INTRAMUSCULAR (IM) + INTRANASAL (IN) RAPAMYCIN - A NEW PARADIGM

AUG 21, 22

A world first for human in vivo rapamycin delivery protocol? A quantum leap in human translation of mTOR longevity science? HIGH

LEVEL SUMMARY

- **57 yo male, very high muscular/aerobic fitness, one meal a day, strict plant based ketogenic diet for last 6 years, high daily exerciser, TRT, 8 week regular blood donation • No metabolic response to oral rapamycin dosing, deep dive rapamycin literature, exploring alternate drug delivery routes/formulations for superior AUC/mTOR inhibition/lesser side effects profile**
- **6 week update, weekly dosing of a novel human rapamycin drug delivery protocol • Experimentation with Intramuscular (IM) rapamycin (rapamycin powder at 30mg/mL in DSMO) @ 15mg/week**
- **Experimentation with Intranasal (IN) rapamycin (rapamycin powder at 30mg/mL in DSM) @ 3mg/nasal cavity/week**
- **Trough level Sirolimus 11.1 ng/mL, AUC @ 168 hrs = 6600 ng*hr/mL • IM + IN delivered 10X AUC of 15mg ORAL and 20X AUC of 6mg/week ORAL • Elevation in TG (TC, LDL, HDL same/lower), slight increase FG, hbA1c, no low WBC counts**
- **Crossover into clinical iron deficiency anemia (but largely manifested by long term chronic phlebotomy as a targeted low level iron store management, a separate n=1 protocol). High dose rapamycin appears to have induced the crossover.**
- **NO SIGNIFICANT SYMPTOMATIC SIDE EFFECTS (100% normal daily activities, including continued daily high resistance/aerobic protocol)**
- **Short term, high dose parenteral rapamycin is unsurprisingly quite safe • Ongoing dosing protocol for metabolic/side effects impact discovery with longer intervention looking for side effects, CBC dysregulation, and any transiency in markers *Is bypassing 1st pass metabolism/liver gene expression of oral rapamycin delivery with a fundamentally parenteral IM + IN drug delivery leading to a new "paradigm" of higher pan tissue rapamycin penetration (including brain) and lower side effects (symptomatic and biomarker) equating to a much higher lifespan extension mTOR inhibition translation in humans?***

****THIS IS NOT MEDICAL ADVICE. I AM NOT A MEDICAL PROFESSIONAL. THIS IS N=1 HIGH RISK EXPERIMENTATION. REPLICATING ANY OF THE INTERVENTIONS DISCLOSED HEREIN MAY CAUSE SEVERE (AND IRREVERSIBLE) BODILY HARM****

SEMINAL RODENT/RAPAMYCIN LONGEVITY LITERATURE

Dosing of rapamycin is critical to achieve an optimal antiangiogenic effect against cancer

https://sci-hub.se/10.1111/j.1432-2277.2004.00026.x/

"Here we investigated effective dosing schedules against tumors and angiogenesis. Growth of established CT-26 colon adenocarcinoma tumors was measured in Balb/c ***mice treated with total equivalent rapamycin doses (1.5 mg/kg/day) given once a day, once every 3 days, or by continuous infusion***. ***Tumors were most inhibited with continuous rapamycin infusion, and less by bolus dosing. Interestingly, however, continuous dosing produced the lowest rapamycin blood levels (15 ng/ml***). As rapamycin-sensitive p70S6-kinase intracellular signaling is critical for angiogenesis, p70S6-kinase activation was measured in endothelial cells by Western blotting. Maximal p70S6-kinase inhibition occurred from 1–5 ng/ml rapamycin. These same rapamycin concentrations optimally blocked vessel-sprouting from cultured aortic rings. Therefore, low level rapamycin dosing most effectively controls tumors in mice. Importantly, antiangiogenic rapamycin levels are compatible with immunosuppressive doses, supporting its potential use in transplant patients with cancer. ***It is interesting to consider the observation that continuous delivery of rapamycin provided the most effective in vivo treatment regimen. This finding is similar to what has been observed with other known antiangiogenic agents, where low-level continuous-delivery methods induce the greatest anti-tumor effect***. The advantage of this type of therapy is that side effects tend to be low, making it possible to use this approach over a long period of time to maintain constant 'pressure' against tumor expansion. It is notable that as antiangiogenic effects are directed against normal vessel cells, and not cancer cells, drug resistance to this form of therapy is less likely."

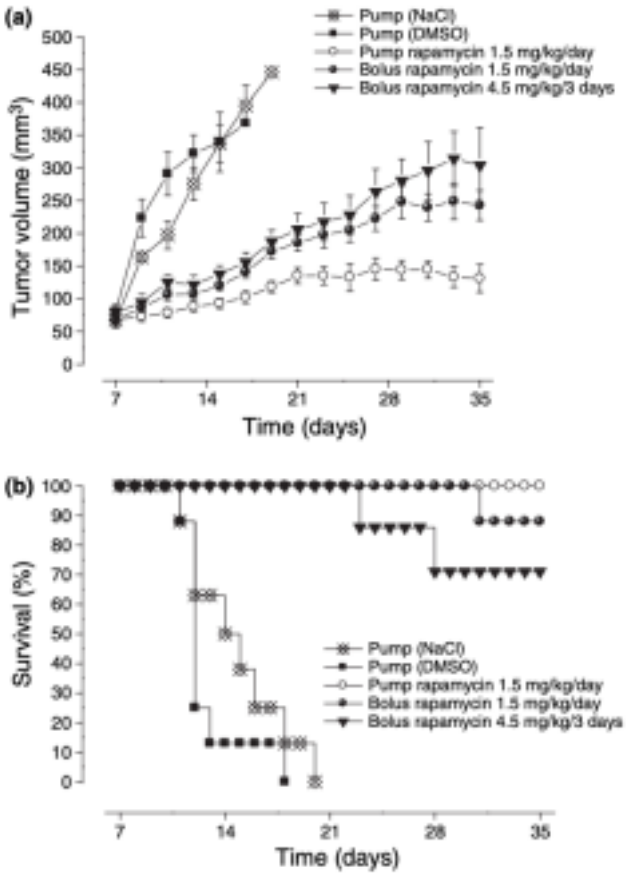


Figure 1 Effect of different rapamycin delivery schedules on CT-26 colon adenocarcinoma growth in Balb/c mice. Subcutaneous CT-26 tumors were established for 7 days before beginning treatment with rapamycin. Treatment regimens consisted of rapamycin given i.p. at 1.5 mg/kg/day ($n = 8$), 4.5 mg/kg once every 3 days ($n = 8$), or 1.5 mg/kg/day by a continuous infusion pump ($n = 7$). Controls for i.p. injections were given saline each day ($n = 8$), or for pump controls, pumps with DMSO carrier solution were placed in mice ($n = 8$). (a) The tumor volume (mean ± SEM) measured every other day after treatment; (b) animal survival in each of the test groups. There was a significant difference in tumor volume in continuous infusion mice after day 11, compared with either of the two bolus injection groups ($P \leq 0.03$).

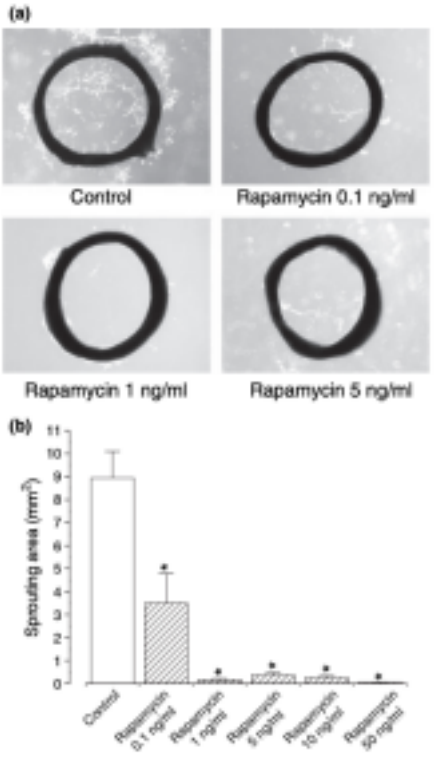


Figure 2 Dose-response effect of rapamycin on *in vitro* angiogenesis. Different rapamycin doses were tested in an assay measuring vascular-sprouting from cultured rat aortic rings. (a) Photomicrographs of aortic rings 4 days after culture with increasing concentrations of rapamycin. Rapamycin blocked vessel sprouting at concentrations ≥1 ng/ml. (b) Quantitative computer-assisted analysis of the sprouting area around the aortic rings. Results from the complete range of rapamycin concentrations tested is shown (0.1–50 ng/ml); *$P < 0.05$, versus control. Values are expressed as the mean ± SEM obtained from four separate experiments.

Figure 3 Effect of rapamycin on p70S6-kinase activation. HUVEC were cultured with increasing concentrations of rapamycin, and VEGF-induced phosphorylation of p70S6-kinase at the Thr389 site was measured by Western blotting. 'Full' stimulation refers to culturing of HUVEC with medium containing fetal bovine serum and supplemental growth factors. 'Diet' conditions refer to culturing without either fetal bovine serum or supplements. In the experiment, one control HUVEC culture was maintained under 'full' culture conditions, and the remainder were put under 'diet' conditions for 10 h, then rapamycin was added for 1 h, as indicated. To induce p70S6-kinase phosphorylation in the presence or absence of rapamycin, VEGF (50 ng/ml) was added to selected cultures for 20 min, and all cells were subsequently harvested for Western blotting. Blots were probed with an antibody against phospho(Thr389 site) p70S6-kinase, and loading of protein was controlled with a β-actin-specific antibody.

The above paper back in 2003 showed that a continuous low dosing protocol in vivo lead to the highest survival rate in this rodent model of adenocarcinoma. So this paper suggested that for cancer mitigation, maintaining a constant low dose of rapamycin was OPTIMAL. ***Thus, dosing that causes (allows) mTOR inhibition to dip below a biological threshold (ie. likely the vast majority of many current oral intermittent regimes) may NOT provide maximum prophylactic longevity benefit.***

A seminal study on mice/rapamycin back in 2009 that initiated the interest with rapamycin as a lifespan extending intervention.

Rapamycin fed late in life extends lifespan in genetically heterogeneous mice (2009)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2786175/pdf/nihms127666.pdf

"Inhibition of the TOR signalling pathway by genetic or pharmacological intervention extends lifespan in invertebrates, including yeast, nematodes and fruit flies. However, whether inhibition of mTOR signalling can extend life in a mammalian species was unknown. We report here that

rapamycin, an inhibitor of the mTOR pathway, extends median and maximal lifespan of both male and female mice when fed ***(ORALLY***) beginning at 600 days of age. Based on age at 90% mortality, rapamycin led to an increase of 14% for females and 9% for males. Disease patterns of rapamycin treated mice did not differ from those of control mice. In a separate study, rapamycin fed to mice beginning at 270 days of age also increased survival in both males and females, based on an interim analysis conducted near the median survival point. Rapamycin may extend lifespan by postponing death from cancer, by retarding mechanisms of ageing, or both. ***These are the first results to demonstrate a role for mTOR signalling in the regulation of mammalian lifespan, as well as pharmacological extension of lifespan in both genders. These findings have implications for further development of interventions targeting mTOR for the treatment and prevention of age-related disease"***

Table 1
The effect of rapamycin on maximum lifespan

Comparison	Sites	Age at 90th Percentile for Controls (UL*)	Age at 90th Percentile for Rapa	Percent Increase
Females, Rapa vs Control	All sites	1094 (1136)	1245	14%
Females, Rapa vs Control	TJL	1100 (1165)	1282	17%
Females, Rapa vs Control	UM	1094 (1149)	1250	14%
Females, Rapa vs Control	UT	1089 (1159)	1179	8%
Males, Rapa vs Control	All sites	1078 (1111)	1179	9%
Males, Rapa vs Control	TJL	1035 (1091)	1142	10%
Males, Rapa vs Control	UM	1141 (1177)	1188	4%
Males, Rapa vs Control	UT	1020 (1101)	1179	16%

* Note: "UL" = upper limit of the 95% confidence interval for control mice. For example, top row, for females pooled across sites, the 95% confidence interval for controls goes up to 1136 days, and the estimate for 90th percentile survival for the rapamycin-treated mice is 1245 days. This gives good evidence that the 90th percentile survival for Rapa (1245) is substantially above that for Controls (1094).

mTOR Inhibition Alleviates Mitochondrial Disease in a Mouse Model of Leigh Syndrome
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4055856/pdf/nihms584331.pdf

"***We first examined the effects of delivering rapamycin (8 mg/kg) every other day by intraperitoneal injection beginning at weaning [approximately postnatal day 20 (P20)]. This treatment reduces mTOR signaling in wild-type mice and provided significant increases in median survival of male (25%) and female (38%) knockout mice*** (Fig. 1A). A slight reduction in maximum body size and a delay in age of disease onset were also observed (Fig. 1B and fig. S2). ***Although these results showed that Ndufs4−/− mice benefit from rapamycin treatment, we noted that by 24 hours after injection, rapamycin levels in blood were reduced by more than 95% (fig. S3).*** We therefore performed a follow-up study delivering rapamycin (8 mg/kg) daily by intra-peritoneal injection starting at P10, which resulted in blood levels ranging from >1800 ng/ml immediately after injection to 45 ng/ml trough levels (fig. S3). ***For comparison, an encapsulated rapamycin diet that extends life span in wild-type mice by about 15% achieves steady-state blood levels of about 60 to 70 ng/ml, and trough levels between 3 and 30 ng/ml are***

recommended for patients receiving rapamycin (10). In the daily-treated cohort, we observed a striking extension of median and maximum life span; the longest-lived mouse survived 269 days. Median survival of males and females was 114 and 111 days, respectively (fig. S2C)

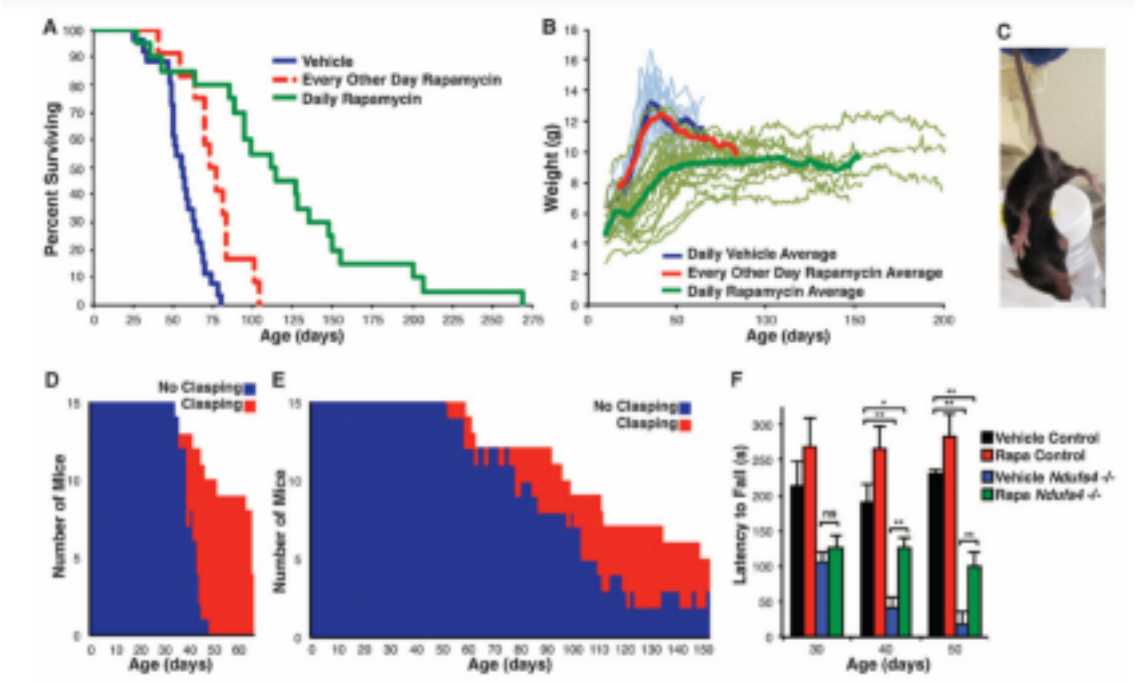


Fig. 1. Reduced mTOR signaling improves health and survival in a mouse model of Leigh syndrome

(**A**) Survival of the *Ndufs4*$^{-/-}$ mice was significantly extended by rapamycin injection every other day; life span more than doubled with daily rapamycin treatment (log-rank $P = 0.0002$ and $P < 0.0001$, respectively). (**B**) Body weight plots of *Ndufs4*$^{-/-}$ mice. (**C**) Representative forelimb clasping behavior, a widely used sign of neurological degeneration. Clasping involves an inward curling of the spine and a retraction of forelimbs (shown here) or all limbs toward the midline of the body. (**D** and **E**) Clasping in vehicle-treated (D) and daily rapamycin-treated (E) *Ndufs4*$^{-/-}$ mice as a function of age. A total of 15 mice were observed for clasping daily for each treatment. Age of onset of clasping behavior is significantly delayed in rapamycin-treatedmice (**P<0.001 by log-rank test). (**F**) *Ndufs4*$^{-/-}$ mice show a progressive decline in rotarod performance that is rescued by rapamycin (*$P <$ 0.05, **$P < 0.005$, Student's t test; error bars are ± SEM). (See also fig. S5, which indicates replicate numbers.)

