effective than against HIV-reverse transcriptase. The mechanism of inhibition of DNA polymerases by the tea polyphenols was, in most cases, competitive with respect to the template-primer and noncompetitive to the nucleotide substrate. The inhibition of cellular polymerases by green tea catechins seems to cause their cytotoxicity to cultured cells, and might explain the epidemiological finding in Japan that the mortality of digestive tract cancer is significantly lower in areas where green tea ingestion is high.

4. **Suramin**


Abstract

This review article discusses the opportunities and possibilities of antipurinergic therapy (APT) with suramin on mice models with ASD and fragile X syndrome. Suramin is an APT mediator that triggers the mechanism linked to mitochondria, influences immunity, and has the ability to stabilize locomotor function and coordination, social behavior, normalize brain synapse structure, cell-to-cell signaling, and recover mitochondrial metabolism in mice with autistic-like behaviors and genetics. Suramin may provide new therapeutic strategies for ASD subtypes. Many clinical studies have explored the scope of APT with suramin to assist with the genetic, metabolic, and environmental risk factors of ASD. The pharmacological components of suramin allow binding against purinergic receptors without release, serving as an antagonist of extracellular APT, and other nucleotides. In recent studies, APT and suramin have been investigated to help correct behavior, genetic irregularities, and metabolism stemmed from neurodevelopmental disorders. This review article looks at APT with suramin under the following conditions in animal models:

- Gene-environment interaction
- APT with poly(I:C) mouse model
- Fragile X mental retardation syndrome 1 (Fmr1) knockout mouse model

This review article investigates studies by Naviaux et al. and the influence of suramin and APT in relation to ASD and fragile X syndrome. It was concluded that APT with suramin assists in correcting genetic abnormalities and environmental predispositions that may impact social behaviour related to ASD.


Abstract

Objective

No drug is yet approved to treat the core symptoms of autism spectrum disorder (ASD). Low-dose suramin was effective in the maternal immune activation and Fragile X mouse models of ASD. The Suramin Autism Treatment-1 (SAT-1) trial was a double-blind, placebo-controlled, translational pilot study to examine the safety and activity of low-dose suramin in children with ASD.

Methods

Ten male subjects with ASD, ages 5–14 years, were matched by age, IQ, and autism severity into five pairs, then randomized to receive a single, intravenous infusion of suramin (20
mg/kg) or saline. The primary outcomes were ADOS-2 comparison scores and Expressive One-Word Picture Vocabulary Test (EOWPVT). Secondary outcomes were the aberrant behaviour checklist, autism treatment evaluation checklist, repetitive behaviour questionnaire, and clinical global impression questionnaire.

**Results**

Blood levels of suramin were $12 \pm 1.5$ μmol/L (mean ± SD) at 2 days and $1.5 \pm 0.5$ μmol/L after 6 weeks. The terminal half-life was $14.7 \pm 0.7$ days. A self-limited, asymptomatic rash was seen, but there were no serious adverse events. ADOS-2 comparison scores improved by $-1.6 \pm 0.55$ points ($n = 5$; 95% CI = −2.3 to −0.9; Cohen’s $d = 2.9$; $P = 0.0028$) in the suramin group and did not change in the placebo group. EOWPVT scores did not change. Secondary outcomes also showed improvements in language, social interaction, and decreased restricted or repetitive behaviors.

**Interpretation**

The safety and activity of low-dose suramin showed promise as a novel approach to treatment of ASD in this small study.

**5. Reishi Mushroom**


抄録

Two new lanostane-type triterpenes, lucidumol A and ganoderic acid β, were isolated from the spores of Ganoderma (G.) lucidum, together with a new natural one and seven that were known. The structures of the new triterpenes were determined as (24S)-24, 25-dihydroxylanost-8-ene-3, 7-dione and 3β, 7β-dihydroxy-11, 15-dioxolanosta-8, 24(E)-dien-26-oic acid, respectively, by chemical and spectroscopic means. The quantitative analyses of 5 fruiting bodies, antlered form and spores of G. lucidum were performed by high performance liquid chromatography and demonstrated that ganoderic alcohol and acid contents were quite high in the spore. Of the compound isolated, ganoderic acid β, (24S)-lanost-9(11)-dien-3β, 24, 25-triol (called lucidumol B), ganodermanondiol, ganodermanontriol and ganolucidic acid A showed significant anti-human immunodeficiency virus (anti-HIV)-1 protease activity with IC₅₀ values of 20-90 μM.


Four new lanostane triterpenes, colossolactone V (1), colossolactone VI (2), colossolactone VII (3), and colossolactone VIII (4), were isolated from the fruiting bodies of the Vietnamese
mushroom *Ganoderma colossum*, together with the known compound colossolactone E (5). The structures of 1–4 were assigned on the basis of spectroscopic evidence, and their absolute configurations were determined by CD spectroscopy and the Mosher ester method. Compounds 1–5, as well as two previously isolated compounds [schisanlactone A (6) and colossolactone G (7)] from the same mushroom, were evaluated for inhibition of HIV-1 protease, with IC₅₀ values for the most potent compounds ranging from 5 to 13 µg/

6. Bitter Melon


Ng T B, Wong C M, Li W W, Yeung H W. *Isolation and characterization of a galactose binding lectin with insulinomimetic activites from the seeds of the bitter gourd Momordica charantia (Cucurbitaceae)*. International Journal of Peptide and Protein Research. 1986; 28 163-72

Title: Alpha-momorcharin inhibits HIV-1 replication in acutely but not chronically infected T-lymphocytes

Alternative Title: α库瓜子蛋白抑制急性感染而不抑制慢性感染T淋巴细胞中HIV－1的复制

Author: Zheng YT(郑永唐); Bi KL(贲昆龙); Jin SW(金善炜)

Corresponding Author: 贲昆龙


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Indexed Type: SCI

Department: 中国科学院昆明动物所; 中国科学院上海有机化学研究所

English Abstract: To identify the anti-human immunodeficiency virus type 1 (HIV-1) activities of α-momorcharin (α－MMC) from momordica charantia in acutely and chronically infected Tlymphocytes. METHODS: The anti-HIV activities of 1－MMC were examined by 1) the inhibition of syncytia formation induced by HIV-1 III B; 2) reduction of p24 core antigen expression level and decrease in numbers of HIV antigen positive cells in acutely and chronically infected cultures. The cytotoxic effects of α－MMC was tested by trypan blue dye exclusion or colorimetric MTT
assay. RESULTS: α-MMC was found to obviously inhibit HIV-1-IIIb-induced C8166 syncytia formation and markedly reduced both expression of p24 core antigen and the numbers of HIV antigen positive cells in acutely but not chronically HIV-1-infected culture. The median effective concentration (EC=50) in these assays were 0.016, 0.07, and 0.32 mg.L⁻¹, respectively. CONCLUSION: α-MMC is a unique component of momorcharin with anti-HIV activity, and markedly inhibited HIV-1 replication in acutely but not chronically HIV-1-infected T-lymphocytes.

