Intranasal Administration of Toxicants and Therapeutics

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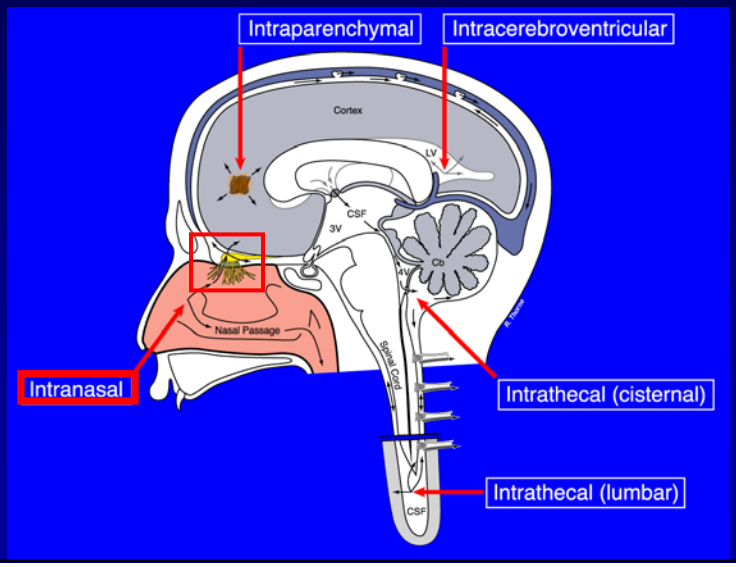
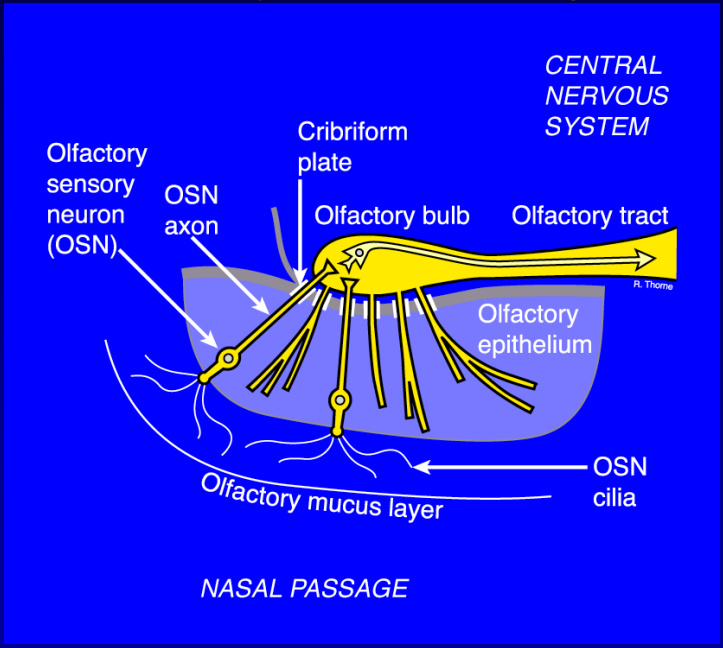
Invasive and Noninvasive Routes



Extracellular Delivery of Intranasal Drugs to the CNS



Robert Thorne, Ph.D. (2002) University of Minnesota Thesis.

INTRANASAL DELIVERY TO THE CNS

- Is non-invasive.
- Bypasses the blood-brain barrier.
- Results in rapid **extracellular delivery** to the brain and spinal cord along both the **olfactory and trigeminal pathways** involving perineural and perivascular channels.
- Reduces or eliminates systemic exposure.
- Does not require modification of the therapeutic or toxic agent.
- Works best for potent compounds active in the nanomolar range or very low micromolar range.