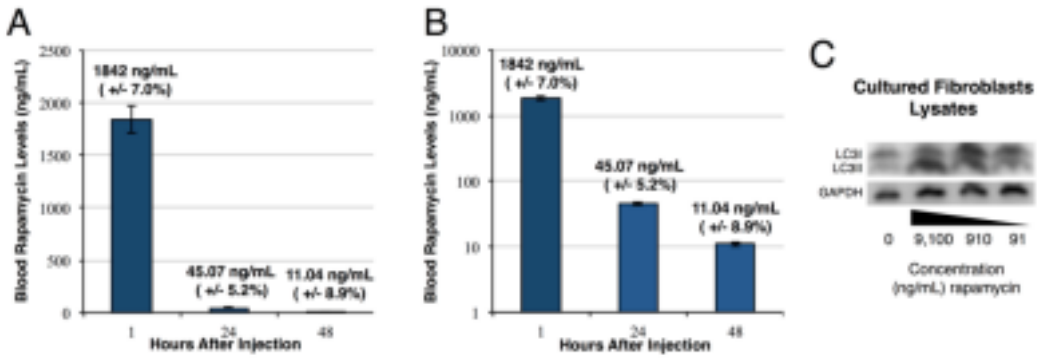


Fig. S3

Blood levels of rapamycin following injection of 8mg/kg. (A-B) Analysis of blood levels of rapamycin show a rapid decrease in blood levels by 24 hours post-injection (shown in linear (A) and log (B) scale). (C) Western blotting for LC3, a marker of mTOR inhibition, treated with rapamycin for 6 hours suggests that levels of rapamycin in mice at 24 hours post-injection are below levels necessary to induce pathways downstream of mTOR. Error bars represent +/- standard error of the mean (SEM), n=3 animals per data point.

More than 100% lifespan extension with daily injection…but critically, ONLY with higher AUC, avoiding the low trough levels, and maintaining higher steady state levels of rapamycin. A couple of key FUNDAMENTAL TRANSLATION notables…method of delivery (IP injection), high systemic AUC exposure, and maintenance of a minimum threshold of rapamycin blood level.

A subsequent study in 2014 of mice/rapamycin at HIGHER oral doses showing further increases in lifespan extension.

Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4032600/pdf/acel0013-0468.pdf

"Rapamycin (***fed ORALLY at 42 ppm***), an inhibitor of mTOR kinase, increased median lifespan of genetically heterogeneous mice by 23% (males) to 26% (females) when tested at a dose threefold higher than that used in our previous studies; maximal longevity was also increased in both sexes. It seems likely that rapamycin-mediated increases in lifespan reflect both broad spectrum antitumor effects as well as deceleration of aging processes more generally. There is now good evidence that rapamycin can alter glucose metabolism after various times and levels of exposure in mice. These results argue that rapamycin can slow the effects of aging on many cell and tissue types and suggest that postponement of lethal neoplasia could be mediated by direct effects both on the tumor cells and on age sensitive antitumor defenses. There are, however, age dependent changes in male C57BL/6 mice which are not prevented by rapamycin, and some of

the protective effects of this drug appear to reflect immediate benefits rather than delay in age dependent change”

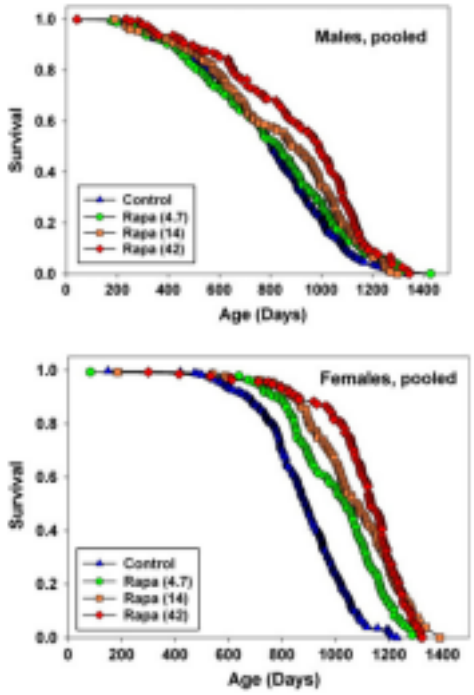


Fig. 1 Survival curves at varying doses of rapamycin. Survival curves for male (top) and female (bottom) mice exposed to varying doses of rapamycin from 9 months of age. Data are pooled across the three test sites. At the time of analysis, fewer than 1% of the mice were still alive. Significance tests, median age, age of 90% mortality, and numbers of mice are shown in Table 1.

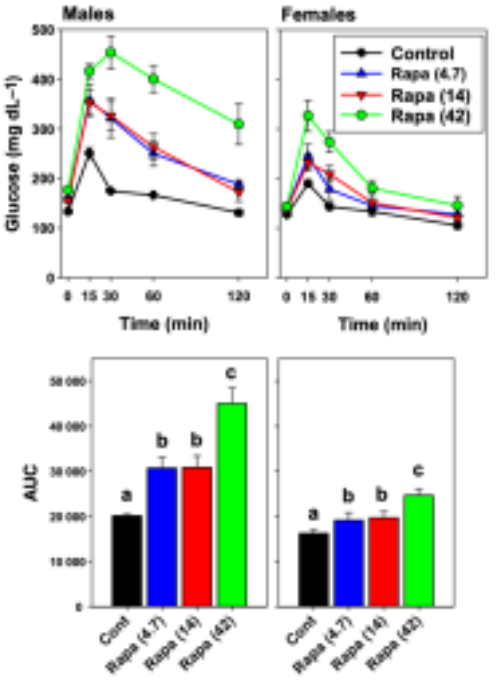


Fig. 4 One-month exposure to rapamycin impairs glucose tolerance. UM-HET3 mice, at 4 months of age, were placed onto diet containing the indicated doses of rapamycin at UT and evaluated 1 month later for responses to intraperitoneal glucose injection. $N = 5$ of each treatment group for each sex. The upper panels show mean ± SEM for plasma glucose at the indicated times. The bottom panels show integrated area under the glucose curve as mean ± SEM; bars that share a letter code are not statistically significant by t-test.

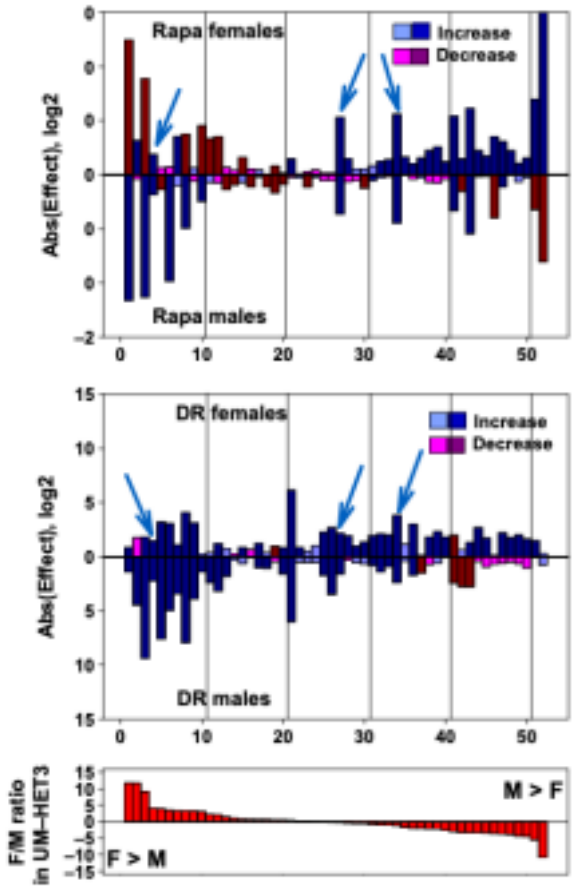
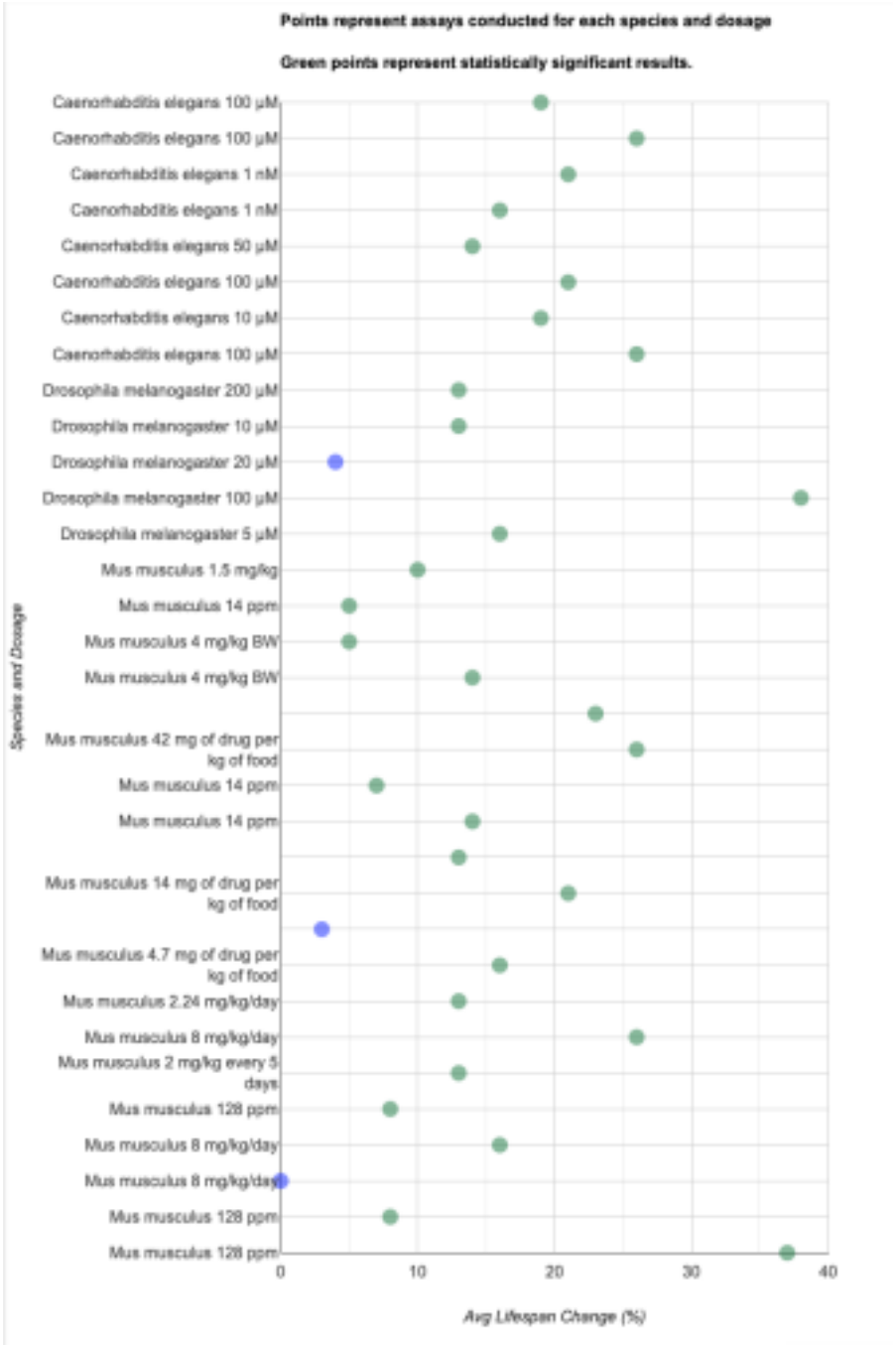


Fig. 5 Expression of mRNA for liver genes involved in xenobiotic metabolism. Effects of rapamycin (top panel) and DR diet (middle panel) on hepatic mRNA levels for 52 genes related to xenobiotic metabolism. Top and middle panels: mice were placed on rapamycin (14 ppm) or DR diet at 4 months of age and euthanized at 12 months of age. There were six males and six females in the control group and in each of the two treatment groups. All mice were housed at UM. Data from treated female mice are shown in the top of each panel, bars pointing upwards, and data from treated male mice are shown in the bottom half, with bars pointing downwards. The length of each bar is shown on a log2 scale, as the ratio of treated mice divided by control mice. Bars shown in blue are increases compared with control, and bars shown in red are decreases compared with control mice. Thus, a blue bar with a value of eight represents an increase of 2^8=256-fold above control levels, and a red bar with a value of eight represents a decline of 256-fold below control levels. Dark blue and dark red bars show genes for which the nominal (unadjusted) *P*-value is $P < 0.05$. Lighter blue and red bars shown genes that do not reach this arbitrary significance threshold. The first gene, for example, Sult2a2 (see Table S3), is increased by DR, slightly but significantly, in both males and females, is dramatically increased (by $2^{11.6}$ = 3100-fold) by rapamycin in males, but shows a large ($2^{12.4}$ = 5400-fold), significant decline in rapamycin-treated females. The cyan arrowheads point out the three mRNA that are elevated by both DR and rapamycin in both sexes.The bottom panel shows female/male ratios, for untreated UM-HET3 controls; genes at the left are expressed at levels approximately 2^{12}-fold higher in female controls, and genes at the right are expressed roughly 2^{11}-fold higher in male controls.

A higher dose (actually AUC) leads to larger lifespan extension, a consistent theme in rodent/rapamycin longevity studies.

DrugAge: The Database of Ageing-related Drugs

https://genomics.senescence.info/drugs/drug_details.php?compound_name=Rapamyci



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The database of longevity enhancement by rapamycin spans a great many animal models, indicative of a central pan species longevity controlling pathway.

Rapamycin for longevity: opinion article

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6814615/

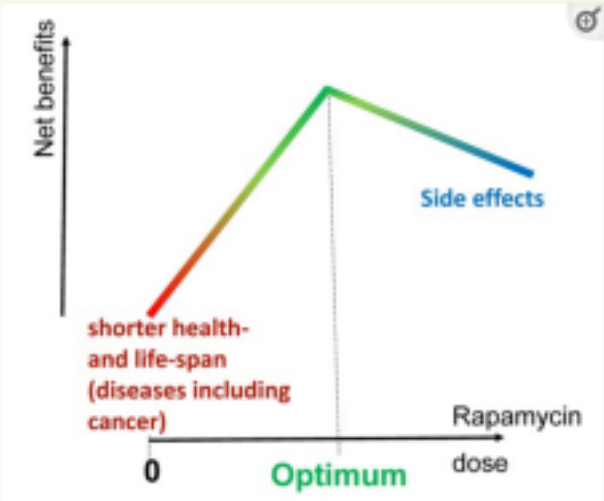
This seminal 2019 "call to arms" opinion piece by Mikhail Blagosklonny.

"In conclusion, the side effects of rapamycin are well-known and reversible. ***When used on an "anti-aging schedule", side effects may be absent*** but, if not, they may be mitigated by combining rapamycin with other anti-aging drugs (metformin, statins) or by temporarily discontinuing it. Noteworthy, the alternative to the reversible (and avoidable) side effects of rapamycin/everolimus are the irreversible (and inevitable) effects of aging. But ***the fear of nonexistent side effects*** is not the only reason the use of mTOR inhibitors for life extension has been questioned. ***The tailored optimal dose (see Figure 2, below) should be determined individually for each patient and may vary widely***. For antiaging purposes, however, rapamycin may be used either intermittently (e.g., once a week) or at low daily doses and ***can be discontinued if any unpleasant effects occur***. Doses and frequencies should be limited by the side effects: stomatitis/mucositis, anemia, thrombopenia, leukopenia, edema, and pneumonitis. ***To be safe, even mild hyperglycemia should be avoided*** or mitigated with metformin. Do we need new or safer rapalogs to start aging prevention? ***There is currently no consensus around the short-term markers of anti-aging effects. Therefore, rapamycin trials should be focused on its potential side effects rather than anti-aging effects***"

Wow, what do the highlighted sections even mean re longevity translation? So taking rapamycin up to some random individual n=1 side effect limits is THE "OPTIMAL" FUNDAMENTAL PHYSIOLOGICAL anti-aging dosing schedule? Is this REALLY what we learned from rodent studies?

This sadly seems more like a "rationalization for human impediment" piece than fundamental rapamycin mTOR longevity translation.

In Figure 2 from the paper, the peak "maximal net benefits" is considered "optimum"…optimum for what, lifespan extension or some truly meaningless dose limiting toxicity? And this longevity "escapism" point was arrived using what principles of mTOR translation biological science?



Exactly how is this human dose limiting toxicity (DLT) defined peak the "optimum" in an article that promotes taking rapamycin for animal model translation longevity? There is a crucial disconnect

between animal studies (no DLT) and human translation.

We need a new paradigm of thinking…a bridge between biological science and "applied engineering"/pharmacological translation. In short, oral dosing has reached the castle wall, and there is a moat…how do we scale the castle walls? Perhaps it's time for the basic oral rapamycin science to be re-imagined by "escapism entrepreneurs/engineers"?

Fast forward to 2022, 20+ years since rapamycin was FDA approved, and 13 years since the 2009 mice/rapamycin longevity study, and where are we clinically and as a biohacking community with human prophylactic translation of volumes of longevity studies in mice??