**Language:** 英语

### 7. Stinging Nettle


Abstract: A series of four mannose (Man)-, three N-acetylglucosamine (GlcNAc)ₙ-, ten N-acetylgalactosamine/galactose(GalNAc/Gal)-, one 5-acetyleneuraminic acid(α-2,3-Gal/GalNAc) - and one 5-acetyleneuraminic acid(α-2,6-Gal/GalNAc)-specific plant agglutinins were evaluated for their antiviral activity in vitro.

The (GlcNAc)ₙ-specific lectin from *Urtica dioica* (UDA) was inhibitory to HIV-1-, HIV-2-, CMV-, RSV- and influenza A virus-induced cytopathicity at an EC₅₀ ranging from 0.3 to 9 μg/ml. The GalNAc/Gal-, α-2,3-Gal/GalNAc- or α-2,6-Gal/GalNAc-specific lectins were not inhibitory to HIV or CMV at non-toxic concentrations. CA, EHA and UDA proved to be potent inhibitors of syncytium formation between persistently HIV-1- and HIV-2-infected HUT-78 cells and CD4⁺ Molt/4 (clone 8) cells (EC₅₀:0.2–2 μg/ml). Unlike dextran sulfate, the plant lectins CA, EHA and UDA did not interfere with HIV-1 adsorption to MT-4 cells and RSV- and influenza A virus adsorption to HeLa and MDCK cells, respectively. They presumably interact at the level of virion fusion with the target cell.

### 8. Olive Leaf

Sulforaphane treatment of autism spectrum disorder (ASD)

Significance

Autism spectrum disorder (ASD), encompassing impaired communication and social interaction, and repetitive stereotypic behavior and language, affects 1–2% of predominantly male individuals and is an enormous medical and economic problem for which there is no documented, mechanism-based treatment. In a placebo-controlled, randomized, double-blind clinical trial, daily oral administration for 18 wk of the phytochemical sulforaphane (derived from broccoli sprouts) to 29 young men with ASD substantially (and reversibly) improved behavior compared with 15 placebo recipients. Behavior was quantified by both parents/caregivers and physicians by three widely accepted measures. Sulforaphane, which showed negligible toxicity, was selected because it upregulates genes that protect aerobic cells against oxidative stress, inflammation, and DNA-damage, all of which are prominent and possibly mechanistic characteristics of ASD.

Abstract

Autism spectrum disorder (ASD), characterized by both impaired communication and social interaction, and by stereotypic behavior, affects about 1 in 68, predominantly males. The medico-economic burdens of ASD are enormous, and no recognized treatment targets the core features of ASD. In a placebo-controlled, double-blind, randomized trial, young men (aged 13–27) with moderate to severe ASD received the phytochemical sulforaphane (derived from broccoli sprout extracts—or indistinguishable placebo (n = 15). The effects on behavior of daily oral doses of sulforaphane (50–150 µmol) for 18 wk, followed by 4 wk without treatment, were quantified by three widely accepted behavioral measures completed by parents/caregivers and physicians: the Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS), and Clinical Global Impression Improvement Scale (CGI-I). Initial scores for ABC and SRS were closely matched for participants assigned to placebo and sulforaphane. After 18 wk, participants receiving placebo experienced minimal change (<3.3%), whereas those receiving sulforaphane showed substantial declines (improvement of behavior): 34% for ABC (P < 0.001, comparing treatments) and 17% for SRS scores (P = 0.017). On CGI-I, a significantly greater number of participants receiving sulforaphane had improvement in social interaction, abnormal behavior, and verbal communication (P = 0.015–0.007). Upon discontinuation of sulforaphane, total scores on all scales rose toward pretreatment levels. Dietary sulforaphane, of recognized low toxicity, was selected for its capacity to reverse abnormalities that have been associated with ASD, including oxidative stress and lower antioxidant capacity, depressed glutathione synthesis, reduced mitochondrial function and oxidative phosphorylation, increased lipid peroxidation, and neuroinflammation.
Suramin


Abstract
This review article discusses the opportunities and possibilities of antipurinergic therapy (APT) with suramin on mice models with ASD and fragile X syndrome. Suramin is an APT mediator that triggers the mechanism linked to mitochondria, influences immunity, and has the ability to stabilize locomotor function and coordination, social behaviour, normalize brain synapse structure, cell-to-cell signaling, and recover mitochondrial metabolism in mice with autistic-like behaviours and genetics. Suramin may provide new therapeutic strategies for ASD subtypes. Many clinical studies have explored the scope of APT with suramin to assist with the genetic, metabolic, and environmental risk factors of ASD. The pharmacological components of suramin allow binding against purinergic receptors without release, serving as an antagonist of extracellular APT, and other nucleotides. In recent studies, APT and suramin have been investigated to help correct behaviour, genetic irregularities, and metabolism stemmed from neurodevelopmental disorders. This review article looks at APT with suramin under the following conditions in animal models:
• Gene-environment interaction
• APT with poly(I:C) mouse model
• Fragile X mental retardation syndrome 1 (Fmr1) knockout mouse model This review article investigates studies by Naviaux et al. and the influence of suramin and APT in relation to ASD and fragile X syndrome. It was concluded that APT with suramin assists in correcting genetic abnormalities and environmental predispositions that may impact social behaviour related to ASD.