Intranasal Delivery Bypasses the BBB to Target the Following Classes of Agents to the CNS:

Neuropeptides: Melanocortin, exendin(9-39), hypocretin-1

Neurotrophins: NGF, IGF-I, FGF-2, EGF, ADNF, GDF-5

Cytokines: Interferon beta-1b, erythropoietin

Hormones: Insulin, leptin, progesterone, estradiol

Small Drugs: Methotrexate, carbamazepine, deferoxamine

Polynucleotides: GRN-163 (glioma therapeutic agent)

Genes: DNA plasmids & viral vectors

Therapeutic Cells: Stem Cells

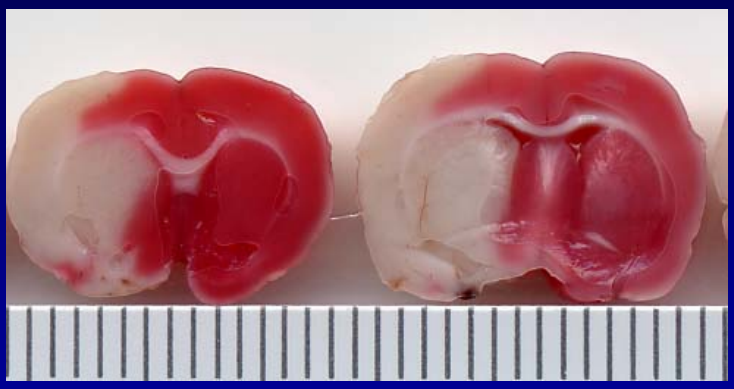
Toxicants: MPTP, DEET?, permethrin?

Intranasal Delivery to the Primate CNS

Monkeys	***Humans***
Estrogen	Insulin
Progesterone	NAP (neuroprotective peptide)
Dopamine	Bremelanotide
Colloidal Gold	L-Name
Viruses	MSH/ACTH$_{4\text{-}10}$
Interferon beta	Atrial Natriuretic Peptide
Prostaglandin D2 Analog	Oxytocin
Hypocretin-1	Cholecystokinin
	Cocaine, Heroin

Our lab at the SFVAH has a long-term interest in neuroprotection, focusing on two different models relevant to the VA Healthcare System: stroke and traumatic brain injury.

We use a model of stroke in rats. To create a stroke, the middle cerebral artery is occluded using a suture. The occlusion lasts for two hours, at which time the suture is withdrawn and blood flow is restored. At different time points afterwards, we conduct behavioral tests, and approximately one week following stroke, the animals are euthanized and the amount of tissue damage caused by the stroke (infarct volume) is measured.



Generally, rats subjected to stroke suffer significant weight loss, and our Animal Use and Care Committee regulations state that a weight loss of >15% requires that the animal be sacrificed. This stroke model is relatively severe, and the infarct extends into brain areas that control appetitive behavior. Animals are weighed daily, and 100% of those subjected to stroke will require euthanasia between one and two weeks post-surgery.

AN EARLIER PAPER HAD DEMONSTRATED THAT I.P. DEFEROXAMINE (DFO) (100 MG/KG) PROVIDED NEUROPROTECTION AGAINST STROKE SURGERY PERFORMED 48-72 HOURS LATER.

SINCE IT IS DOUBTFUL THAT DFO WILL BE ADMINISTERED I.P. TO HUMANS, WE DECIDED TO TRY AND ADMINISTER DFO INTRANASALLY (I.N.) TO PROTECT AGAINST SUBSEQUENT STROKE (PATIENT POPULATION WOULD BE THOSE UNDERGOING VASCULAR SURGERY).

DFO HAS A SHORT PLASMA HALF LIFE (0.3 HOURS), AND ACUTE I.V. ADMINISTRATION CAUSES A RAPID DROP IN BLOOD PRESSURE THAT CAN BE LIFE-THREATENING. IT HAS BEEN USED CLINICALLY (ADMINISTERED SUBCUTANEOUSLY) FOR OVER 4 DECADES FOR THE TREAT-MENT OF CHRONIC TRANSFUSION-RELATED IRON OVERLOAD.