Basically, we have a disperse global community of retail biohackers taking rapamycin ORALLY/intermittently (typically weekly). Rapamycin oral dosing appears to be n=1 deviations of the clinical patient population of Dr Allan Green (https://rapamycintherapy.com/), generally 5 mg/week +/-. Individual dosing exploration appears to be following the mantra of Mikhail Blagosklonny (a long time leading proponent of rapamycin for human anti-aging), who espouses "take as much as you can tolerate". Mild lipid and glucose dysregulation and various dose limiting side effects are generally the status quo reported with low weekly intermittent dosing. Very little sirolimus blood testing being reported re trough/AUC benchmarks. Some small human trials and a large dog trial are commencing.

Some of the leading rapamycin advocates in the field such as Dr Allan Green, Matt Kaberlein, and Peter Attia are reportedly taking 5-10mg/week or other close iterations +/-, but there is no

LONGEVITY based rationale other than dosing below side effects? Some are taking higher oral doses but longer intermittency for perhaps some undefined "spike benefit", yet minimizing side effects?

Is THIS the current state of human rapamycin longevity translation science, throwing a dart at dosage? What are the longevity biomarkers?

Shouldn't we be measuring mTOR1/2 assays (blood/tissue), pharmacodynamics, radionuclide rapamycin tracer imaging across tissues/brain vs dose, tissue gene expression analysis, autophagy? In short, scaling from rodents to humans, mapping the complete physiological pathways. Biology does not care about human and commercial impediments!

Let's be crystal clear…this is a very potent immunosuppression drug, and can cause n=1 harm with elevated chronic dosing, although with over 20+ years of clinical use, it is a relatively safe drug even with short term high doses, with symptoms reversing with discontinuation.

Sirolimus and mTOR Inhibitors: A Review of Side Effects and Specific Management in Solid Organ Transplantation

https://sci-hub.se/https://doi.org/10.1007/s40264-019-00810-9

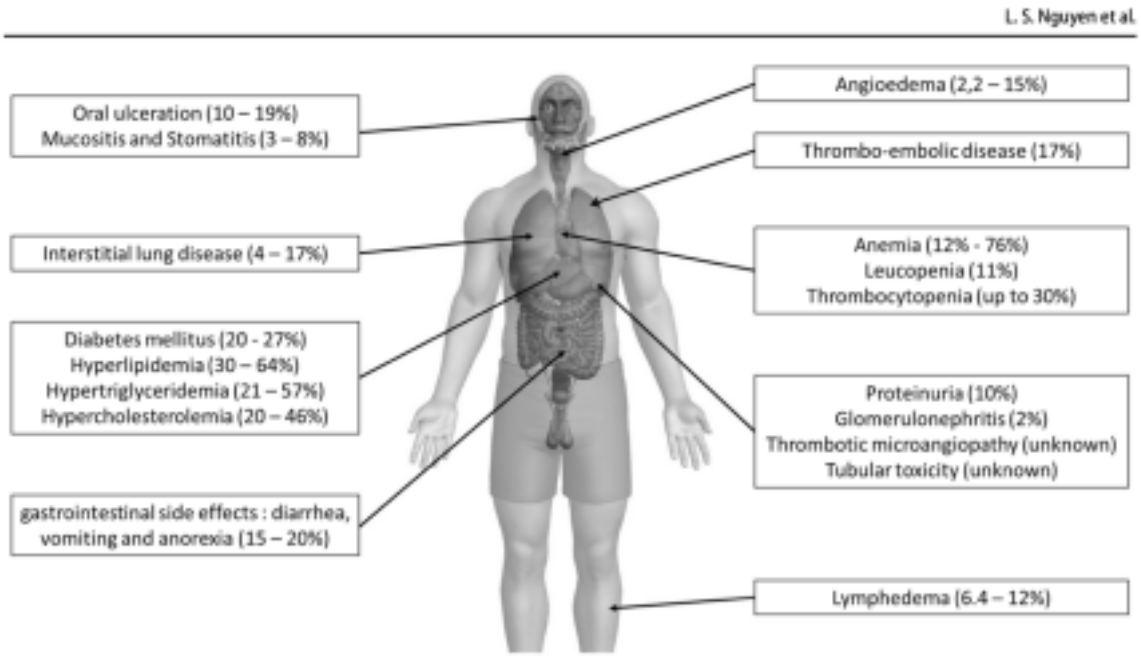


Fig. 2 Known side effects associated with mechanistic target of rapamycin (mTOR) inhibitors

Rodents in research labs typically die from cancer (90%+). Rapamycin delays cancer, thus resulting in lifespan extension, quite a simple relationship revolving around the central thesis of mTOR1/mTOR2 inhibition. We can speculate about whether rapamycin might defer other leading

causes of human mortality such as heart disease, stroke, respiratory, or Alzheimer, but rapamycin extending longevity beyond cancer suppression is wishful thinking and not strongly supported by many wild type mice and human studies to date (setting aside transgenic mouse/rapamycin models which greatly weaken the human translation physiological fundamentals). Neurodegenerative diseases are far more complex, many pathways, unclear

etiology, polygenic, and mTOR inhibition may not be only pathway to deferral, although I am hopeful of efficacy translation.

A seminal paper in 2012, albeit in wild type mice was a data point marker discovery re cognitive amelioration with rapamycin.

Chronic inhibition of mTOR by rapamycin modulates cognitive and non-cognitive components of behavior throughout lifespan in mice

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3454865/pdf/nihms-390701.pdf

"To explore the effect of chronic rapamycin treatment (**ORAL**) on normal brain aging we determined cognitive and non-cognitive components of behavior throughout lifespan in male and female C57BL/6 mice that were fed control- or rapamycin supplemented chow. Our studies show that rapamycin enhances cognitive function in young adult mice and blocks age-associated cognitive decline in older animals. In addition, mice fed with rapamycin-supplemented chow showed decreased anxiety and depressive-like behavior at all ages tested. Levels of three major monoamines (norepinephrine, dopamine and 5-hydroxytryptamine) and their metabolites (3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5- hydroxyindolacetic acid) were significantly augmented in midbrain of rapamycin-treated mice compared to controls. Our results suggest that chronic, partial inhibition of mTOR by oral rapamycin enhances learning and memory in young adults, maintains memory in old C57BL/6J mice, and has concomitant anxiolytic and antidepressant-like effects, possibly by stimulating major monoamine pathways in brain. ***Our results suggest that an approximate 30% reduction in TOR activity in brain for 16 weeks or longer improves performance of C57BL/6 mice in tasks that involve long-term plasticity and are dependent on hippocampus or on hippocampus and prefrontal cortex. Rapamycin and rapamycin analogs, already used in the clinic, may have potential for therapeutic intervention in cognitive and affective dysfunctions associated with aging.***"

CVD is largely a lifestyle mediated disease (non-genetically predisposed), and with dietary, exercise protocols, and meds, can largely be mitigated or deferred as a primary mortality pathway.

The molecular biological origins of the vast array of lethal cancer types (outside known causative genes) are still largely a mystery and all the interventions in the world can still result in a random cancer diagnosis. ***As such, deferring cancer incidence in humans with rapamycin as prophylactic, building on addition the large history of cancer/transplant therapeutic management, is a profoundly worthy longevity intervention deserving of greater clinical human translation research.***

From my read of the vast rodent rapamycin longevity literature, as well as more importantly the hundreds of studies on human cancer cohorts on daily rapamycin where trough levels of rapamycin are typically 5 ng/ml minimum for cancer suppression, confirmed mTOR inhibition,

and consistent biomarker dysregulation, ***I have crystallized to a beliefthat for human translation equivalency, a few mg/week intermittent dosing, the resultant very low trough sirolimus levels, and a large majority of people reporting no ill effects; the sum total clinical status is grossly insufficient dosing levels to produce the pan tissue mTOR1/mTOR2 inhibition for meaningful pharmacological lifespan extension***. With a great many anecdotal n=1 reports of people taking weekly dosing and NO side effects, or higher weekly dosing reporting symptoms of fatigue, canker sores, rashes, and slightly dysregulated lipids, infrequent glucose dysregulation, and subsequent immediate scaling back, taking pauses, or terminating rapamycin experimentation, there is something fundamentally limiting in the current human prophylactic translation with standard ORAL rapamycin dosing.

Rapamycin in aging and disease: maximizing efficacy while minimizing side effects (2016)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5216691/pdf/oncotarget-07-44876.pdf

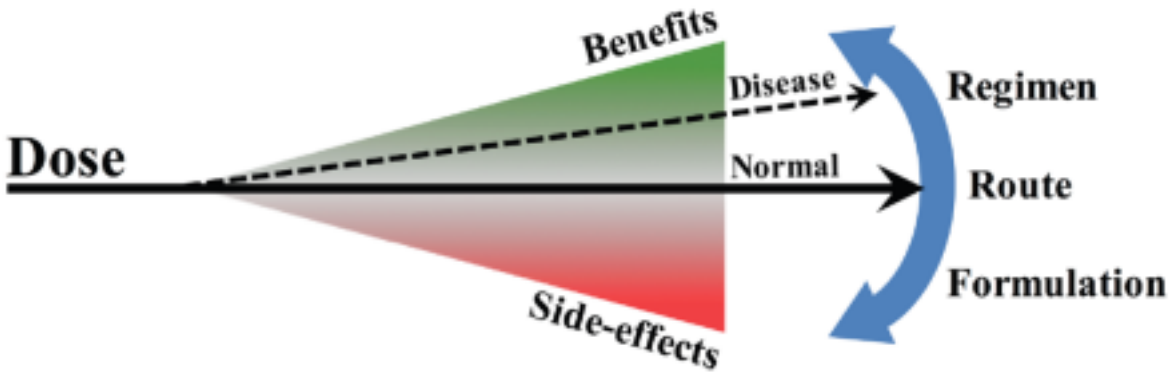


Figure 1: Maximizing the benefits of rapamycin. Rapamycin is associated with positive outcomes and side effects which both increase with dose. Optimization of dosage regimen, drug delivery route, and formulation may provide maximum benefits while reducing off-target effects. In the setting of diseases, such as mitochondrial disease, the benefits of rapamycin may outweigh the side effects even at high dose.

The above animation captures the essence of the challenge, which is indirectly a critique of the woeful FAILINGS of oral rapamycin delivery.

"Optimization of dosage regimen, drug delivery route, and formulation may provide maximum benefits while reducing off-target effects". YES!

"We had previously shown that daily intraperitoneal (IP) injection of rapamycin at 8 mg/kg/day dramatically attenuates disease progression and enhances survival by more than 100%. More recently, we tested a range of dietary eRAPA concentrations in this mouse model, finding that lower amounts of the drug comparable to those tested for effects on lifespan in wild type mice

had no effect in the KO mice. ***Instead, much higher levels of eRAPA or IP rapamycin were needed to attenuate disease and increase survival***. Based on relative phenotypic outcomes in both the KO and wild type mice, as well as serum concentrations of the drug, ***we conclude that***

dietary eRAPA must be provided at approximately 27-fold higher levels than has been commonly used (378 ppm) in order to achieve similar biological activity to daily injection of 8 mg/kg. In addition, 14 ppm eRAPA had little impact on developmental growth rate in control or diseased mice, a phenotypic readout of rapamycin activity, ***while higher doses robustly reduced growth***. While eRAPA and injected rapamycin are not directly comparable, as the delivery methods will have different pharmacokinetic parameters and may result in dramatically different tissue distributions***, these results provide an initial foray into examination of dosing and delivery of rapamycin in a preclinical model of a medically relevant class of disease***."

Without meaningful translation science, we should be maximizing rapamycin tissue partition coefficients whilst reducing side effects. Why think otherwise from rodent studies?

How can we better leverage rapamycin pharmacologically for our ultimate objective of lifespan extension…is there an alternate modality, a new paradigm of drug delivery?

We know from abundant literature (20+ years) that oral rapamycin undergoes very potent 1st pass metabolism effects, with rapamune tablet bioavailability massively reduced to approx. 10-15%. Compare this to IV drug delivery which is 100%. ***In addition to massive destruction of rapamycin into metabolites via the oral route, the liver is a central axis of systemic influencing gene expression, typically manifested as lipid, insulin and other core metabolic and systemic regulatory functions. This immediate oral post gastro hepatic processing/absorption focal point and subsequent "flared" systemic biomarkers dysregulation, I believe precludes a more beneficial pan tissue distribution (improved target tissue partition coefficients) and greater systemic efficacy of unmetabolized rapamycin via alternate parenteral delivery routes***.

ORAL RAPAMYCIN: 1ST PASS METABOLISM

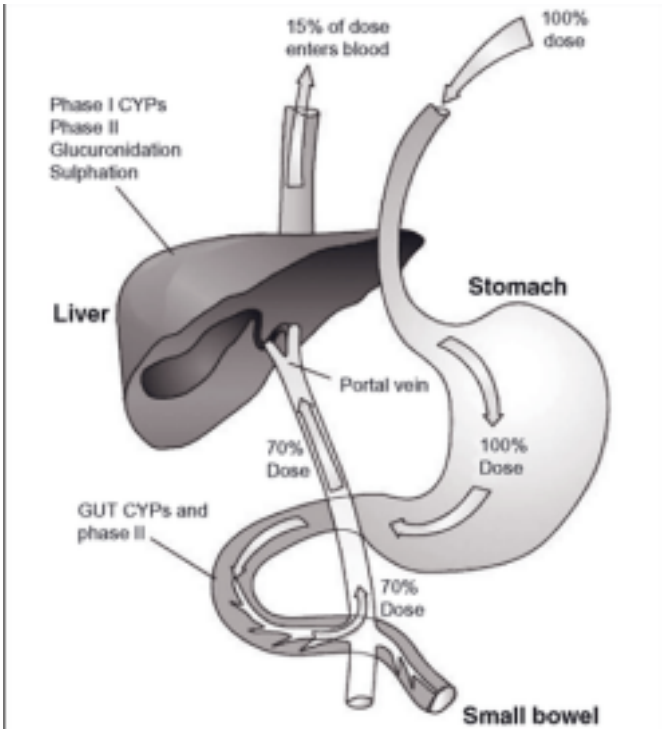
First-Pass Elimination Basic Concepts and Clinical Consequences (1984)

https://sci-hub.se/https://doi.org/10.2165/00003088-198409010-00001

Individual Variation in First-Pass Metabolism (1993)

https://sci-hub.se/https://doi.org/10.2165/00003088-199325040-00005

This 1st pass metabolism effect is the "moat" of rapamycin pharmacological translation. We must explore alternate routes of delivery and formulations…move the translation needle.



A 2014 paper utilizing both ip injections and oral rapamycin delivery in

mice. **Comparison of rapamycin schedules in mice on high-fat diet**

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4614913/pdf/kccy-13-21-970491.pdf

"Here we demonstrated that i.p. injections of rapamycin prevented weight gain on high fat diet, whereas rapamycin by oral gavash did not. Orally administrated rapamycin has poor bioavailability. The i.p. route of its administration ensures high peak of systemic levels of rapamycin. ***It may seem paradoxical that intermittent administration (by i.p.) is more effective than everyday administration (by oral gavash).*** One plausible explanation is that at high peak levels, rapamycin may affect cell types that are not sensitive to low concentrations of rapamycin. In fact, the effective concentrations of rapamycin vary broadly in cell lines in culture. Although i.p. injections are not suitable for prolonged treatment in humans, we suggest that the development of highly bioavailable oral preparations of rapamycin in humans. Alternatively, existing formulations of rapamycin could be used in high doses (as single dose) to reach desired peak concentrations in humans. Although high doses of rapamycin can cause side effects if used daily, intermittent administration (one a week or 3 times per 2 weeks, for example) may be sufficient for anti-obesity effects"

Here we see quite clearly that injection delivery fundamentally alters the physiological response or rapamycin vs oral. Why are injections "not suitable for prolonged treatment in

humans"?

https://en.wikipedia.org/wiki/Injection_(medicine)

"***Injections are among the most common health care procedures, with at least 16 billion administered in developing and transitional countries each year***. Many injections are designed to administer a medication which has an effect throughout the body. Systemic injections may be used when a person cannot take medicine by mouth, or when the medication itself would not be absorbed into circulation from the gastrointestinal tract. Medications administered via a systemic injection will enter into blood circulation, either directly or indirectly, and thus will have an effect on the entire body. Methods include intravenous, intramuscular, subcutaneous, intradermal, intraosseous". I have been taking injection TRT for years, zero delivery issues. All that is needed is the proper safety/formulation/storage for rapamycin to make the leap from oral to a superior systemic delivery protocol. This endeavour is an engineering problem, supported by a large body of pharmacological translation.

Let's look at some studies of non-oral (parenteral) delivery of rapamycin.

In a rat study of rapamycin tissue distribution, comparing daily oral vs continuous IV, we can see both the far higher bioavailability of IV vs oral (compare tissue SRL levels vs dose), and the far higher SRL levels in intestine and liver of oral vs IV (brain not tested). Oral bioavailability in this study was assessed at < 5%.