Keywords
Antipurinergic therapy; Autism spectrum disorder; Fmr1; Fragile x syndrome; Maternal immune activation; Poly(I:C); Purinergic; Suramin

List of Abbreviations
ASD: Autism Spectrum Disorders; APT: Antipurinergic Therapy; APT; Fmr1: Fragile X Mental Retardation Genelocus 1; P2Y: G-Protein Coupled Purinergic Receptors; P2X: Lonotropic Purinergic Receptors; MIA: Maternal Immune Activation; Poly(I:C): Polyinosinic

Background
This review article discusses the opportunities and possibilities of suramin on mice models with ASD and fragile X syndrome. According to the CDC's Autism and Developmental Disabilities Monitoring (ADDM) Network, 1-2% of children in the United States are affected by ASD [1]. Behaviors of ASD, as described by the ADDM Network, include
limited social behavior and communication, repetitive movements, limited and heightened interest in selective activities and interests, including preferences related to taste, color, clothing, and social activities [1]. These preferences range from high-functioning to functioning, and independent to nonindependent [2]. Genetic risk factors include various metabolic, environmental and genetic conditions, although these factors influence each child differently [1].

The objective of this review article is to determine if controlled doses of suramin alleviate ASD conditions, taking a specific look at clinical studies and research conducted by Naviaux et al. This review article includes findings by Naviaux et al. that discuss suramin and the relationship between MIA mouse model and poly(I:C) and the effect signaling molecules of the mitochondria [2-5]; MIA and fragile X models, as related to genetic impact of ASD [6]. It can be concluded that suramin doses had a positive effect on ASD and other neurodevelopment disorders.

Content for this review article was gathered using search terms “suramin and autism,” “suramin therapy and autism,” “autism therapy,” and “autism,” through public electronic databases such as ProQuest, PubMed, Sciencedirect, and Google Scholar. Publications included Molecular Autism, Clinical Therapeutics, and PLoS ONE. Exclusion and inclusion of criteria included clinical studies tested alternative therapeutic methods using APT with suramin to repair genetic abnormalities as related to neurodevelopmental disorders, including ASD, and fragile X syndrome. Research included studies and research that supported recent clinical studies conducted by Naviaux et al. on autism drug treatment. The following gene strains on mouse models found APT with suramin to repair damaged synapses and improve behavior:

- APT with polyinosinic:polycytidylic acid (poly(I:C)) mouse model
- FVB and Fragile X (Fmr1) knockout mouse model

Controlled doses of suramin were found to repair damaged synapse and improve behavior related to ASD.

Tests with fragile X mouse model found that APT with suramin positively influenced the synapse structural irregularities, metabolism, and behavior. APT with suramin also showed positive results in environmental MIA model. APT provides new research opportunities into pathogenesis and new drug development for human ASD and other spectrum disorders. The repairing methods and capabilities of APT with suramin can be better understood with additional clinical studies. Recommendations for future research include clinical and preclinical trials on suramin as a novel therapy.

Review

APT

APT is a nucleoside triphosphate that moves chemical energy from cell to cell and assists with metabolism production. Comprised of a purine base, APT is a significant player in metabolic processes. APT is also responsible for extracellular and intracellular signaling. The majority of APT synthesis takes place in the mitochondria [2]. Combination APT and suramin determine suramin’s ability to alter purinergic activity and purine metabolism [3-5]. This is significant because purinergic triggers change under metabolic conditions and purine metabolism is a key translator for biochemicals altered by various treatments, including treatments in the maternal immune activation mouse model (MIA) mouse model of ASD [3-6].
**Suramin** may provide new therapeutic strategies for ASD subtypes [7]. Many clinical studies have explored the scope of APT with suramin to assist with the genetic, metabolic, and environmental risk factors of ASD. The pharmacological components of suramin allow binding against purinergic receptors without releasing it, serving as an antagonist of extracellular APT, and other nucleotides. Suramin has the strongest impact on purine metabolism [3-5].

In recent studies, APT and suramin have been investigated to help correct behavior, genetic irregularities, and metabolism stemmed from neurodevelopmental disorders. A large portion of this review article looks at studies by Naviaux et al., the analysis of suramin and APT, and opportunities for suramin therapy. It was concluded that APT with suramin does assist in correcting genetic abnormalities and environmental predispositions that may impact social behavior related to ASD.

**Gene-environment Interaction**

A variety of genetic risk factors have been identified that link rare mutations with ASD. Using genome-wide techniques and sequence-based tools assist in identifying the influence of rare genetic mutations and ASD, the effect of rare mutations and ASD is better understood. The Autism Genome Project genome-wide association conducted a study investigating the influence of common factors that may increase the risk for ASD, called the genome-wide association study (GWAS) [7]. The Autism Genome Project studied the genomic regions of nucleotide polymorphisms of approximately 1,400 families, including an analysis of genetic family history. The GWAS found a connection of SNP rs4141463 in MACROD2, and an SNP at 5P14.1 and 5P15.2 [7]. Previously, the GWAS did not find a similar link when studying nucleotide polymorphisms. In a similar study of 1,170 individuals of European heritage with ASD and 35307 controls (without ASD), there was no link between the MACROD2 identifier and rs4141463 [7]. To develop an inclusive analysis of ASD risk factors, approximately 1,301 families with ASD and their nucleotide polymorphisms were studied. Using an allele-score method to records the individual’s number of alleles and associated alleles helped determine the variance level of ASD and the genomic regions of nucleotide polymorphisms [8].

The Autism Genome Project genome-wide association studied a total of 2,705 families to assess the individual single nucleotide polymorphisms and other common denominators to better understand the risk factors of ASD, but no single nucleotide polymorphism was identified in relation to ASD [8]. The identified single nucleotide polymorphism found was rs1718101 in a gene called CNTNAP2 [9]. This gene is vulnerable for ASD.

Similarly, a study determining the variances in genes of patients with ASD looked at the genotype arrays by comparing 996 individuals with ASD of European decent to 1,287 individuals (controls) [9]. Although ASD is known to be heritable, Pinto et al. aimed to find genic copy number variants and how this affects other genetic causes of ASD. A high number of unique, genic copy number variants were found de novo and inherited events, respectively, among a single family [9]. This finding complicates ASD genes SHANK2, SYNGAP1, DLGAP2 and DDX53-PTCHD1 [9]. This finding may provide a useful link to understanding the complex connected genetic pathways of ASD.