ANEMIAS THAT REQUIRE TRANSFUSION

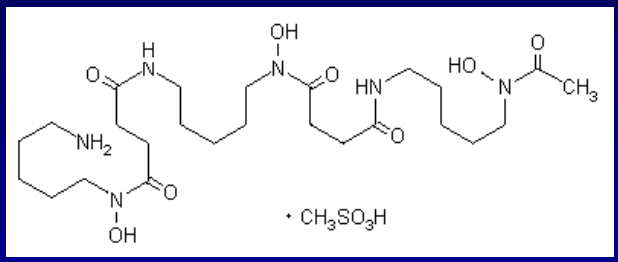
PATIENT POPULATION:
B-THALASSEMIA
SICKLE CELL DISEASE
MYELODYSPLASTIC SYNDROMES

B-THALASSEMIA—ANEMIA THAT REQUIRES TRANSFUSION OF 1-2 UNITS OF BLOOD EVERY 2-3 WEEKS STARTING AS EARLY AS 3 YOA. EACH UNIT OF BLOOD CONTAINS ~ 225 MG OF IRON.

IRON ACCUMULATION

THE HUMAN BODY HAS NO METHOD TO EXCRETE IRON EXCEPT TO BLEED, BUT ANEMIC PATIENTS CAN NOT BE BLED. THEREFORE, IRON IS REMOVED BY CHRONIC IRON-CHELATION THERAPY.

DEFEROXAMINE (DFO) OR DESFERAL



DEFEROXAMINE IS A COMPOUND SECRETED BY *STREPTOMYCES PYLOSIS* TO SECURE FE(III) FROM THE ENVIRONMENT. ITS AFFINITY FOR FE(III) IS 10^{31}, AND IT FORMS A HEXADENTATE CHELATE WITH IRON, OCCUPYING ALL SIX COORDINATION POSITIONS.

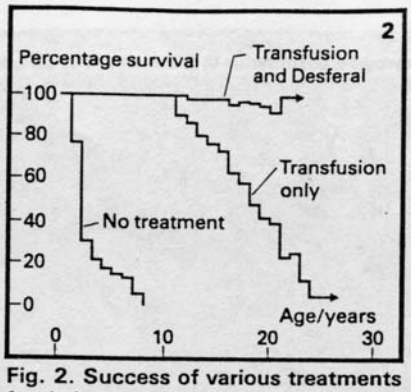


Fig. 2. Success of various treatments for thalassaemia.

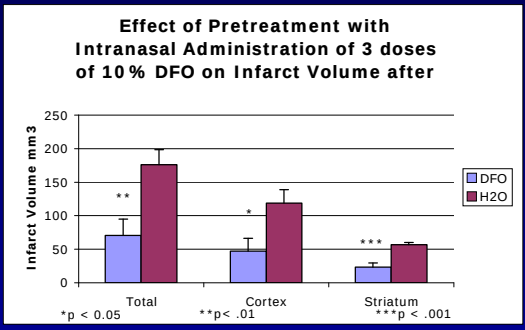
PRETREATMENT PARADIGM:

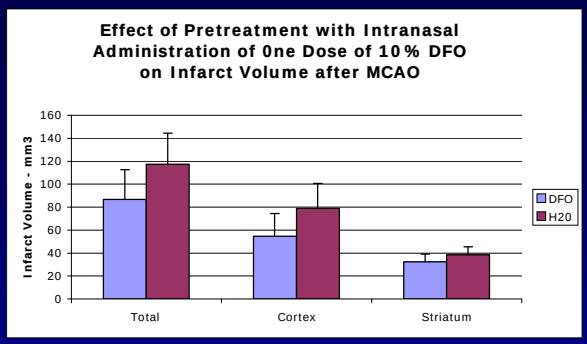
1 DOSE OF DFO = 6 MG (I.N. 60 MICROLITERS), GIVEN OVER A 20-MIN PERIOD TO ALTERNATE NOSTRILS, 6 MICROLITERS AT A TIME EVERY 2 MINUTES, ALTERNATING NOSTRILS. THREE DOSES ARE GIVEN, THREE HOURS APART.

DOSE, WAIT THREE HOURS
DOSE, WAIT THREE HOURS
FINAL DOSE.