Distribution of Sirolimus in Rat Tissue (1997)

https://sci-hub.se/10.1016/s0009-9120(96)00157-9

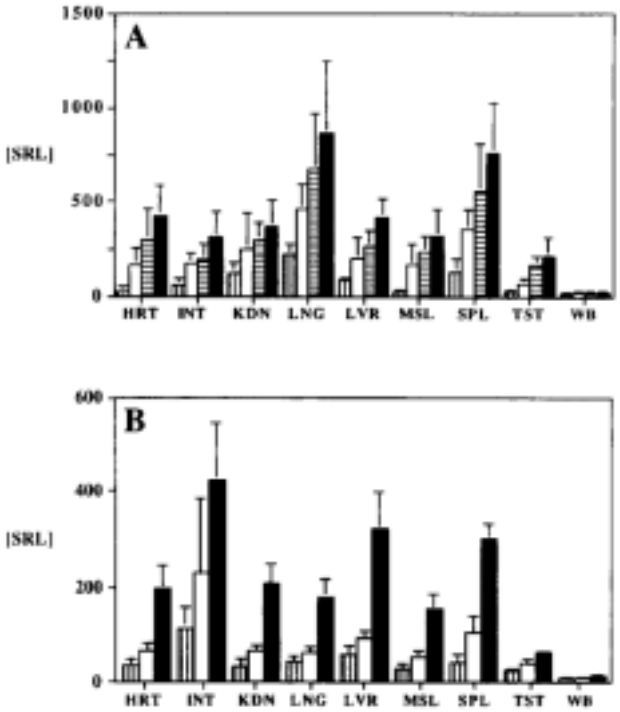


Figure 1 — Mean WB and tissue SRL concentrations ([SRL], ng/ml or ng/gm) following CIVI (A) or daily PO (B) administration for 14 d. CIVI dose groups at 0.04, 0.08, 0.16, and 0.4 mg/kg/d are indicated by vertically hatched, unshaded, horizontally hatched, and shaded bars, respectively. PO dose groups at 0.4, 0.8, and 1.6 mg/kg/d are indicated by vertically hatched, unshaded, and shaded bars, respectively. Error bars indicate one unit of standard deviation. As assessed by one-way ANOVA, all tissues and WB showed significant differences ($p < 0.05$) among group mean SRL concentrations with respect to dose.

In a rapamycin study of dogs (much larger animal model closer to human physiology and mortality pathways), rapamycin was delivered by intramuscular (IM) injections.

Rapamycin Pharmacokinetic and Pharmacodynamic Relationships in Osteosarcoma: A Comparative Oncology Study in Dogs (2010)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2882366/pdf/pone.0011013.pdf

"Rapamycin may be safely administered to dogs and can yield therapeutic exposures." This is a much larger animal model vs rodent and quite conclusively demonstrates the conceptual safe crossover from oral to IM delivery.

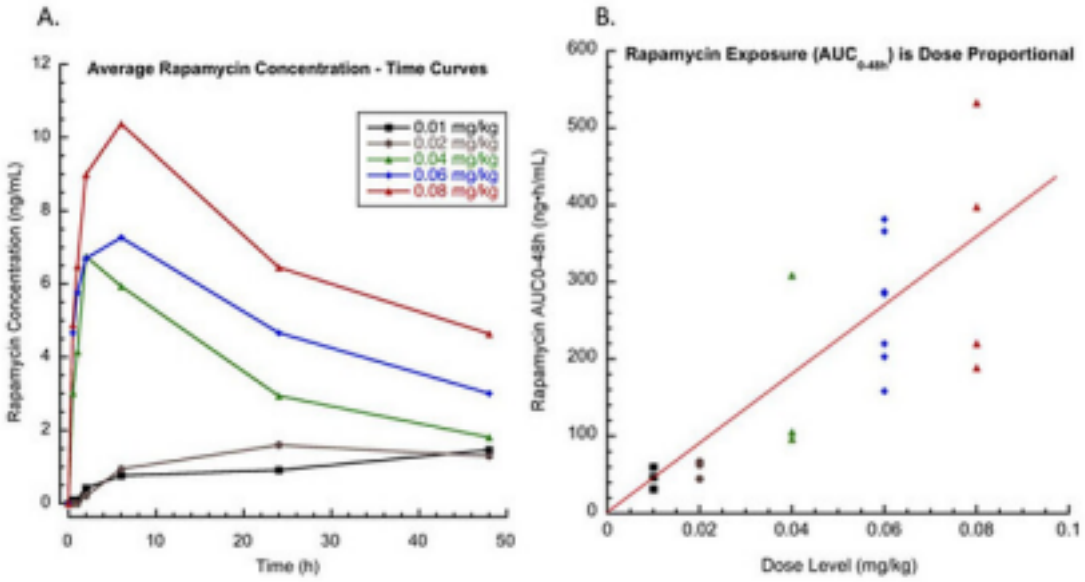
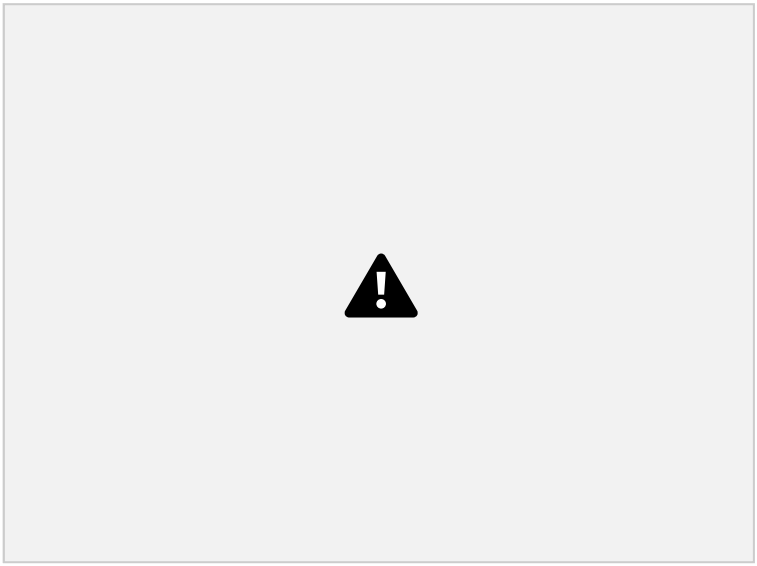


Figure 2. Rapamycin exposure in dogs with osteosarcoma is dose dependent. Serial rapamycin whole blood concentrations (ng/ml) were measured by HPLC with MS/MS detection for all dogs that completed study (n = 19). After a single parenteral dose of rapamycin, 7-point PK analysis (samples collected at 0, 30 minutes, 1,2, 6, 24 and 48 hours) was performed. Over the dose range studied, **A.** average concentration – time curves for each dose level, and **B.** rapamycin exposure (AUC_{0-48h}) increased proportionally to dose.
doi:10.1371/journal.pone.0011013.g002

In a similar study of rapamycin in dogs, but via oral administration, and looking at the dose vs time curve vs IM administration, ***one can immediately observe the far slower clearance of rapamycin and higher AUC of IM vs. oral.*** ***This study clearly demonstrates a FUNDAMENTALLY altered systemic pharmacological response vs. oral in a much larger animal model (closer to human).***

Pharmacokinetics of orally administered low-dose rapamycin in healthy dogs: A pilot study (2016)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5642271/pdf/nihms869823.pdf



Clinical Pharmacokinetics of Sirolimus (2001)

https://sci-hub.se/10.2165/00003088-200140080-00002

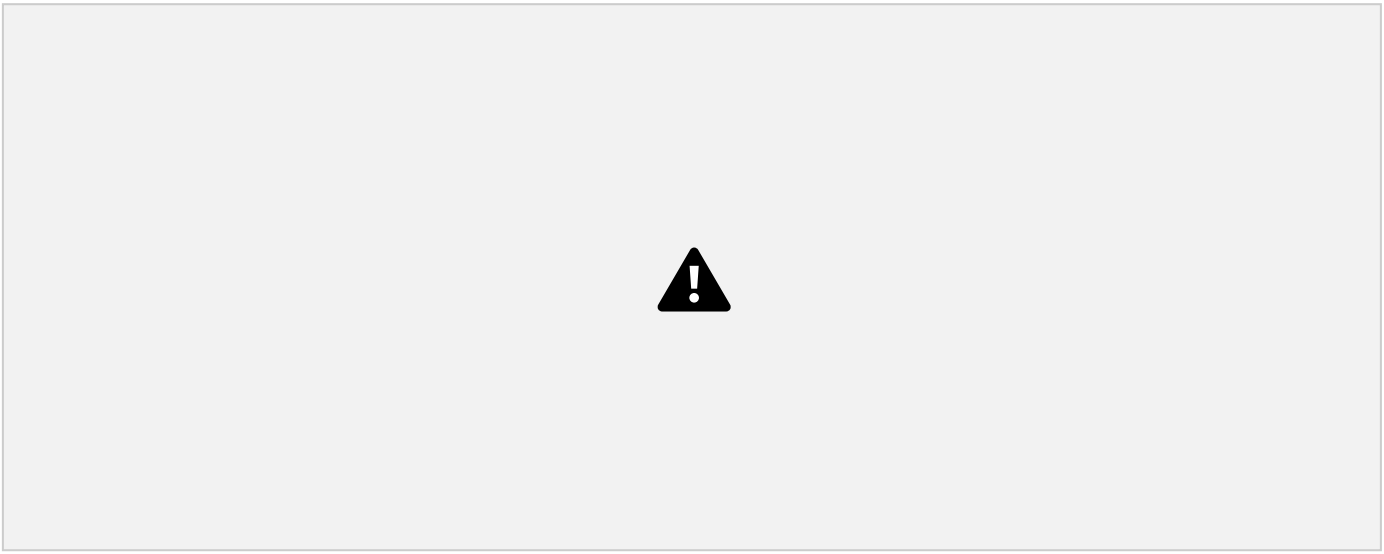
"Because of its hydrophobic nature, sirolimus is widely distributed in lipid membranes of body tissues as well as erythrocytes, showing a large apparent volume of distribution (5.6 to 16.7 L/kg. Following 14 days of intravenous or oral administration, spleen, kidney, liver, intestine and heart tissue-to blood partition coefficients were as high as 40. Because the tissue distribution of sirolimus has not formally been studied in humans, the possible relationship of these concentrations to the efficacy and toxicity of the drug is currently unknown. (This paper was in 2001, and venture to say we still do not know today the tissue distribution of rapamycin in humans using different delivery/formulation protocols). This is a vastly UNEXPLORED avenue to leveraging and unleashing the FULL pharmacological benefits of rapamycin.

In another study of tacrolimus of intramuscular vs oral administration in monkeys.

Reduced variability in tacrolimus pharmacokinetics following intramuscular injection compared to oral administration in cynomolgus monkeys: Investigating optimal dosing regimens (2019)

https://sci-hub.se/10.1016/j.jphs.2018.05.013

"The dose of tacrolimus were 0.1 mg/kg (3-4 kg monkeys = 0.4mg) for IM injections and 5 mg/head for oral administrations (***difference 12.5X dosing***). ***The mean oral bioavailability of tacrolimus relative to the IM injection was estimated be 4.8% while mean relative bioavailability of IM injection was assumed to be 100%***. In the case of tacrolimus, we have shown that IM injections reduce the variability for parameters that have importance in the therapeutic exposure of the drug. ***Furthermore, this study indicates that long-acting IM***



injections could prove useful for tacrolimus. The advantages of reduced variability combined

with sustained release shown in this study for the IM injection of tacrolimus could warrant a new strategy for administering tacrolimus."

So the parenteral rapamycin delivery route story so far, a plethora of studies on utilizing different non oral drug delivery methods, showing altered systemic pharmacology, yet not explored in humans!

I too was sleep walking when I started taking rapamycin, following the herd of oral rapamycin delivery until this paper was posted on intranasal delivery of rapamycin.

Intranasal rapamycin ameliorates Alzheimer like cognitive decline in a mouse model of Down syndrome (2018)

https://translationalneurodegeneration.biomedcentral.com/track/pdf/10.1186/s40035-018-0133-9.pdf

"The aberrant modulation of the mTOR signalling in DS and AD age-related cognitive decline affects crucial neuronal pathways, including insulin signaling and autophagy, involved in pathology onset and progression. Within this context, the therapeutic use of mTOR-inhibitors may prevent/attenuate the neurodegenerative phenomena. ***By our work we aimed to rescue mTOR signalling in DS mice by a novel rapamycin intranasal administration protocol (InRapa) that maximizes brain delivery and reduce systemic side effects***."

"Our analysis demonstrates that InRapa treated mice showed a brain concentration of rapamycin of 5.0 ± 1.0 ng/g after 4 h, while the plasma concentration was 6.7 ± 1.3 ng/ml. In contrast, animals treated by i.p. injection showed a rapamycin brain concentration of 11.1 ± 1.7 ng/g and a plasma concentration of 890.5 ± 98.1 ng/ml"

For whatever reason, perhaps the shear simplicity of the delivery route, simple formulation, and PROFOUND pharmacodynamic benefit triggered an "aha" moment! All of my inventions/patents have been "aha" moments, so my experience in engineering, my gut and the vast literature readings: all urging me to run with it.

Putting aside the transgenic mouse model, this intranasal rapamycin delivery paper completely reset my thinking of the exploration of a new paradigm for rapamycin "to rescue mTOR signalling by a novel administration protocol and reduce systemic side effects".

Since neurodegeneration/cognitive prevention is largely the primary motivation for my lifestyle interventions and the addition of rapamycin, this study showing a superior delivery of rapamycin to the brain but without the systemic side effects completely changed my thinking of oral rapamycin as a woefully inferior delivery drug delivery protocol. ***I was determined to explore parenteral alternatives.***

So I began a deep dive into all things drug delivery routes, CNS vs systemic, pharmacodynamics,

anatomical differences rodents vs humans (re intranasal), drug vehicles/excipients, formulations, dosing, mechanical delivery routes, safety, storage, etc.

EFFECT OF ROUTE OF ADMINISTRATION AND DISTRIBUTION ON DRUG

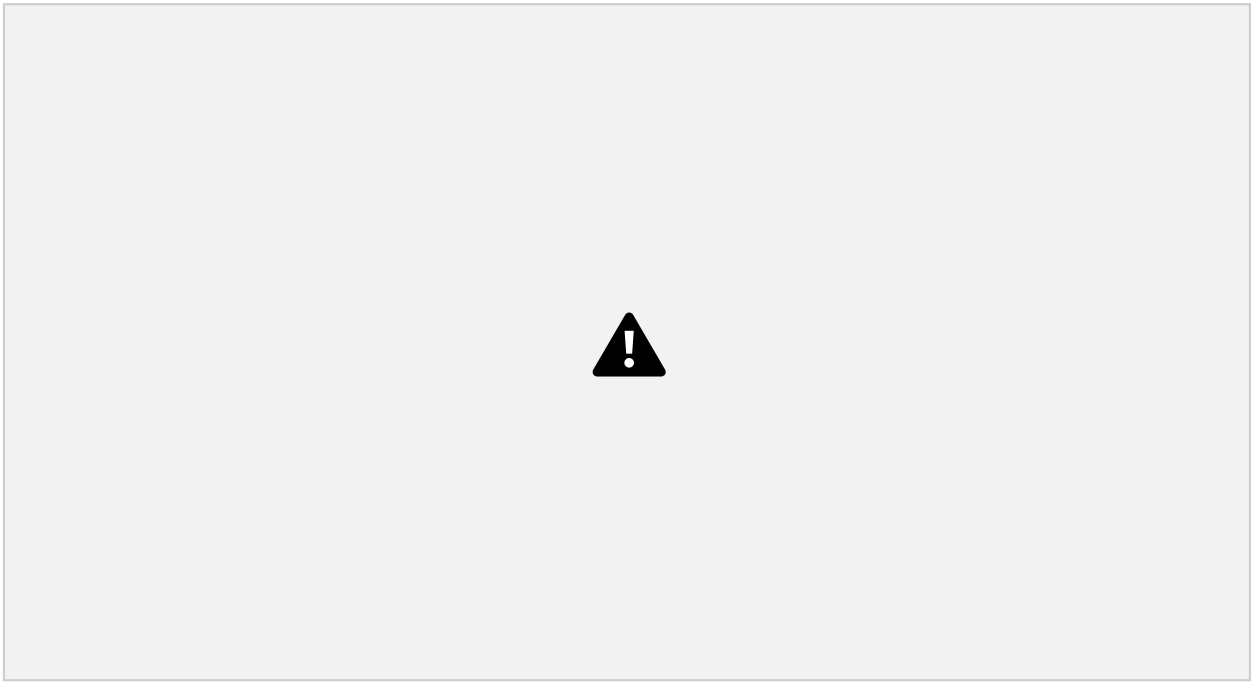
ACTION https://sci-hub.se/https://doi.org/10.1007/BF01062110

PHARMACOKINETICS

https://sci-hub.se/https://doi.org/10.1016/B978-0-323-07445-2.00001-X **DRUG ABSORPTION,**

DISTRIBUTION AND ELIMINATION; PHARMACOKINETICS

http://www.columbia.edu/itc/gsas/g9600/2004/GrazianoReadings/Drugabs.pdf



INTRANASAL (IN) DRUG DELIVERY

The nasal approach to delivering treatment for brain diseases: an anatomic, physiologic, and delivery technology overview

https://www.future-science.com/doi/epub/10.4155/tde.14.41

NASAL DELIVERY – A Promising Route of Drug Delivery to the Brain: Scientific Considerations

https://drug-dev.com/nasal-delivery-a-promising-route-of-drug-delivery-to-the-brain-scientific considerations-2/

Transport of drugs from the nasal cavity to the central nervous system
https://sci-hub.se/10.1016/s0928-0987(00)00087-7

Physicochemical and physiological considerations for efficient nose-to-brain targeting

https://sci-hub.se/10.1517/17425247.2012.636801

Intranasal Drug Delivery Potential Advantages and Limitations from a Clinical Pharmacokinetic Perspective

https://sci-hub.se/10.2165/00003088-198917050-00001

Nasal-to-CNS drug delivery: where are we now and where are we heading? An industrial perspective

https://sci-hub.se/https://doi.org/10.4155/tde.11.149

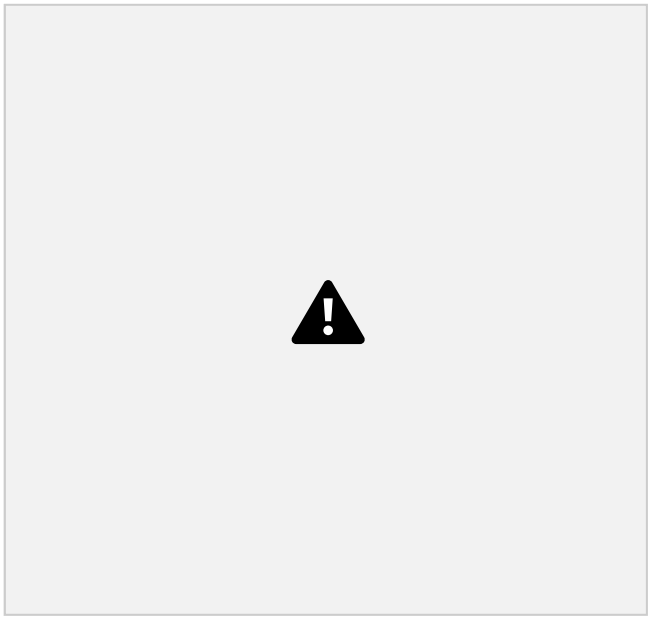
The Upper Nasal Space—A Novel Delivery Route Ideal for Central Nervous System Drugs

https://touchneurology.com/wp content/uploads/sites/3/2020/07/touchNEURO_US_16.1_p25-31.pdf

Evaluation of Intranasal Vaccine Delivery Using Anatomical Replicas of Infant Nasal Airways

https://sci-hub.se/10.1007/s11095-020-02976-9

A brief summary of the vast a and fundamental challenges of anatomical challenges translating intranasal delivery in mice to humans.