Casey et al. used homozygous haplotype mapping to determine the genomic variation as the genetic influence of ASD as novel studies have suggested copy number variation as the causation of the genetic influence of ASD [10]. Using homozygous haplotype mapping to explore how and why identical haplotype structures are more common in individuals with ASD compared to individuals without ASD (controls). The study was conducted on
1,402 Autism Genome Project trios, respectively, and 1 million single nucleotide polymorphisms were coded. Findings included 1,218 new genes that may be linked to ASD, and 25 known genes that are linked to ASD. This finding supports the idea that homozygous haplotype regions have genes that linked to ASD and the significance of homozygous haplotype mapping to achieve comprehensive genome-wide data [10]. Genetic predisposition can also identify the possible cause and effect of internal and external factors that may influence gene-environment relations that impact the clinical results of patients with ASD.

Using a method of DNA polymorphism analysis to categorize the different causes and influences in ASD subtypes, Lao investigates genomic analysis as a comprehensive solution for ASD and other neurodevelopment disorders [7]. Lao looked at three genetic pathways to better understand the prognostic significance for individuals with ASD. Mixed sequences of these pathways determine the clinical result of a patient and related medical conditions. Accordingly, the three pathways include:

1. Genetic factors associated with the immune system and inflammatory responses related to ASD pathogenesis;
2. Genetic factors that impact the behavior and function of the main cellular defense tools that assist in reversing oxidative stress;
3. Pharmacogenetics and doses of medical prescription.

Identifying the environmental and genetic causes of a patient with ASD is difficult due to non-determined environmental conditions that may alter the genetic function and capabilities over time. The genetic test proposed by Lao suggests ASD diagnoses based on environmental and genetic factors. By analyzing the neuroplasticity potential and prognosis modifiers, and gaining a better understanding of the nine different clinical subtypes of ASD, a comprehensive treatment plan can be designed specific to each patient and patient needs.

The combination of genetic and environmental influences also plays a large role in the fruition of ASD. Metabolic function influences the genetic disposition of ASD during pregnancy. Metabolic function can be negatively influenced with the additional stress of diabetes, hypertension and obesity [11]. Krakowiak et al. investigated the metabolic conditions during pregnancy and the link to ASD, developmental impairments and developmental delays. Children born in California between the ages of 2-5 participated in the Childhood Autism Risks from Genetics and the Environment study, between January 2003 and June 2010. ASD, developmental impairments and/or developmental delays were assessed via a standardized test. Information about the mother’s health was collected from either medical records, or via an interview [11]. It was found that maternal metabolic function may be linked to neurodevelopmental problems appearing in children, as diabetic mothers of children with ASD scored lower on Mullen Scales of Early Learning. Results were compared to mothers without metabolic conditions. Although this is a small sampling, it may be helpful in developing a stronger understanding of maternal care [11].

In a case-control study from California, Volk et al. found a correlation between areas with high air pollution and increased risk factor for autism [12]. In areas with high air population, prenatal and early childhood exposure was negatively impacted. This includes exposure to traffic pollution during pregnancy and during the first year of life. Maternal infection during pregnancy has also been linked to autism [13]. Studies have linked a high rate of rheumatoid
arthritus, type 1 diabetes, and celiac disease in mothers of children with ASD [14]. This may link autoimmune connections, or abnormal immune systems, as hereditary.

Antipurinergic Therapy

Antipurinergic therapy: Poly(I:C) mouse model
To assess the behavior of purinergic triggers in mice models of ASD, Naviaux et al. investigated the impact of APT with suramin on poly(I:C) mouse models [2-6]. The aim of the study was to better understand the shared mechanisms of ASD and the mitochondrial pathways that influence the metabolic functions of non-infectious cellular stress. To do this, extracellular nucleotides (mitochondrial ATP) and APT were studied [2]. The study was conducted at the University of California, San Diego.

Purinergic triggers and behaviors where tested on the maternal immune activation mouse model (MIA) of ASD [3-5]. Mitochondrial ATP and ADP are part of a series of mitokines that function both inside and outside the cell. Outside the cell, ATP and ADP monitor purinergic receptors that live on the surface of every cell in the body [3-6]. P2X and P2Y are subsets of purinergic receptors that help regulate a spectrum of biological behaviors and mannerisms that impact known genetic dispositions of autism, including taste and senses related to taste [3-6], food allergies [3,7], proper food absorption and proper function of the gastrointestinal tract [2,15-17], and chronic inflammation [2].

The test was designed to understand if increased cellular danger response (CDR) helps manage the metabolic responses to cellular pathogens in MIA adult female mice that were exposed to a synthetic, double strand of poly(I:C), at specific points during pregnancy [2]. Pregnant mice were injected with poly(I:C) to yield offspring with neurodevelopment traits shared in autism and schizophrenia [2]. MIA mouse models were used because of the high levels of cytokines in the fetal environment and fetal brain, which increase the risk of autism and schizophrenia in the offspring [2].

Pregnant mouse models exposed to doses of poly(I:C) at specific points during pregnancy imitate traditional pregnancy risks for offspring. In a study, pregnant mice susceptible to poly(I:C) at two different points during pregnancy (stage E12.5 and stage E17.5) found two different results: the first mouse model exhibited biochemical and metabolic dysfunctions and weakened behavioral and functioning abilities [2]; the second mouse model was shown to have intensified biochemical and metabolic dysfunctions that allowed for a stronger analysis of the traits and behaviors that resembled autism [2]. Accordingly, the biochemical and metabolic dysfunctions created by poly(I:C) were solved with suramin treatment [2,7]. When MIA mouse models were attended with suramin, brain mitochondrial activity was lowered and oxygen consumption in the body was raised [2,7]. Body temperature was also stabilized [2,7].