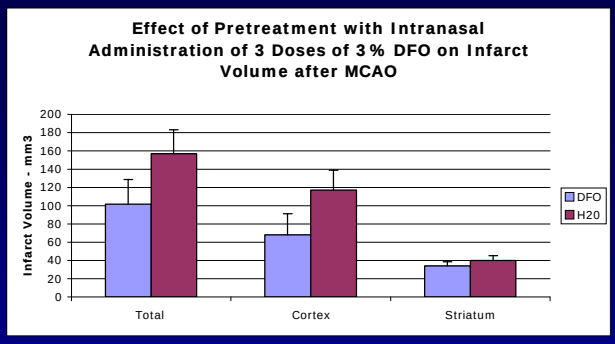
48 HOURS LATER, A 2-HR MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO) IS PERFORMED. ANIMALS ARE SACRIFICED 5 DAYS FOLLOWING MCAO, AND THE BRAINS ARE REMOVED, SECTIONED IN A BRAIN MATRIX, AND STAINED WITH TTC. THE INFARCTED AREAS ARE QUANTIFIED USING NIH IMAGE AND EXPRESSED AS TOTAL INFARCT, CORTICAL INFARCT, OR STRIATAL INFARCT (TOTAL = CORTICAL + STRIATAL).

INFARCT SIZE IS REDUCED 65% BY I.N. DFO PRETREATMENT

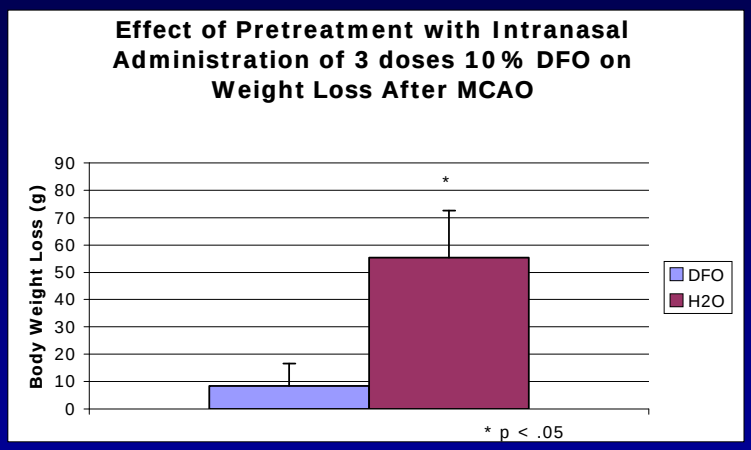




RESULTS WERE NOT SIGNIFICANT WHEN COMPARED TO CONTEMP. CONTROLS, BUT SIGNIFICANCE WAS OBTAINED WHEN TESTED AGAINST POOLED CONTROLS FROM ALL EXPERIMENTS, N = 34

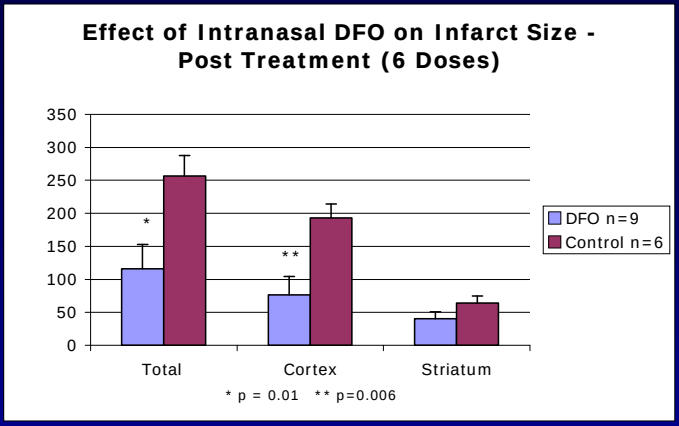


RESULTS WERE NOT SIGNFICANT WHEN COMPARED TO EITHER CONTROL GROUP.