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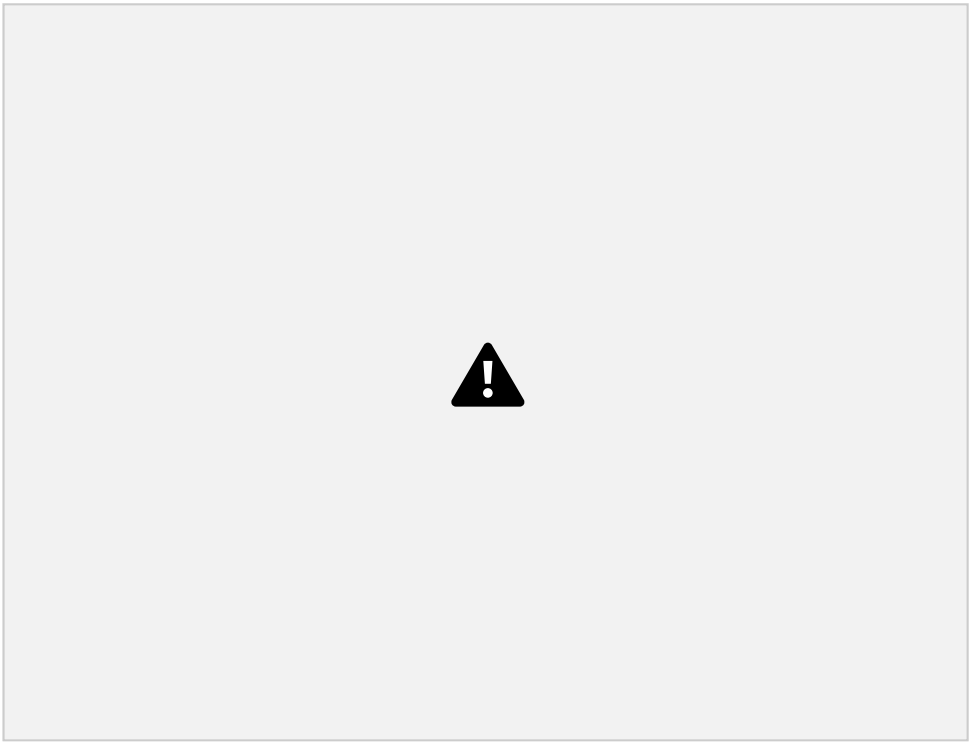
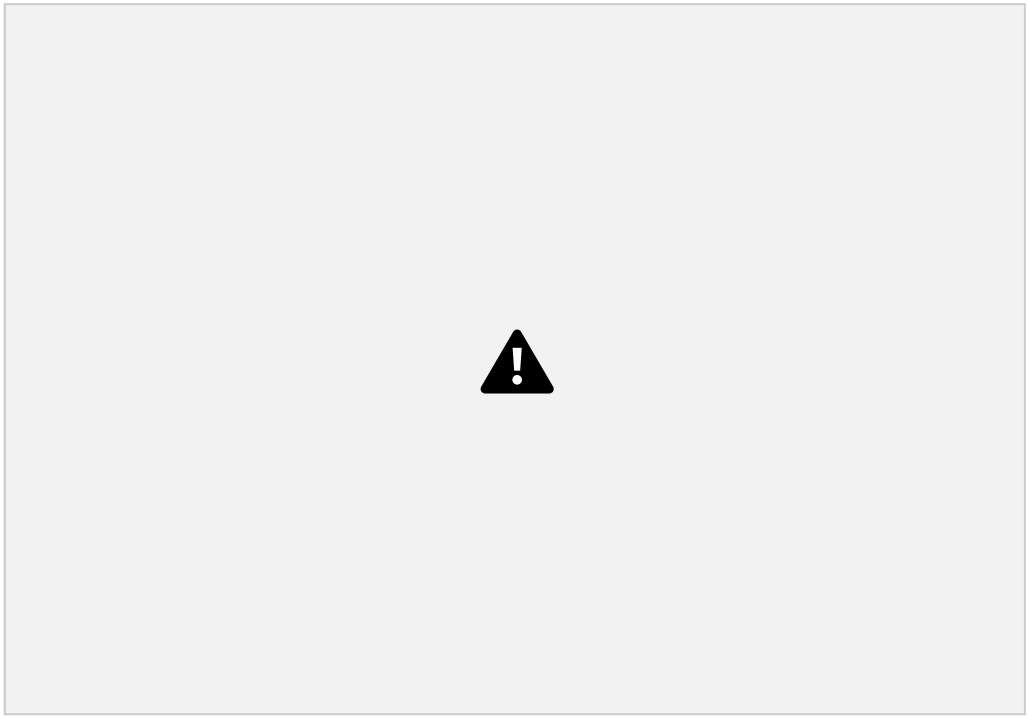


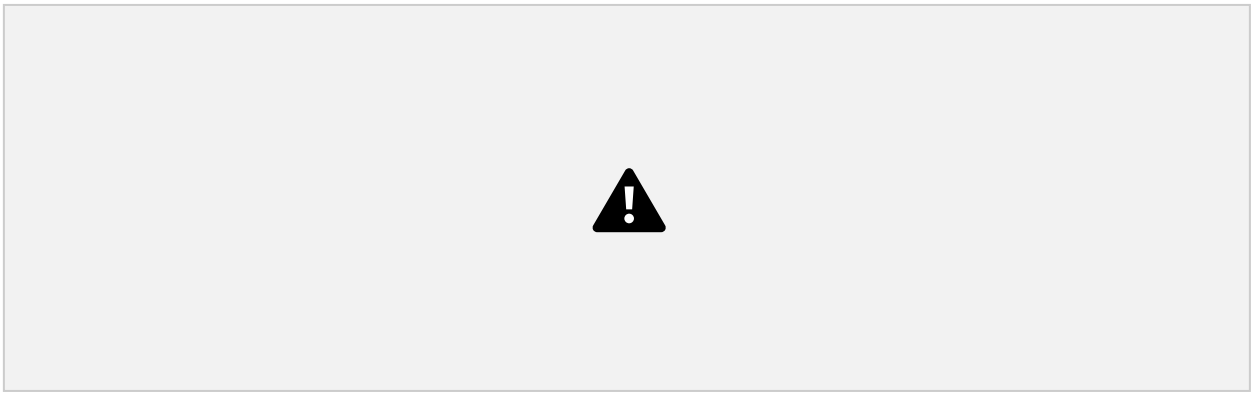


*"**The ratio between the 10 cm^2 olfactory region and 70 kg average body weight in man provides a ratio of 0.14, which is 100–200-times lower than the ratio for animals such as rats and dogs where the olfactory epithelium covers roughly 50% of the nasal mucosa**. **Preclinical species have more olfactory receptor cells than humans (12 million in man vs 1 billion in dogs) This may***

be an important factor in both facilitated absorption of molecules and also mediating or reversing potential toxicity associated with drug delivery to this region. The mucociliary clearance rate in humans is three- to four-times slower than in rats, potentially leaving more time for absorption to occur It is also conceivable that other differences between animal and human physiology offset the potential disadvantages of human anatomy; for example, the CSF turnover rate in rats is five- to six-times higher compared with man, which may be important for drugs that enter the brain via the CSF. This is extremely important particularly because of the limitations of conventional nasal delivery devices in reaching beyond the nasal valve to the upper and posterior regions of the nasal cavity with the richest cranial nerve innervation, the target region that is likely to be the optimal target for nose to brain delivery. ***Consequently, only a very small fraction of the inspired air actually reaches the olfactory region during nasal respiration***. ***It is known that the average portion of the nasal dose that reaches the CNS after nasal administration can be as little as 0.1% or less***. The distance from the nostril to the olfactory epithelium is only a few centimeters, but its location in the slit-like olfactory cleft behind the narrow nasal valve and at the end of a complex labyrinth of passages severely complicates access. In a study evaluating the olfactory deposition of a liquid dye delivered with this type of syringe and-tube performed by a trained physician, after decongestion and in recumbent subjects, delivery to the olfactory cleft was achieved in 11 out of 15 subjects. In the same study, delivery of drops and of a traditional spray by the subjects themselves, also after

decongestion, showed olfactory deposition in only one subject after spray and in none after drops, clearly illustrating the inadequacy of these traditional methods in reaching the olfactory cleft. This demonstrates that although olfactory delivery is achievable with an impractical medical procedure it is unsuited for routine drug delivery at home (and even in most clinics). These data, and other data, also show that commonly used nasal delivery devices are relatively useless for targeting drug delivery to the olfactory region as would be desired for nose to brain delivery.

Respiratory epithelium is the major type of epithelium present in the human nasal cavity and represents 85% of total area. Due to its highly vascular nature, the respiratory epithelium is the surface that is responsible for the majority of absorption, which leads to systemic exposure following nasal dosing. ***It is known that by weight percentage, the olfactory bulb makes up a larger percentage of weight in the rodent brain (cited as 4–20%) than in humans, where the olfactory bulb makes up only approximately 0.064% of the total mass. This type of allometric asymmetry will need to be factored into interpretation of rodent efficacy studies and human dose projections"***



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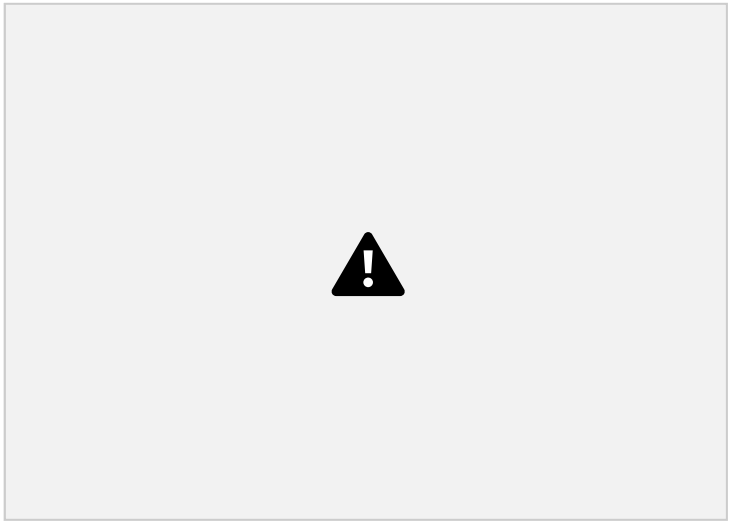
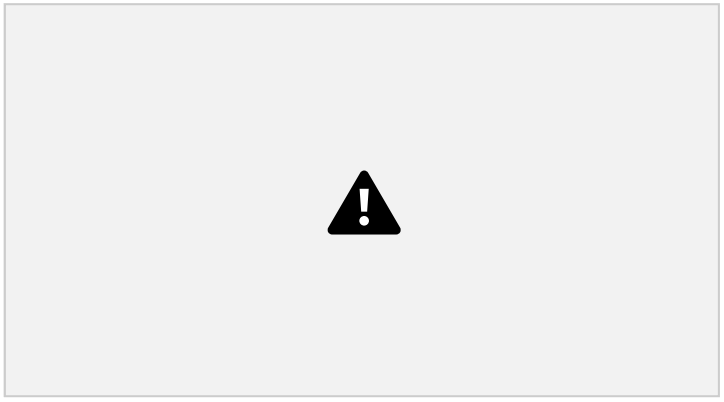
By restricting drop size in nasally administered sprays to a diameter >10 µm deposition is restricted to the nasal cavity and lung exposure is essentially zero. The total spray volume that can be reliably delivered to each naris is limited by the size of the nasal cavity and generally thought to be no more than 150 µl and the upper limit of a drug dose has been suggested to be 25 mg/dose.

The diffusion of drugs from the surface of the brain into the brain tissue is slow with the rate of diffusion, expressed as diffusion coefficient (D), inversely related to the molecular weight of the drug





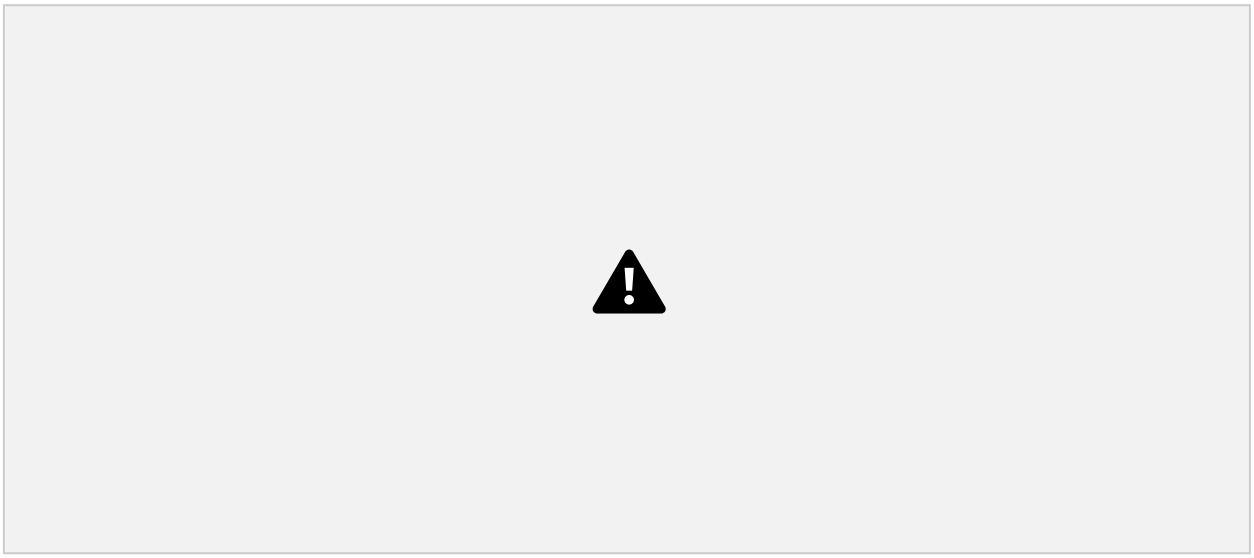
"Hydrophobic and charged hydrophilic molecules have been shown to diffuse poorly through mucus, whereas uncharged hydrophilic molecules are able to diffuse rapidly through the mesh of mucins nearly the speed of water for smaller molecules. ***Drugs larger than 500 Da in size will be especially prone to poor mucus diffusion and becoming stuck, though most drugs will be smaller than 500 Da in size***, thus it is not an important issue. Optimizing a formulation to maximize crossing into the lamina propria will be crucial for any therapy. This size limit is not entirely inhibiting though, as molecules as large as wheat germ agglutinin–horseradish peroxidase (80 kDa) and even whole stem cells have been transported to some degree. Nonpolar compounds are thought to be transported poorly to the CNS intranasally, though there is a growly