Normal activity and function blocks communication of cytokines that are influenced by innate immune activation [2]. As innate immunity is triggered by the maternal immune activation (MIA) mouse model, there is an anticipated reduction in quantity of cells in response to ATP. In the MIA mouse model, approximately 50% of synaptosomal FMRP was reduced and levels were stabilized by way of ATP [2,6,18,19]. The reduction of synaptosomal FMRP is evidence that the multisystem abnormalities existing in the MIA mouse model is reduced by way of ATP, despite mice having no genetic reductions of the Fragile X (Fmr1) gene [2]. This finding proved consistent with the hypothesis that insufficient levels of FMRP is related to general CDR by way of hyperpurinergia in this specific view of ASD [2,6,18,19].
In one study, APT in various ASD in the Fmr1 knockout mouse model was tested to determine if APT alleviates anxious social behavior, influences desires and inclinations in new situations, and synapse formation and metabolism [6,18,19]. Methodology included western analysis, behavioral analysis, electron microscopy, mass spectrometry and metabolomics [6,19,20]. Purinergic antagoniste **suramin** was administered at doses of 20 mg/kg via weekly intraperitoneal injections, beginning at 9 weeks of age. Social actions and behavior improved, and metabolism and synaptosomal structure in the brain improved [3-6,18,19]. Specifically, the following irregularities were positively changed: amyloid β precursor protein (APP), complement C1q, TDP43, endocannabinoid, purinergic synaptosomal glutamate, and IP3 receptor expression [4]. A review of the metabolic activity revealed 20 biochemical translations in accompany with improved symptoms, seventeen of which were found in conjunction with traits of ASD in humans. Eleven were in conjunction with the MIA mouse model. The described metabolic translations were also recognized as mediators that assisted the mechanics of preserved cell danger response (CDR) [3-6]. Although MIA and fragile X mental retardation syndrome 1 mouse models of ASD have stark genetic and environmental determinants of ASD, **suramin** and APT treatment was found to reduce 50% of Fmr1 proteins (FMRP) and revive normal FMRP and normal behaviors in the MIA mouse model [6]. When FMRP activity is eliminated genetically, fragile X syndrome develops [6].

In prior studies, the dynamic components and functions of purinergic signaling proved to be an appropriate test drug for MIA mouse models of **autism** [6]. Fragile X mental retardation syndrome 1 was selected as a test because the irregularities in purinergic signaling may be the cause of environmental MIA and the genetic conditions that shape and impact fragile X mental retardation syndrome 1 models [6]. Results concluded that APT aides in alleviating aspects of environmental MIA and genetic fragile X mental retardation syndrome 1 models in ASD mouse models. Environmental and genetic irregularities are related to mitochondria and controlled by purinergic triggers [6].

In a different study by Naviaux et al. APT alleviated social behavior, anxiety during new experiences, and disruption to metabolic function in mouse models with ASD. Naviaux et al. used the MIA mouse model to determine the effect of controlled, single dose of **suramin** on social behavior and metabolic reaction of adult mice [20]. The MIA mouse model was administered with a controlled, single dose of **suramin**. Surmain helped repair social behavior, metabolism, and alleviate **anxiety** during new experiences. Purine metabolism was identified as the most significant regulatory pathway, determined through a metabolomic review. Naviaux et al. reported that 17 out of 18 broken metabolic were normalized by way of purine metabolism [20]. Two days following the **suramin** treatment, the level of **suramin** mixture in the plasma and brainstem was 7.64 μ? [20] Five weeks following the experiment and post drug washout, the positive results seen via APT diminished. Naviaux et al. concluded that purine metabolism had a significant role in the behavior and metabolism of MIA mouse model, and a controlled, single dose of APT with **suramin** assists in alleviating metabolic and behavioral irregularities [20]. Metabolism influences neurotransmission and synaptic plasticity, which can cause symptoms of neurodevelopment disorders, including ASD [20]. This is especially significant during the onset or progression of infection, such as fever, during pregnancy. Metabolic changes that are caused due to infection increase the risk of ASD [20].

MIA mouse models exemplify symptoms of ASD and making pregnant mice vulnerable to poly(I:C) reveals symptoms of ASD in offspring by triggering metabolic response to cell danger
response. Continued existence of cell danger response is a common feature of ASD and Naviaux et al. postulate that purinergic signaling helps stabilize cell danger response. To test the affect of purinergic signaling on cell danger response, Naviaux et al. conducted a metabolomic study on APT in MIA mouse models [20]. A single dose of suramin administered to six-month old mice amended over 90% of disturbances in metabolic pathways, as well as behavioral aberrations. This finding helped determine that purine metabolism and purinergic signaling helps stabilize metabolism and behavior in MIA mouse models with disorders like ASD [20].

Poly(I:C) was injected in pregnant mouse models (>99% pure; <1% mononucleotide content), respectively. Behavioral tests were conducted when mice were nine weeks old. Behavioral tests included: social approach, rotarod, light-dark box, and absence of normal behaviors produced by suramin. At 21 or 26-27 weeks, mice were given suramin or saline and tests were conducted again. The study only included male mice.

Social approach testing was defined as percentage of time socializing with stranger mouse compared to Lego blocks during the first 5 minutes of exposure time. Social approach was observed during 2-4 days, and following 5 weeks of suramin injection [20]. Social approach was defined as the time spent exploring chambers of the three-chamber box. During pilot experiments, mice spent most of their time exploring the first half of each block and social preference was calculated based on the social interactions (sniffing) of the mouse during the first 5 minutes of each phase [20]. Stranger mice were exposed to a wire cup during phase I for at least 30 minutes, respectively [20]. When the test mouse was removed from the chamber in phase II and replaced with an unfamiliar mouse, the unfamiliar mouse was set under one of the wire cups, with Lego blocks. At this time, the test mouse was again placed back in the box and allotted 10 minutes of explore time [20]. Explore time through the chambers, and sniff time, was recorded and scored. Saline and poly(I:C) mice groups were in equal number and based on their social approach scores when mice were 2.25 months old before controlled single-dose with saline, or 6.5 months old with suramin [20].

It was found mice groups exposed to saline and poly(I:C) scored the same as single dose of saline or suramin before the T-maze [20]. T-maze tests determined comfort level with newness by testing how mice reacted to impromptu changes in a maze of black plexiglass. The test was designed according to Frye and Walf [21]. Reaction time and decision time was recorded. Alternated choices were observed at day 2 and day 4, and following 5 weeks of suramin administration.

The rotarod test, adapted by Pallier et al., identified sensorimotor coordination and balance [22]. Mice were allotted 3 opportunities and 30 minutes of break time before another 3 opportunities were granted. With 2 periods, all mice were able to hold balance for 30 seconds. When rotarod time was increased, reaction time was recorded in seconds. The mice began at 4 rpm and advanced to 40 rpm across 5 minutes [20].