NEW PARADIGM: POST-TREATMENT

ANIMALS WERE SUBJECTED TO A 2-HR MCAO, AND IMMEDIATELY FOLLOWING THE START OF REPERFUSION, I.N. DFO ADMINISTRATION COMMENCED. SIX DOSES (6 MG) WERE GIVEN, THREE ON THE DAY OF STROKE, AND THREE ON THE DAY FOLLOWING STROKE. AFTER ANIMALS WERE SACRIFICED AND INFARCT VOLUMES WERE QUANTIFIED, THE RESULTS SHOWED:



TREATMENT WITH I.N. DFO FOLLOWING STROKE REDUCED INFARCT SIZE BY 50%.

IN CONCLUSION, DFO DELIVERED TO THE CNS BY I.N. ADMINISTRATION, PENETRATES INTO BRAIN TISSUE AND CONFERS NEURO-PROTECTION, EITHER AS A PRETREATMENT OR AS A POST-TREATMENT.

MORE SIGNIFICANTLY, I.N. DFO MAY FIND UTILITY FOR THE TREATMENT OF CNS DIS-ORDERS SUCH AS AD, PARKINSON'S, MS, STROKE, TBI, OR ICH. OTHER CHELATORS MAY ALSO BE DELIVERED BY THE I.N. ROUTE.

Intranasal Delivery of Toxicants

Documented Insecticides or Pesticides Used in the Gulf War:

The requirement for pesticide use in the Kuwait Theater of Operation (KTO) arose due to the prevalence of pests such as filth flies, sand flies, mosquitoes, fleas, and lice indigenous to this part of the Middle East. These insects carry several infectious diseases, including leishmaniasis, sand fly fever, malaria, and typhus. Unabated, these diseases were believed to be capable of incapacitating a large number of the US and Coalition fighting force.

To combat this threat more than 60 different pesticide products and formulations were used during the Gulf War. This total includes a variety of products that include sprays, powders, baits, pest strips, and flypaper. Depending on its intended use and the pest it was formulated to target, most of the pesticide products consisted of at least one active ingredient and one or more inert ingredients. A complete analysis of all pesticides used during the Gulf War was not practical. Therefore, the Office of the Special Assistant's (OSA) report focused on those considered to have the greatest potential to cause adverse health effects – those that were considered to be the pesticides of potential concern (POPCs).

Many Undocumented Insecticides and Pesticides Were Mailed by Families and/or Friends of Warfighters

e.g. compounds used in veterinary or agricultural applications

We wanted to focus on DEET. The first problem we encountered was finding a commercial source of pure DEET. Most commercial products contain concentrations of DEET ranging between 10 and 35% with other compounds. So we used REI's Jungle Juice (RJJ), which is stated to be 100% DEET.

We administered RJJ to anesthetized rats using the same paradigm as DFO pretreatment studies.

RJJ was diluted with mineral oil, which also served as the control. Rats receiving RJJ in mineral oil immediately awakened from anesthesia and exhibited a variation of a simple partial seizure. They immediately raised their heads and gnashed their teeth, repetitively opening and shutting their jaws.

This behavior was not observed in control rats, treated with mineral oil, alone. These data are consistent with the information from the DEET registry, maintained by Dr. Tom Osimitz. The registry reports that the most commonly reported adverse event from DEET exposure was a seizure. DEET seems to stimulate CNS activity, particularly when administered via the intranasal route.

We could not obtain samples of pure premethrin, but we have since located a source of pure DEET, pure premethrin, and radiolabeled versions of both molecules (Dr. William Reifenrath).

A recent report from Dr. William Frey II and colleagues demonstrated that intranasal administration of bone marrow-derived stem cells to animals that have a chemically-induced version of Parkinson's Disease (PD) effectively reverses the tissue damage and motor deficits observed in the animals with PD.

We recently received funding to administer human embryonic stem cells—neuronal precursor cells—to pig brain. The intranasal administration of such cells to individuals suffering from neurological symptoms associated with Gulf War deployment may significantly improve prognosis.

CAVEAT:

"ANY SUFFICIENTLY ADVANCED TECHNOLOGY IS INDISTINGUISHABLE FROM MAGIC."

ARTHUR C. CLARK

MANY REVIEWERS, INCLUDING THE CHIEF OF NEUROLOGY AT THE SFVAH, SIMPLY SAY "I DON'T BELIEVE IT."

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