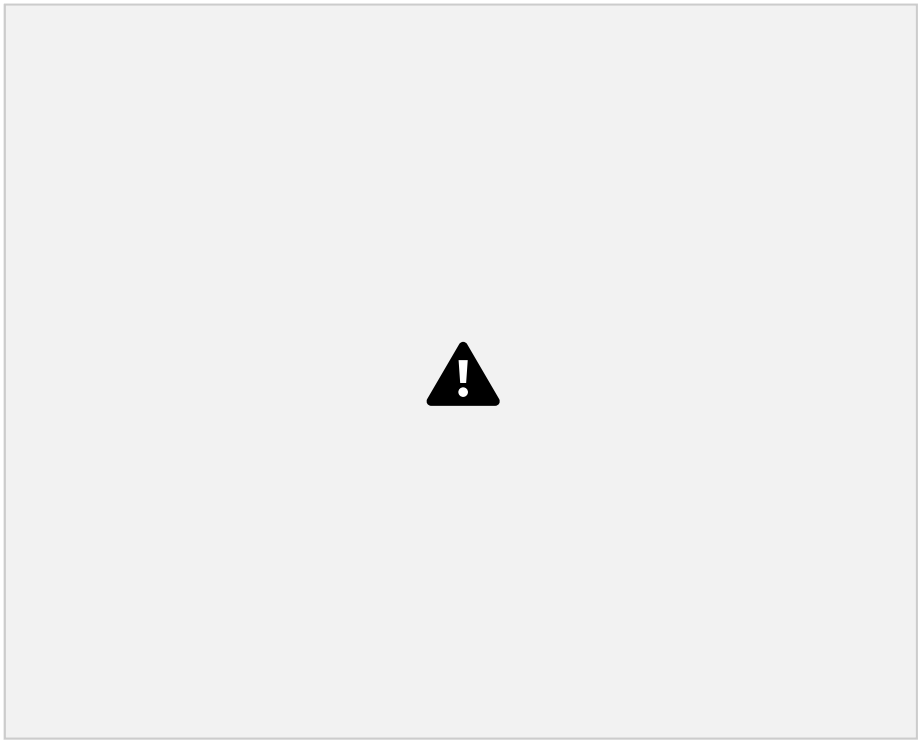
body of evidence that the proper microemulsion formulation can greatly increase the intranasal brain area under curve (AUC) compared to IV administration of the compound. Indeed, there is evidence that with some drugs increasing the hydrophobicity can increase delivery to the CNS. ***It is known that hydrophobic compounds cross biological membranes such as the nasal epithelia, blood vessels, or BBB well. This shows that not only are hydrophobic drugs capable of being administered intranasally with the correct formulation, but this may be an advantage***. Often, researchers are administering doses as low as 25 µL but usually closer to 200 µL in size in these experiments; a size selected because this is the maximum volume of the nasal cavity in the

model rodents. In humans, the nasal cavity is 6–7 mL in volume, which is impractical at best. ***Furthermore, 50% of the rodent nasal cavity is covered in olfactory epithelia, compared to <5% in humans***. This limitation in area will make delivery to the CNS less efficient and ***adds emphasis on making sure administered drugs reach the correct region of the nasal cavity***. Intranasal administration devices are another compelling strategy that will find a role in the clinical use of intranasal drugs. Recall that the olfactory region is <10% of the entire nasal cavity and located on the superior aspect as well as the rapid mucus clearance in the motile respiratory regions. ***Traditional spray pumps tend to only reach the anterior and lateral aspects of the nasal cavity, with <3% of the dose reaching the olfactory region.***"

Intranasal drug administration in psychiatry

https://medium.com/@dsquintana/intranasal-drug-administration-in-psychiatry-80d076f1abdd





“In comparison to intravenous and oral administration, there’s less dosing control with intranasal administration. Sniffing during administration (which is a common behaviour) can reduce treatment efficacy as this draws the medication along the floor of the nose into the throat and gastrointestinal system, which misses the nose-to-brain transfer target in the upper areas of the
nasal cavity”

Numerical Analysis of Enhanced Nano-Drug Delivery to the Olfactory

Bulb https://sci-hub.se/https:/doi.org/10.1080/02786826.2021.1959018

“However, due to the complex structure of the nasal cavity, ***only a minuscule amount reaches the olfactory region naturally***”

Accessing the brain: the nose may know the way

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3652706/pdf/jcbfm201341a.pdf

“The central distribution of [125I]-labeled proteins following IN administration in rats and monkeys has suggested that delivery occurs along olfactory and trigeminal nerve components in the nasal epithelium to the olfactory bulb and brainstem, respectively, with further dispersion to other CNS areas from these initial points of brain entry. ***At least three sequential transport steps are therefore necessary for a substance to be delivered to distant, widespread sites within the CNS following IN administration: (1) transport across the epithelial ‘barriers’ (olfactory or respiratory) in the nasal passages, (2) transport from the nasal mucosa to sites of brain entry***

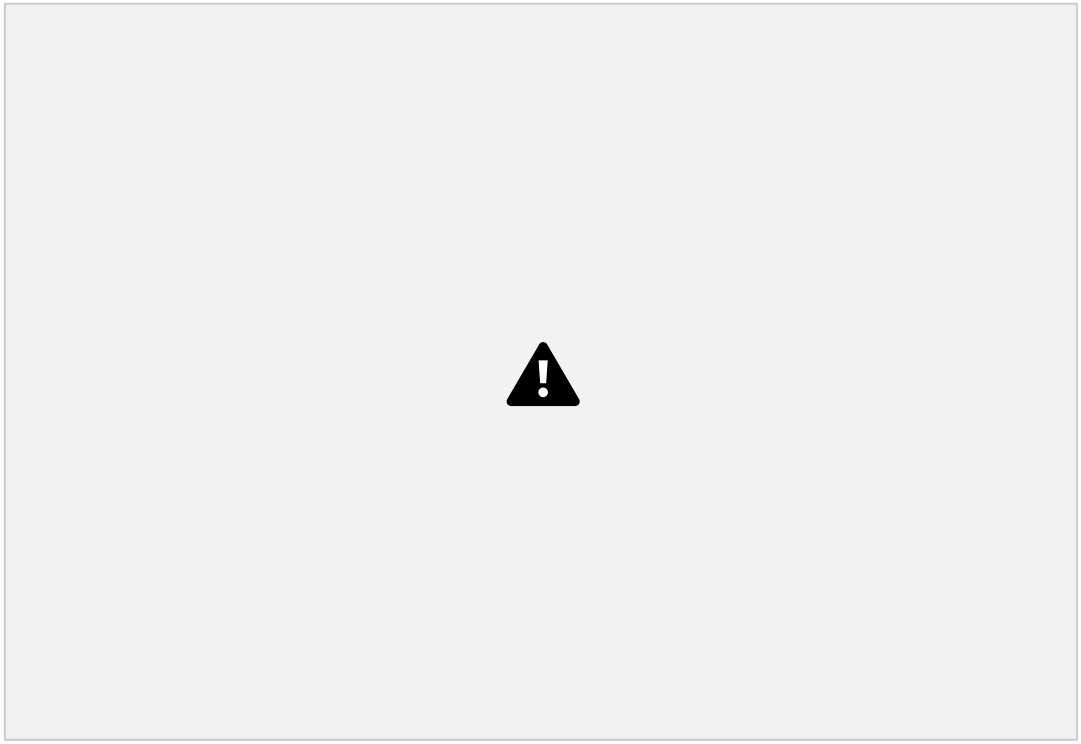
near the pial brain surface in the cranial compartment (i.e. entry points of peripheral olfactory or trigeminal nerve-associated components comprising the delivery pathways) and (3) transport from these initial brain entry sites to other sites within the CNS.

The nasal vasculature may therefore act as a sink for some IN applied substances, effectively preventing them from reaching the CNS. Nevertheless, the observation that the olfactory and respiratory regions of the nasal cavity are stained following IV Evans blue while the brain is not demonstrates that the blood vessels in the nasal mucosa are more permeable than cerebral vessels comprising the BBB. Substances which have crossed the nasal epithelium to reach the lamina propria but escape local absorption into the blood stream and drainage within nasal lymphatics to the deep cervical lymph nodes may be available to enter the CNS. We have previously shown that [125I]-labeled proteins such as insulin-like growth factor-1 (IGF-1) and interferon-β1b (INF-β1b) rapidly distribute along pathways associated with the trigeminal and olfactory nerves to reach both rostral and caudal brain regions in rats and cynomolgus monkeys within 30–60 min after beginning IN application”

Nose-to-Brain Transport Pathways of Wheat Germ Agglutinin Conjugated PEG-PLA Nanoparticles

https://sci-hub.se/10.1007/s11095-011-0641-0

“Nasal absorption of nanoparticles into the systemic circulation was rapid, and the plasma level remained almost constant until 2 h post application The trigeminal nerve showed significantly higher radioactivity than any other tissues and a 38.6-fold radioactivity than the olfactory bulb”



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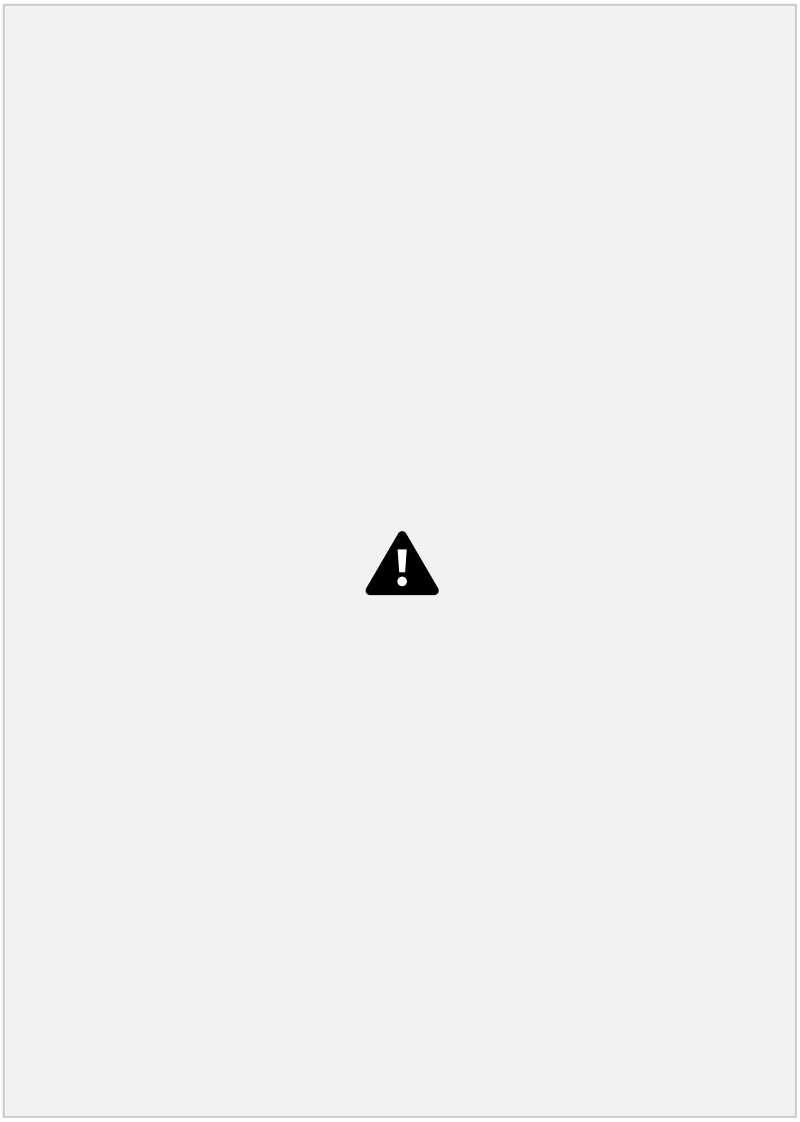
Improvement of Intranasal Drug Delivery with Intravail® Alkylsaccharide Excipient as a Mucosal Absorption Enhancer Aiding in the Treatment of Conditions of the Central Nervous System

https://link.springer.com/content/pdf/10.1007/s40268-021-00360-5.pdf

There is potential for nose-to-brain delivery owing to the presence of the first (olfactory) and fifth (trigeminal) cranial nerves via intracellular, transcellular, and paracellular transport. Although olfactory filaments reach through the nasal mucosal surface, those of the trigeminal nerve are beneath the line of tight junctions

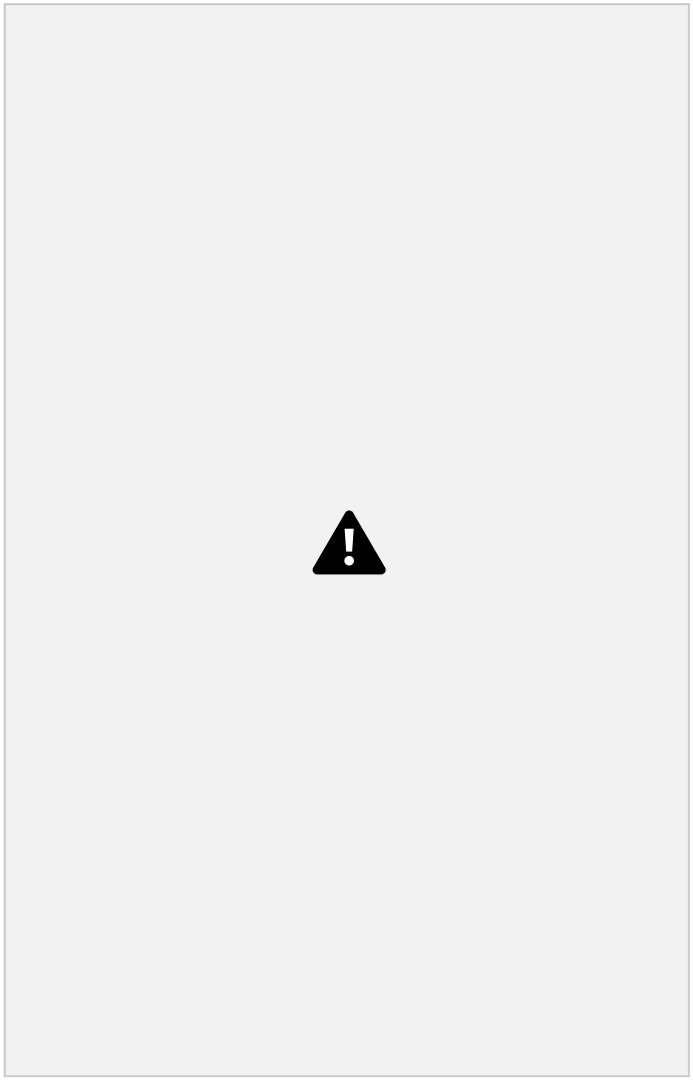


NOSE-TO-BRAIN DRUG DELIVERY: AN UPDATE TO THE ALTERNATIVE PATH TO SUCCESSFUL TARGETED ANTI-MIGRAINE DRUGS

https://www.researchgate.net/profile/Souvik-Chattopadhyay/publication/350040693_Nose-to-brain_drug_delivery_An_update_to_the_alternative_path_to_successful_targeted_anti migraine_drugs/links/604cca5a92851c2b23c8e9ea/Nose-to-brain-drug-delivery-An-update-to the-alternative-path-to-successful-targeted-anti-migraine-drugs.pdf

Intranasal route: The green corridor for Alzheimer's disease therapeutics

https://sci-hub.se/https:/doi.org/10.1016/j.jddst.2021.102791



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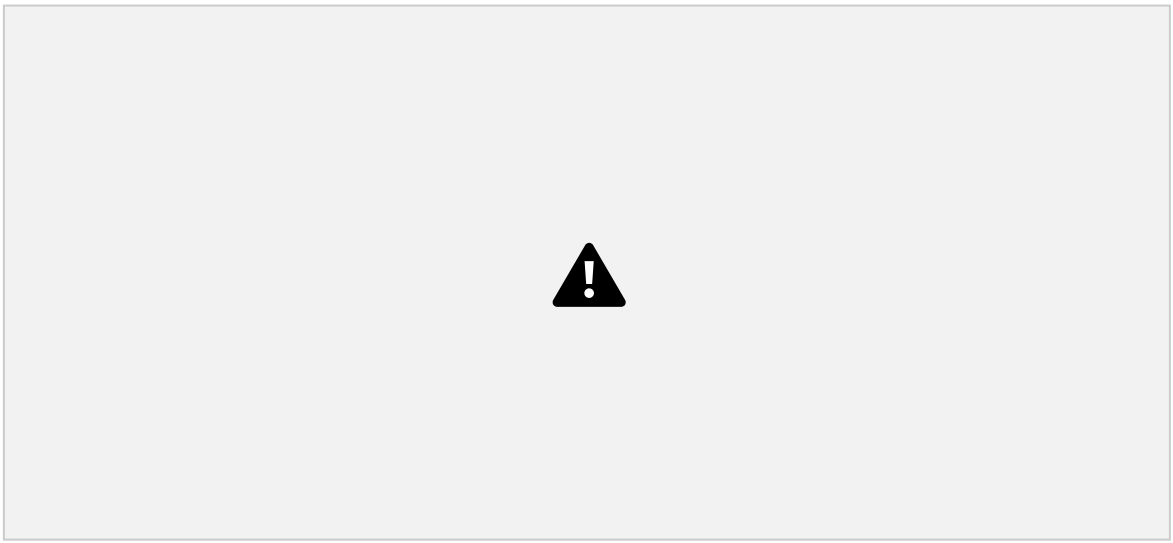