Light-dark box test determined how mice reacted to the change in light and dark and how anxiety affected behavior. The box was divided with a light chamber and a dark chamber and met with a door on either side. Mice were exposed to the light chamber and observed for 10 minutes. Time in the light chamber was observed and recorded [22-25].

Absence of abnormal behavior was conducted on non-MIA control animals; these animals were administered with saline as adults. Male mice were used for the studies. Data analysis was conducted using Student’s t-test to compare suramin and saline treatment groups.

FVB and Fragile X (Fmr1) knockout mouse model
To better determine how APT will positively benefit metabolism, behavior, synaptic irregularities as pertaining to mouse models with fragile X syndrome, the genetic loss of the fragile X protein mouse strains of FVB were used to assess the impact of the Fragile X (Fmr1) knockout on the said strain [6]. Mouse behavior was observed and recorded after mice were administered APT with suramin every week for one month. When mice were 13 weeks old, behavioral assessments were conducted. Behavioral tests were described as repulse inhibition mazes, reactions according to spontaneous noise to evoke a startling sensation, marble burying, sensorimotor function, T-maze and social approach [6].

Using mass spectrometry, 60 pathways with 673 metabolites were assessed via partial least squares discriminate analysis (PLSDA), depicted visually and arranged in numerical order based on metabolic changes according to variable importance in projection (VIP) scores [4]. The small sample size found that suramin was responsible for 30% (20/60 pathways) of the pharmacometabolomic alterations in the aforementioned pathways [6]. Fragile X protein knockout was positive. Distinct social conducts and behaviors are significant traits that resemble autism, as observed in mouse models with ASD [6]. When mouse models (Fmr1 null males) were given the opportunity to interact with a “stranger mouse” or an “inanimate object,” mice were 26% less likely to spend time with a “stranger mouse” and 35% of mice experienced a “reduction in social novelty” [6], as determined by time socializing with a new mouse vs. an acquainted mouse [6]. The aforementioned traits that resemble autism in humans were tested with antipurinergic therapy with suramin in mouse models with ASD [6].

ASD

The main finding of the clinical studies was to advance therapies for purinergic signaling that cause ASD and other neurodevelopmental conditions. The authors noted that antipurinergic therapy provides new research opportunities into pathogenesis and new drug development for human ASD and other neurodevelopmental disorders [2]. Clinical studies focused on the purinergic signaling activities of mouse models with ASD to determine the abnormalities associated with autism to suggest alternative treatments.

APT with suramin was found to positively influence the irregularities in the structure of synapses, metabolism and behavior of fragile X knockout mouse models [6]. APT with suramin also showed positive results in environmental MIA model and recent studies have shown positive results in genetic model of fragile X knockout [6]. APT with suramin created the opportunity for cells to stabilize metabolism and revive cell activity [2].

APT with suramin can serve as a new drug to correct the social and behavioral traits similar in MIA models that resemble traits similar to humans with autism [2]. Limitations of the study reflect the range of genetic and environmental causes and influences of ASD. ASD is also translated differently from person to person; meaning, genetic and environmental influences are translated differently from person to person, although common traits exist across the spectrum. Although a number of strains were tested on mouse models, animal models are not ideal test subjects. With additional clinical trials and research, the properties of suramin and antipurinergic therapy with suramin may be a solution for neurodevelopmental disorders.

Suramin treatments for autistic children are not FDA approved. Long-term therapy with suramin is not recommended as it may cause harmful side effects.
Conclusions

Results by Naviaux et al. concluded the medical conditions of ASD as produced by metabolic influences and caused by conserved cell danger response, respectively [2]. APT with suramin allowed cells to stabilize metabolism and revive cell activity [2]. Mitochondrial ATP, known as mitokines, trigger molecules made in the mitochondria, and exchange information about the health of neighbouring cells and possible cell danger by way of purinergic signalling. The study determined APT as a corrective mechanism for 16 irregularities that characterized ASD and respective phenotypes [2]. This included: ERK1/2 and CAMKII signal transduction abnormalities, P2Y2 and P2X7 purinergic receptor expression, correction of the hypothermia, metabolic, mitochondrial, correction of social and locomotor dysfunctions, blockage of cerebellar Purkinje cell loss and preserving the correct form of ultrastructural synaptic dysmorphology [2].

Purinergic signalling abnormalities are of most significance in fragile X syndrome, as they ranked the highest by the multivariate analysis, and are made up of about 20% of the highest numerical value of lipid metabolites [6]. The high activity of purinergic signalling and irregularities are also associated with behaviours and actions resembling autism in humans. Naviaux et al. note that the “inborn errors” located in purine, and pyrimidine metabolism, are characteristics of autism [6]. These cellular irregularities may cause hyperuricosuria, which has been linked to approximately 20% of children who do not have syndromic autism [6]. In 2012 and 2013, the association between purinergic signalling and purine metabolism in ASD was tested in MIA mouse models [2,6]. Recently, brain purinergic signalling was recognized as the most significant gene activity translation that is linked to abnormal behaviours in autistic children [6].

When Naviaux et al. used APT and suramin in the mouse model with fragile X, positive results were yielded for 17 of the 54 proteins that were classified as cerebral synaptosomes of Fmr1 knockout mice. Suramin lowered PI3 Kinase activity in the synaptosomal PI3/AKT/GSK3β pathway, lowered AKT, and raised the blockage phosphorylation of PI3K/AKT pathway protein glycogen synthase kinase 3β (pGSK3βSer9) approximately 47% [6,19,20]. Suramin also raised p70 S6 kinase Phosphorylation (pS6KThr389), a blocker of insulin receptor substrate 1 (IRS1), 46% [6,18,19]. There were no alterations in mTOR activity or function and phosphorylation in cerebral synaptosomes in the mouse model of fragile X were also left unchanged [6,18,19].

Autistic children and adults with fragile X syndrome have common brain abnormalities in the cerebellum, specifically the cerebellar vermis, which impact lobule VII. It was found that the Purkinje cells that were sustained during antipurinergic therapy at week 16 in the MIA model is determined by environmental influences and can be controlled by environmental influences [2]. Antipurinergic therapy reduced the amount of Purkinje cell loss during weeks 6 to 16 [2,6]. The synaptosomal purinergic receptor response of control animals (non-MIA mouse models) administered with suramin was also observed. It was found that “down regulation” did not cause any irregular behaviour or behavioural traits in mice. More studies must be done on purinergic signalling to understand why antipurinergic therapy had no impact on behaviour in healthy mice and had the opposite biochemical impact in healthy mice and mice with autistic behavioural traits [2,6].

In the reviewed fragile X mouse model test, APT with suramin positively influenced the irregularities in structure of synapses, metabolism and behaviour. APT with suramin also showed positive results in environmental MIA model and recent studies have shown positive results in genetic model of fragile X knockout. APT with suramin has the capacity to repair
irregularities presented in fragile X and MIA models because of the unique location near a foundational vehicle, related to mitochondria and controlled by purinergic signalling. This impacts both the progression and performance in both environmental MIA and genetic fragile X [2,6].

Fmr1 knockout mice used in this study had post-synaptic masses that were hypomorphic. Cerebral synaptosomes that resembled standard appearance and standard post-synaptic densities were evident in mouse models treated with suramin [6]. In other studies by Naviaux et al. with MIA mouse models, MIA mouse models treated with APT had post-synaptic masses that were hypomorphic and unsteady. APT was also heavily populated in a specific area [6]. APT fixed the irregularities seen in the synaptic structure [6].

Behavioural traits were observed in the Fmr1 knockout model and compared to a control FVB model. The mice models, however, were not of the same litter and differences in action and etiquette (behaviour) may be a result of differences in genotype and breed [6]. The focal point of the test was to determine how APT with suramin would influence and amend the irregularities in behaviour, metabolism and synaptic organization. While other treatments have been assessed by distinguishing the synaptic irregularities and/or neurotransmitters that translate into various conditions of fragile X in mice, the reviewed study found that APT with suramin plays the same function as the following, as included by Naviaux et al.: metabolic therapies, blockage of metabolic control enzyme glycogen synthase kinase 3β (GSK3β), drug inhibition of glutamatergic signalling with mGluR5 inhibitors, inhibition of endocannabinoid signalling, and genetic inhibition of amyloid β precursor protein (APP) [6].

References


Scutalaria/Skullcap


Wogonin and baicalein are bioactive flavones in the popular Chinese herbal remedy Huang-Qin (Scutellaria baicalensis Georgi). These specialized flavones lack a 4'-hydroxyl group on the B ring (4'-deoxyflavones) and induce apoptosis in a wide spectrum of human tumor cells in vitro and inhibit tumor growth in vivo in different mouse tumor models. Root-specific flavones (RSFs) from Scutellaria have a variety of reported additional beneficial effects.
including antioxidant and antiviral properties. We describe the characterization of a new pathway for the synthesis of these compounds, in which pinocembrin (a 4-deoxyflavanone) serves as a key intermediate. Although two genes encoding flavone synthase II (FNSII) are expressed in the roots of S. baicalensis, FNSII-1 has broad specificity for flavanones as substrates, whereas FNSII-2 is specific for pinocembrin. FNSII-2 is responsible for the synthesis of 4-deoxyRSFs, such as chrysin and wogonin, wogonoside, baicalein, and baicalin, which are synthesized from chrysin. A gene encoding a cinnamic acid–specific coenzyme A ligase (SbCLL-7), which is highly expressed in roots, is required for the synthesis of RSFs by FNSII-2, as demonstrated by gene silencing. A specific isoform of chalcone synthase (SbCHS-2) that is highly expressed in roots producing RSFs is also required for the synthesis of chrysin. Our studies reveal a recently evolved pathway for biosynthesis of specific, bioactive 4-deoxyflavones in the roots of S. baicalensis.

**Glyphosate and heavy metals**


Abstract: The major pesticides of the world are glyphosate-based herbicides (GBH), and their toxicity is highly debated. To understand their mode of action, the comparative herbicidal and toxicological effects of glyphosate (G) alone and 14 of its formulations were studied in this work, as a model for pesticides. GBH are mixtures of water, with commonly 36–48% G claimed as the active principle. As with other pesticides, 10–20% of GBH consist of chemical formulants. We previously identified these by mass spectrometry and found them to be mainly families of petroleum-based oxidized molecules, such as POEA, and other contaminants. We exposed plants and human cells to the components of formulations, both mixed and separately, and measured toxicity and human cellular endocrine disruption below the direct toxicity experimentally measured threshold. G was only slightly toxic on plants at the recommended dilutions in agriculture, in contrast with the general belief. In the short term, the strong herbicidal and toxic properties of its formulations were exerted by the POEA formulant family alone. The toxic effects and endocrine disrupting properties of the formulations were mostly due to the formulants and not to G. In this work, we also identified by mass spectrometry the heavy metals arsenic, chromium, cobalt, lead and nickel, which are known to be toxic and endocrine disruptors, as contaminants in 22 pesticides, including 11 G based ones. This could also explain some of the adverse effects of the pesticides. In in vivo chronic regulatory experiments that are used to establish the acceptable daily intakes of pesticides, G or other declared active ingredients in pesticides are assessed alone, without the formulants. Considering these new data, this assessment method appears insufficient to ensure safety. These results, taken together, shed a new light on the toxicity of these major herbicides and of pesticides in general.

This is the best paper on *glyphosate toxicity* and its effect on all of us, especially the children:


Abstract
A huge increase in the incidence and prevalence of chronic diseases has been reported in the United States (US) over the last 20 years. Similar increases have been seen globally. The herbicide glyphosate was introduced in 1974 and its use is accelerating with the advent of herbicide-tolerant genetically engineered (GE) crops. Evidence is mounting that glyphosate interferes with many metabolic processes in plants and animals and glyphosate residues have been detected in both. Glyphosate disrupts the endocrine system and the balance of gut bacteria, it damages DNA and is a driver of mutations that lead to cancer.