As for intranasal hardware, there are some pharmaceutical high end devices for enhanced nose

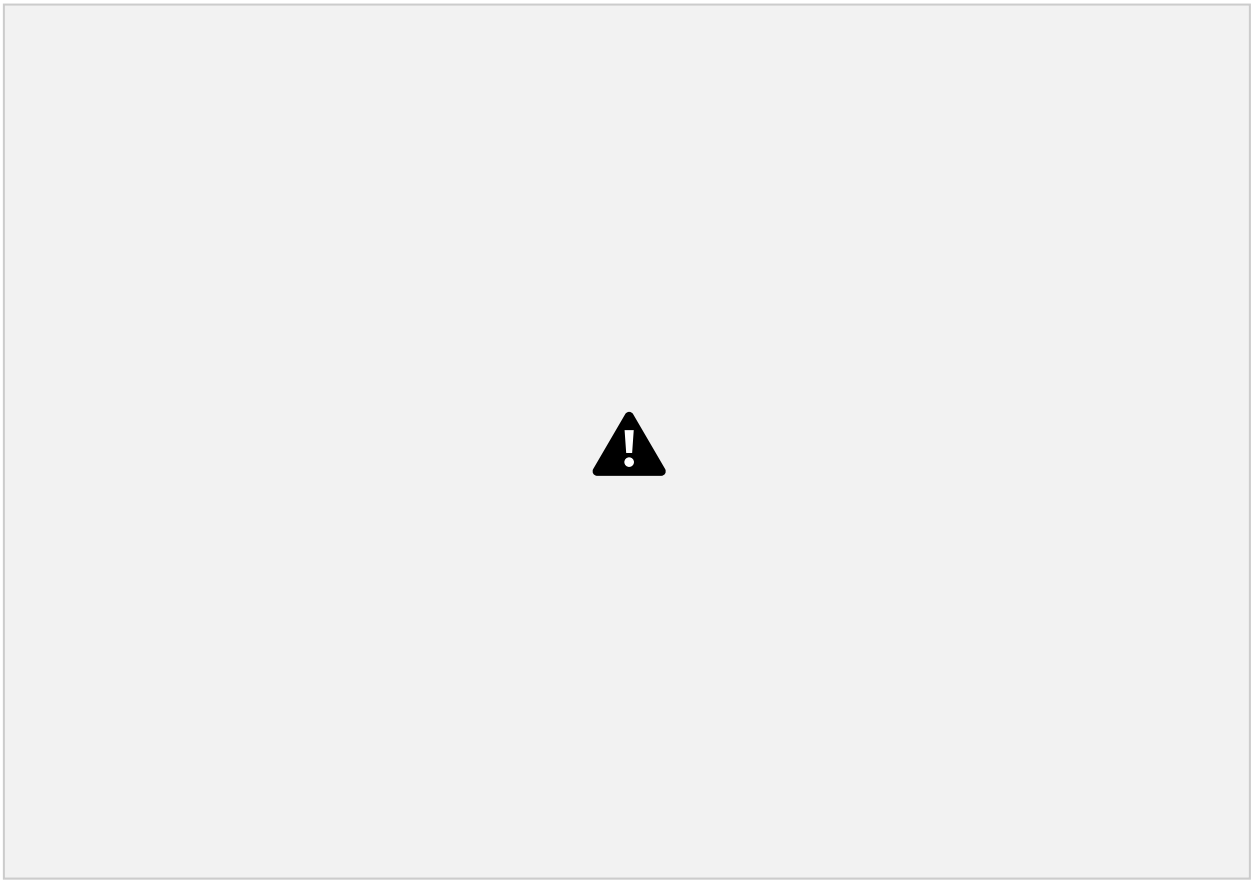
to brain delivery, but not readily retail available, and perhaps not necessary for proof of concept IN delivery.

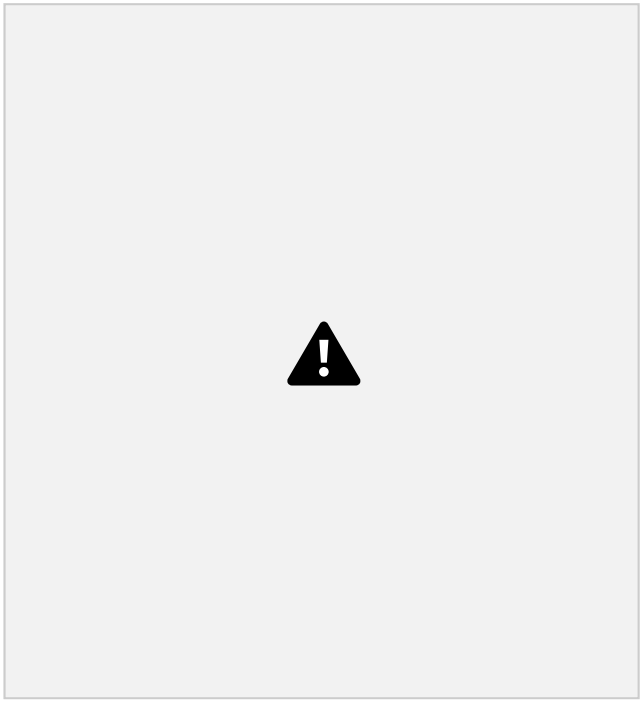
Characterisation of nasal devices for delivery of insulin to the brain and evaluation in humans using functional magnetic resonance imaging

https://sci-hub.se/10.1016/j.jconrel.2019.03.032

"The device with the characteristics that most favoured upper and posterior nasal cavity penetration was selected for an evaluation of nose-to-brain delivery in human volunteers by using an MRI method, arterial spin labelling, to measure CBF as a functional biomarker for central insulin delivery with each shot a single dose of 100 µL was delivered from the device. Two sprays into each nostril for each determination were delivered at an angle of 45° parallel to the septum

Use of a concentrated insulin solution permitted administration of 400 µL spray which is less likely to result in run-off than higher volumes that are sometimes used, e.g. 1.6 mL . ***As a proof of concept we have, through the use of functional MRI, shown that a commercial nasal pump can be used to deliver insulin within the nasal cavity to produce a pharmacological effect in the brain of healthy human volunteers"***





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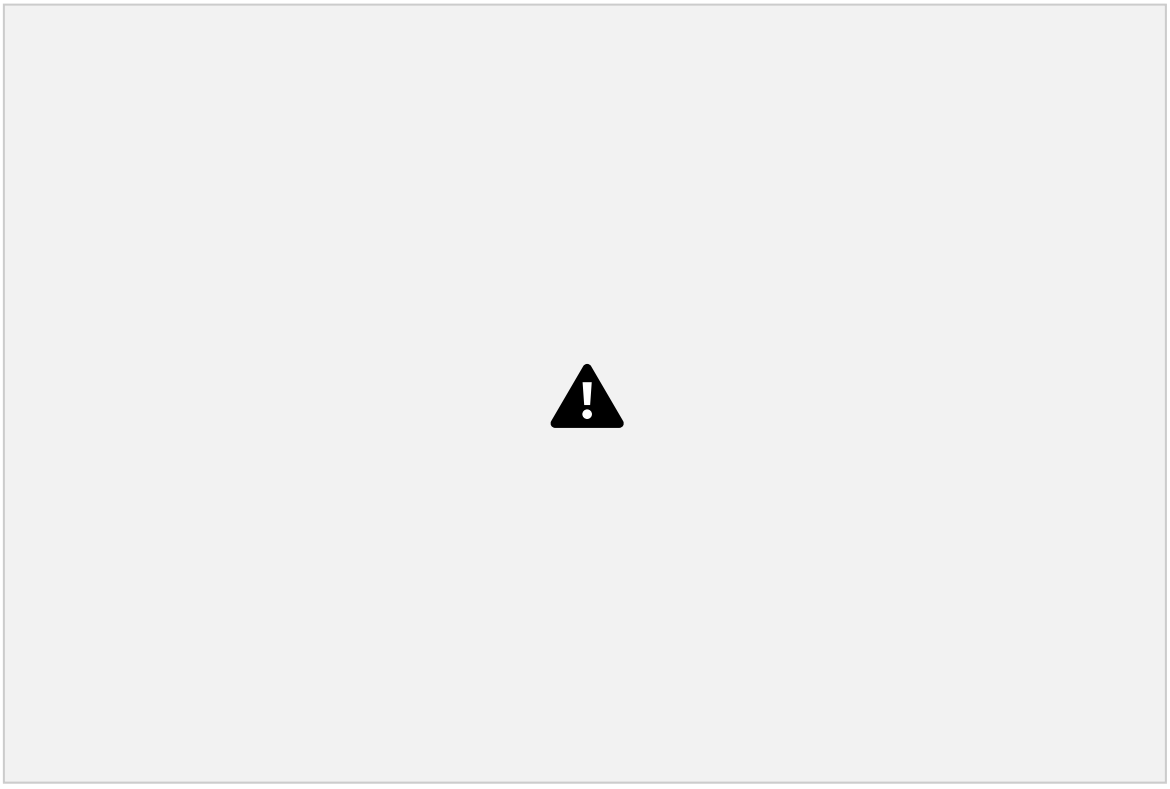

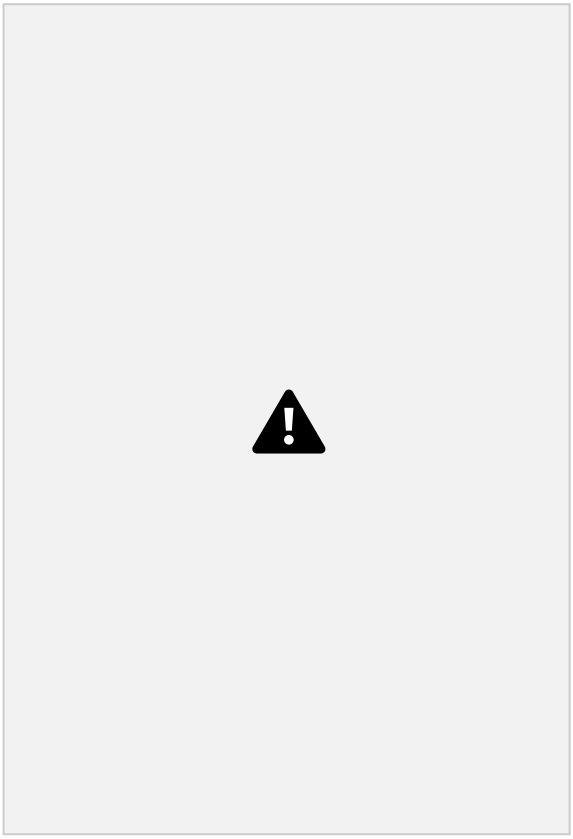

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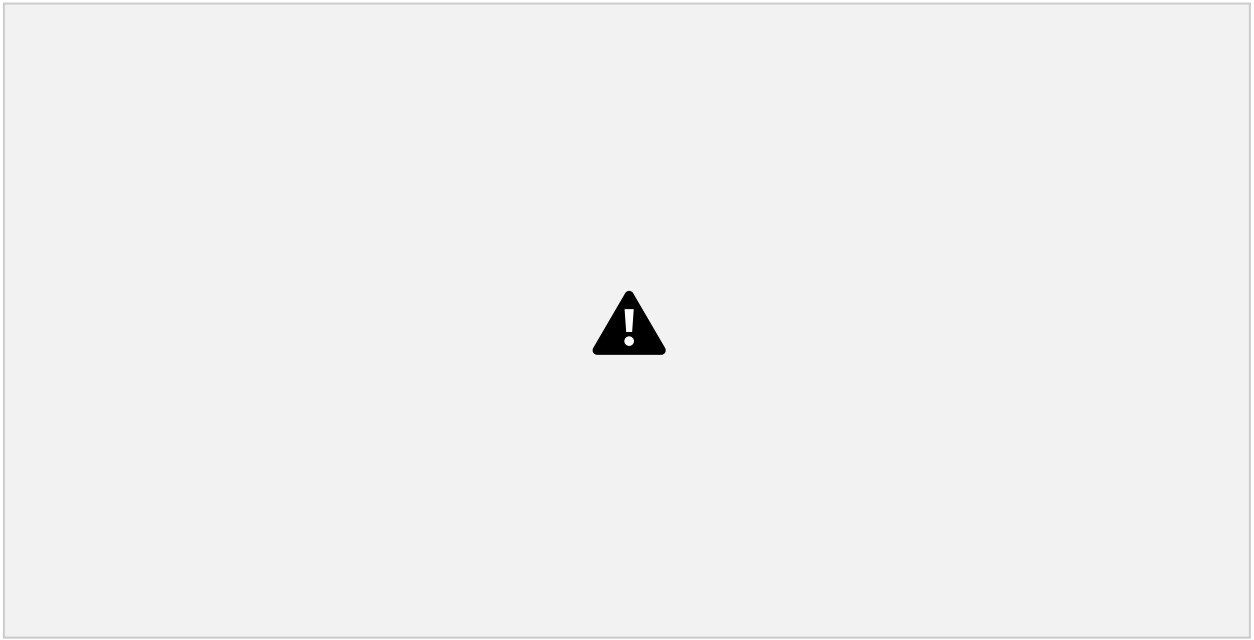


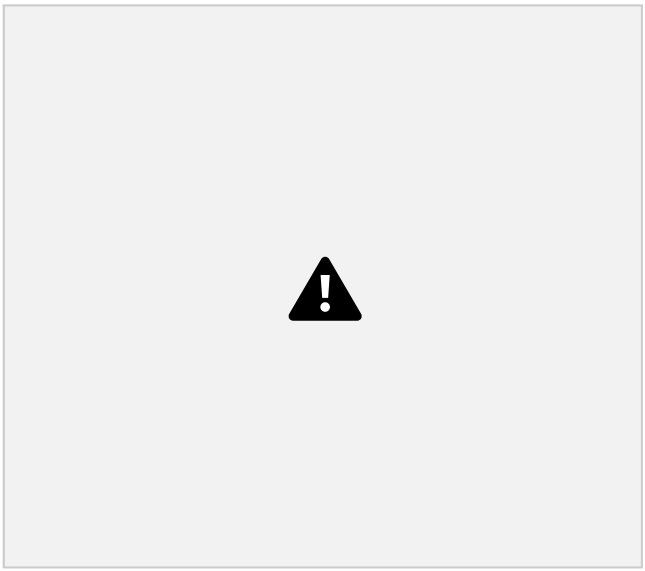
So as you can see, the translation of intranasal from mice to humans is ORDERS OF MAGNITUDE less efficacious, and quite complicated re formulation and delivery method. ***And since rapamycin is highly hydrophobic, formulation and particle size will have to be properly engineered to greatly increase absorption and enhance CNS delivery and brain tissue partition. But the opportunity is orders of magnitude higher direct brain delivery without the***

systemic effects.

The challenge is rapamycin solubility, proper vehicle, nasal mucosal uptake and diffusion, dosing, and safety. What rapamycin excipient to use for actual IM + IN intake?

Nonclinical Vehicle Use in Studies by Multiple Routes in Multiple Species

https://journals.sagepub.com/doi/pdf/10.1080/10915810600961531



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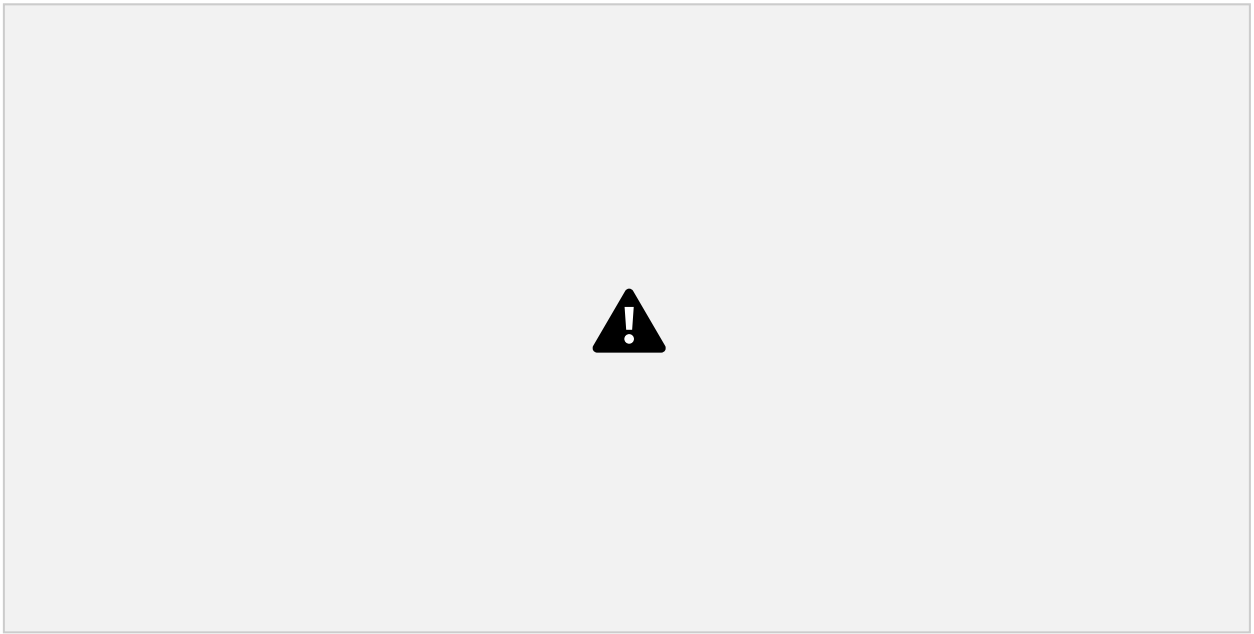


SELECTION OF RAPAMYCIN DRUG DELIVERY EXCEPIENT

Moving from oral physical tablet/powder to parenteral injection requires rapmaycin to be

carried by some type of liquid solution. For intranasal, although there are some powdered drug delivery formulations, they also are typically a liquid formulation. Sublingual, although perhaps an alternate route, does NOT target the CNS/brain and is fraught with enzymatic 1st pass metabolism degradation, and poor solubility. Because rapamycin is hydrophobic, in addition to solubility for drug delivery, the excipient should ideally be lipophilic to aid in wide tissue distribution post delivery. The use of a lipophilic excepient could be a game changer re shifting tissue partitioning/lowering side effects.