In the present study, US government databases were searched for GE crop data, glyphosate application data and disease epidemiological data. Correlation analyses were then performed on a total of 22 diseases in these time-series data sets. The Pearson correlation coefficients are highly significant (< 10^{-5}) between glyphosate applications and hypertension (R = 0.923), stroke (R = 0.925), diabetes prevalence (R = 0.971), diabetes incidence (R = 0.935), obesity (R = 0.962), lipoprotein metabolism disorder (R = 0.973), Alzheimer’s (R = 0.917), senile dementia (R = 0.994), Parkinson’s (R= 0.875), multiple sclerosis (R = 0.828), autism (R = 0.989), inflammatory bowel disease (R = 0.938), intestinal infections (R = 0.974), end stage renal disease (R = 0.975), acute kidney failure (R = 0.978), cancers of the thyroid (R = 0.988), liver (R = 0.960), bladder (R = 0.981), pancreas (R = 0.918), kidney (R = 0.973) and myeloid leukaemia (R = 0.878). The Pearson correlation coefficients are highly significant (< 10^{-4}) between the percentage of GE corn and soy planted in the US and hypertension (R = 0.961), stroke (R = 0.983), diabetes prevalence (R = 0.983), diabetes incidence (R = 0.955), obesity (R = 0.962), lipoprotein metabolism disorder (R = 0.955), Alzheimer’s (R = 0.937), Parkinson’s (R = 0.952), multiple sclerosis (R = 0.876), hepatitis C (R = 0.946), end stage renal disease (R = 0.958), acute kidney failure (R = 0.967), cancers of the thyroid (R = 0.938), liver (R = 0.911), bladder (R = 0.945), pancreas (R = 0.841), kidney (R = 0.940) and myeloid leukaemia (R = 0.889). The significance and strength of the correlations show that the effects of glyphosate and GE crops on human health should be further investigated.

**Sauna Detox:**

Background. Many individuals have been exposed to organochlorinated pesticides (OCPs) through food, water, air, dermal exposure, and/or vertical transmission. Due to enterohepatic reabsorption and affinity to adipose tissue, OCPs are not efficiently eliminated from the human body and may accrue in tissues. Many epidemiological studies demonstrate significant exposure-disease relationships suggesting OCPs can alter metabolic function and potentially lead to illness. There is limited study of interventions to facilitate OCP elimination from the human body. This study explored the efficacy of induced perspiration as a means to eliminate OCPs. Methods. Blood, urine, and sweat (BUS) were collected from 20 individuals. Analysis of 23 OCPs was performed using dual-column gas chromatography with electron-capture detectors. Results. Various OCPs and metabolites, including DDT, DDE, methoxychlor, endrin, and endosulfan sulfate, were excreted into perspiration. Generally, sweat samples showed more frequent OCP detection than serum or urine analysis. Many OCPs were not readily detected in blood testing while still being excreted and identified in sweat. No direct correlation was found among OCP concentrations in the blood, urine, or sweat compartments. Conclusions. Sweat analysis may be useful in detecting some accrued OCPs.
not found in regular serum testing. Induced perspiration may be a viable clinical tool for eliminating some OCPs.

**Glialia**


Autism spectrum disorder (ASD) is a condition defined by social communication deficits and repetitive restrictive behaviors. Association of the fatty acid amide palmitoylethanolamide (PEA) with the flavonoid luteolin displays neuroprotective and antiinflammatory actions in different models of central nervous system pathologies. We hypothesized that association of PEA with luteolin might have therapeutic utility in ASD, and we employed a well-recognized autism animal model, namely sodium valproate administration, to evaluate cognitive and motor deficits.

From the text: co-ultraPEA-LUT®, at a dose of 700 mg+70 mg b.i.d. (Glialia® microgranules, Epitech Group SpA, Italy) for 1 year

**Toxic Metals in Autism**


Genetic and environmental factors contribute to the etiologies of autism spectrum disorder (ASD), but evidence of specific environmental exposures and susceptibility windows is limited. Here we study monozygotic and dizygotic twins discordant for ASD to test whether fetal and postnatal metal dysregulation increases ASD risk. Using validated tooth-matrix biomarkers, we estimate pre- and post-natal exposure profiles of essential and toxic elements. Significant divergences are apparent in metal uptake between ASD cases and their control siblings, but only during discrete developmental periods. Cases have reduced uptake of essential elements manganese and zinc, and higher uptake of the neurotoxin lead. Manganese and lead are also correlated with ASD severity and autistic traits. Our study suggests that metal toxicant uptake and essential element deficiency during specific developmental windows increases ASD risk and severity, supporting the hypothesis of systemic elemental dysregulation in ASD. Independent replication in population-based studies is needed to extend these findings.

**Why we are not well** (I suggest you get the whole article)

Abstract
Medicine and public health are compromised by the highest echelons of science, industry and public administration for the geopolitical objectives of international cohabitation, preservation of resources, environmental conservation and decarbonization, all of which hinge on depopulation. Under the cover of reproductive health involuntary sterilizations are implemented throughout the developing world through adulterated vaccines while in the developed world flu immunization programs weaken the immune systems of the old and civil servants to shorten lifespans and spare governments from meeting insolvent health care and pension plan obligations in the last stage of the demographic transition. Endocrine disruptors inserted in the basic elements of life to presumably prevent caries chronically subvert the human reproductive system to lower the total fertility rate of every country to replacement level. In the name of sustainable development, experimental carbon capture and sequestration methods as well as solar radiation management methods double as weapons against longevity by subjecting billions to unnaturally high exposure levels of heavy metals so the world’s decarbonization goals are tackled from two directions, by reducing greenhouse gases in the atmosphere and increasing morbidity and mortality among the general population to proactively lower future emissions. Poverty and hunger are used as fronts for the deployment of GMO crops that purportedly increase yields, improve nutrition and require fewer fertilizers and pesticides, but that in fact misuse the latest bioengineering advances to cause subfertility, immune deficiencies and crop failures and thus lower the population by limiting births and increasing deaths. Unless stopped, this engineered genocide will damage the genetic and intellectual endowment of humanity and cause population collapse within 20 years, time during which the incidence and severity of NCDs will grow exponentially irrespective of health system investments and medical breakthroughs. Only a political solution can restore our health as individuals and as a civilization.


2 Vaccine studies indicating a 14 fold increase in chronic allergic conditions and neurological disorders in the vaccinated vs unvaccinated children:

1. “Pilot comparative study on the health of vaccinated and unvaccinated 6- to 12-year-old U.S. children”
Anthony R Mawson, Brian D Ray, Azad R Bhuiyan and Binu Jacob
J Transl Sci, 2017 Volume 3(3): 1-12 (full text on the KI website – can strangely not be found anymore on Google search engine)

**Important and relevant other recent helpful references**


Environment


Diet against inflammation


Sulphoraphane/Diet


Fever


More on Diet