Strategies to Improve Drug Strength in Nasal Preparations for Brain Delivery of Low Aqueous Solubility Drugs

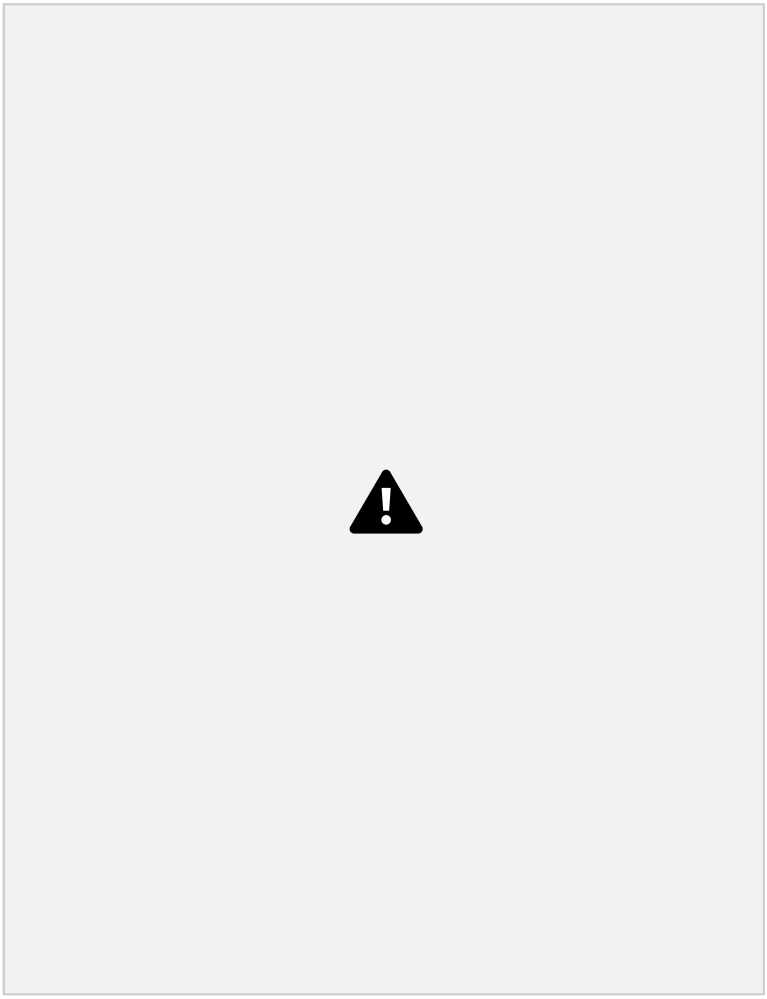
https://www.pharmaexcipients.com/?attachment_id=281597

Despite its numerous advantages, intranasal delivery also has its limitations, the most important being: the fact that only a low volume can be administered (maximum 150–200 µL in humans), therefore requiring potent drugs or high drug strengths; a short residence time in the nasal cavity due to mucociliary clearance, which can limit the time available for drug absorption to occur; enzymatic degradation and efflux transporters, which could likewise reduce drug absorption [2,3]. Furthermore, it is also very important to carefully consider formulation aspects, since none of the formulation's components should be irritant to the nasal mucosa, its pH should be similar to the nasal mucosa's (5.0 to 6.5), and it should be isotonic to slightly hypertonic, all in order to avoid causing sensations of discomfort or toxicity in the nasal epithelium and/or enhanced mucociliary clearance. Moreover, preparation's viscosity and/or

bioadhesiveness should be carefully considered, since a high viscosity/adhesiveness can increase contact time with the nasal mucosa, but if the viscosity is too high the drug diffusion from the formulation might be reduced, which can lead to decreased absorption. ***Independently of the administration route, it is difficult to formulate drugs that have low water solubility at high strength without having to use substantial amounts of cosolvents or surfactants, which are potentially toxic excipients. This is an even bigger problem in intranasal delivery, since the drug has to be administered in a small volume, as mentioned before, and thus even higher drug strengths are required"***

SOLUBILIZATION OF RAPAMYCIN

https://sci-hub.se/10.1016/s0378-5173(00)00617-7



https://www.dmso.org/

“The literature on this agent is voluminous. There are just shy of 1 million scientific articles”

Dimethyl Sulfoxide (DMSO) In Trauma And Disease

https://drive.google.com/file/d/13s_ZYxUc5EG70hVfsa3PVRq2gXmtmW_P/view

PHARMACOLOGY OF DMS0

https://sci-hub.se/10.1016/0011-2240(86)90014-3

CLINICAL TOXICOLOGY

https://1library.net/article/clinical-toxicology-dmso-clinical-pharmacology.q588grwq

“A short-term study (14 days) and a long-term study (90 days) were undertaken by Brobyn who

reported his findings on 213 healthy human volunteers. ***These volunteers were periodically examined by specialists after being administered 1 g/kg/day 80% DMSO daily***. This dose was considered 3–30 times higher than the typical DMSO dose used or recommended. The specific clinical examinations included complete physical examinations with laboratory examinations of blood and urine, ophthalmologic, dermatologic, cardiologic, neurologic, pulmonary function, and bone marrow studies. The results of this study concluded that no significant adverse effects from either the short-term or long-term administration of DMSO were observed after a very extensive toxicology study was conducted. DMSO is a relatively safe drug. Side effects to DMSO are common, but these are usually minor and are related to the concentration of DMSO used and the route of administration"

https://en.wikipedia.org/wiki/Dimethyl_sulfoxide

"DMSO is a non-toxic solvent with a [median lethal dose] higher than ethanol (DMSO: LD50, oral, rat, 14,500 mg/kg; LD50, oral, rat, 7,060 mg/kg"

Dimethyl Sulfoxide USP, PhEur in Approved Pharmaceutical Products and Medical Devices

https://www.pharmtech.com/view/dimethyl-sulfoxide-usp-pheur-approved-pharmaceutical products-and-medical-devices

Application of nasal spray containing dimethyl sulfoxide (DMSO) and ethanol during the COVID-19 pandemic may protect healthcare workers: A randomized controlled trials

https://www.medrxiv.org/content/10.1101/2021.07.06.21259749v2.full.pdf

DMSO in parenteral formulations: a review of opportunities and challenges

https://www.gaylordchemical.com/blog/procipient-in-parenteral-formulations/

Histopathological evaluation of insulin-DMSO formula designed for direct nose-to-brain delivery (2022)

https://www.hh.um.es/pdf/Vol_37/37_5/Maher-37-431-439-2022.pdf

"***The findings presented herein showed no signs of treatment-related lesions or behavioral changes in Sprague Dawley rats following a three-month successive treatment with two strengths of the DSMO formula***. Numerous animal and human studies have underscored the fact that therapeutics, including macromolecules like insulin, can reach directly to the brain through the roof of the nose. bypassing the blood brain barrier (BBB) and the systemic circulation as they travel along the olfactory, trigeminal pathways and nerve fibers. Therapeutics, including both small and macromolecules, like insulin, may be targets for rapid and direct delivery to the CNS owing to the unique connections that the olfactory and

trigeminal nerve fibers possess. ***Moreover, by virtue of the fast onset and bypassing the systemic circulation it offers, DN2BD is considered a sought-after alternative to the conventional routes targeting brain disorders, in general, and neurodegenerative diseases, in particular***. Multiple factors contribute to the success of DN2BD, however, among the most important ones are drug stability, solubility, absorbability, and penetrability. ***DMSO dissolves both polar and nonpolar compounds and is miscible with a wide range of organic solvents as well as with water and has an inhibitory effect on beta-amyloid peptides (Aβ) by altering its biological activity through its solubilizing potential. Additionally, the efficacy of DMSO in CNS-related disorders including AD was previously suggested based on its ability to enhance cranial blood flow, inhibit cholinesterase activity along with inhibiting β-amyloid deposits. As previously reported, intranasal administration of insulin-DMSO delivers it directly to the brain and spinal cord (Publication No US8987199B2).*** This method does not target either the lungs (like inhalational products for bronchial asthma), the nose (like antiallergics) or the blood stream in the nasal mucosa. Alternatively, intranasal administration of the present pharmaceutical composition reaches the brain and spinal cord along the olfactory and trigeminal fibers as it travels through the roof of the nose. ***DMSO was approved for use in pharmaceutical formulations in the U.S. and other countries. It was also placed in the safest category, namely, class 3 solvents, with low toxic potential. Class 3 includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. Solvents in Class 3 may be regarded as less toxic and of lower risk to human health. It is considered that amounts of these solvents of 50 mg/day or less, corresponding to 5,000 ppm or 0.5%, would be acceptable without justification***. Higher amounts may also be acceptable provided they are realistic in relation to manufacturing capability and good manufacturing practice (GMP). Despite being toxic in high concentrations, DMSO has a well established safety profile in low concentrations and was successfully included in formulations at higher concentrations than the one used in the current study without demonstrating any signs of toxicity. Also, our findings showed that no drug-induced morphological changes were microscopically observed after intranasal administration of insulin-D using the formulae for three successive months. Thus, we can conclude that even the higher strength of insulin-D (with higher insulin and DMSO concentrations) did not elicit histological changes that might imply toxicity when compared to the normal untreated group"

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Looking at the high solubility solvents, and considering additional pharmacological aspects (safety in humans/animal models AND highly absorbed in both hydrophylic and lipophilic systemic tissues), DSMO looked like a very promising vehicle for rapamycin delivery. I was not satisified with the safety aspects of benzyl alcohol.

Therefore, I decided on using DSMO for both IM + IN.

INTRAMUSCULAR (IM) DRUG INJECTION

DELIVERY Intramuscular Injection

https://www.ncbi.nlm.nih.gov/books/NBK556121/

Advantages

- Rapid and uniform absorption of the drug, especially the aqueous solutions • Rapid onset of the action compared to that of the oral and the subcutaneous routes • IM injection bypasses the first-pass metabolism of the drug
- It also avoids the gastric factors governing drug absorption
- ***Has efficacy and potency comparable to that of the intravenous drug delivery system (IV is 100% biovailable, and IM is near to 100% as well)***
- Highly effective for emergency scenarios such as acute psychosis and status epilepticus • Depot injections allow slow, sustained, and prolonged drug action
- A large volume of the drug can be administered compared to the subcutaneous route

Disadvantages

- The absorption of the drug is determined by the bulk of the muscle and its vascularity • The onset and duration of the action of the drug are not adjustable
- Inadvertent injection within the subcutaneous plane can lead to delayed action of the drug
- Suspensions, as well as oily drugs, cannot be administered

IV vs. Oral vs. Intramuscular vs. Intranasal Ketamine: Why Route Matters

hsps://www.principiumpsychiatry.com/iv-vs-oral-vs-intramuscular-vs-intranasal-ketamine why-route-masers/

Intravenous: 100%
Intramuscular: 93%
Intranasal: 25-50%
Sublingual (under the tongue): 30%
Orally (by mouth): 16-24%
42

Drug absorption, distribution, metabolism and excretion (pharmacokinetics)

https://www.medicilon.com/drug-absorption-distribution-metabolism-and-excretion pharmacokinetics/

Absorption of drugs outside the digestive tract

Injection administration includes intramuscular injection, subcutaneous injection and intravenous injection. ***The absorption speed of injection administration is generally faster than oral administration, and the bioavailability is higher. After administration, the drug first diffuses to the surrounding water-rich tissues, and then enters the blood circulation through the capillaries. The water solubility of the drug and the blood flow at the injection site affect the absorption rate of the drug during injection.*** Drugs with high water solubility are easy to diffuse in the injection site, increasing the absorption area, which is conducive to absorption;

suspension absorption is slow and lasting. Some drugs are absorbed less quickly by injection, such as ampicillin, tetracycline, diazepam, and phenytoin. In injection sites with rich blood flow, such as skeletal muscle, the drug is absorbed quickly. Intravenous administration has no absorption process, which can make 100% of the drug enter the human circulation. Systemic circulation includes blood circulation and lymphatic circulation. ***Since the blood flow rate is 200 to 500 times faster than that of the lymph fluid, the distribution of drugs in the body is mainly completed by the blood system, but the transport of drugs in the lymph system is also of great significance.***

When interstitial administration such as intramuscular or subcutaneous injection or intra organ or intratumor injection, the drug has two transport routes: capillary and lymphatic capillaries. At this time, the nature of the drug, especially the size of the molecular weight and the permeability of the tube wall determine the transport route of the drug. Drug molecules with a molecular weight of less than 5kDa can be entered in both ways, but since the blood flow is much larger than the lymph flow, almost all of them are apparently transported by blood. On the contrary, macromolecular drugs with a molecular weight greater than 5 kDa have an increasing tropism to the lymphatic system as the molecular weight increases. ***In order to increase the tropism of the drug to the lymph, the drug molecules can be formed into various polymer complexes, or made into water-in-oil (W/O) emulsions, liposomes, microspheres, etc***. **(eg. use of DSMO).** The blood flow distribution in the organs of the human body is the liver the most, followed by the kidney, brain, and heart. To enter the tissues and organs, the drug must first pass through the blood vessel wall (epithelial cell membrane), and finally penetrate the tissue cell membrane. The cell membrane is a protein-containing phospholipid bilayer, and the mechanism of drug penetration through the cell is consistent with the transmembrane transport mechanism. Drugs generally pass through the cell membranes by passive diffusion, and undissociated drugs and fat-soluble drugs are easier to pass. That is, the pKa and oil/water partition coefficient of the drug can affect its permeability to the cell membrane. The liver is the main metabolic organ for most drugs due to its high blood flow and most metabolic enzymes The digestive tract is the most common site of extrahepatic metabolism. ***When the route of administration is changed, the absorption, distribution, and excretion of the drug will also change, and attention should be paid to the difference in dosages for different routes of administration***."

Subcutaneous nanotherapy repurposes the immunosuppressive mechanism of rapamycin to enhance allogeneic islet graft viability

https://www.researchgate.net/publication/346806099_Subcutaneous_nanotherapy_repurposes_the_immunosuppressive_mechanism_of_rapamycin_to_enhance_allogeneic_islet_graft_viability

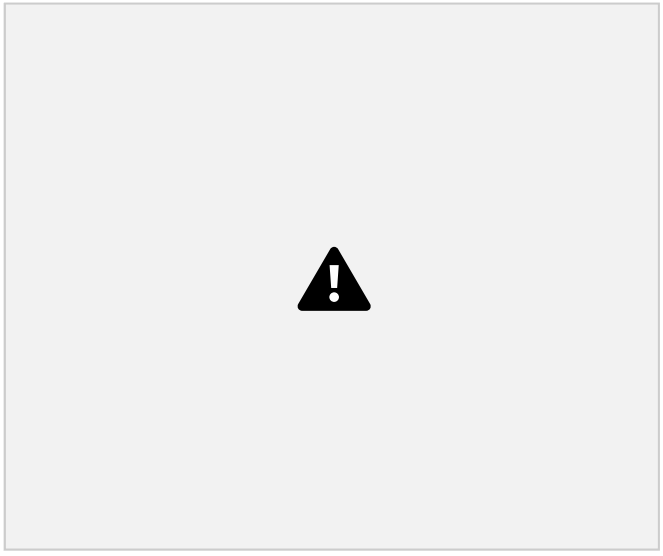
"Repurposing mTOR inhibition significantly improved maintenance of normoglycemia in a clinically relevant, MHC-mismatched, allogeneic, intraportal (liver) islet transplantation model. These results demonstrate the ability of engineered nanocarriers to repurpose drugs for alternate routes of administration by rationally controlling cellular biodistribution. Side effects

related to oral rapamycin administration stem primarily from poor and inconsistent bioavailability and the wide cellular biodistribution. These side effect occur due to the ubiquitous expression of mTOR in diverse cell types, resulting in unintended cell populations also experiencing cell cycle arrest. Clinically, this can lead to malignancy, enhanced susceptibility to infection, impaired wound healing, thrombopenia, alopecia, gastrointestinal distress, gonadal dysfunction, hypertension, hyperlipidemia, nephrotoxicity and peripheral edema. Subcutaneous administration would avoid bioavailability issues that plague Rapamune including first pass metabolism, elimination by intestinal cytochrome P450 and p-glycoprotein, and variability associated with food content. Importantly, the subcutaneous route of administration provides the advantage of targeting lymphatic drainage."

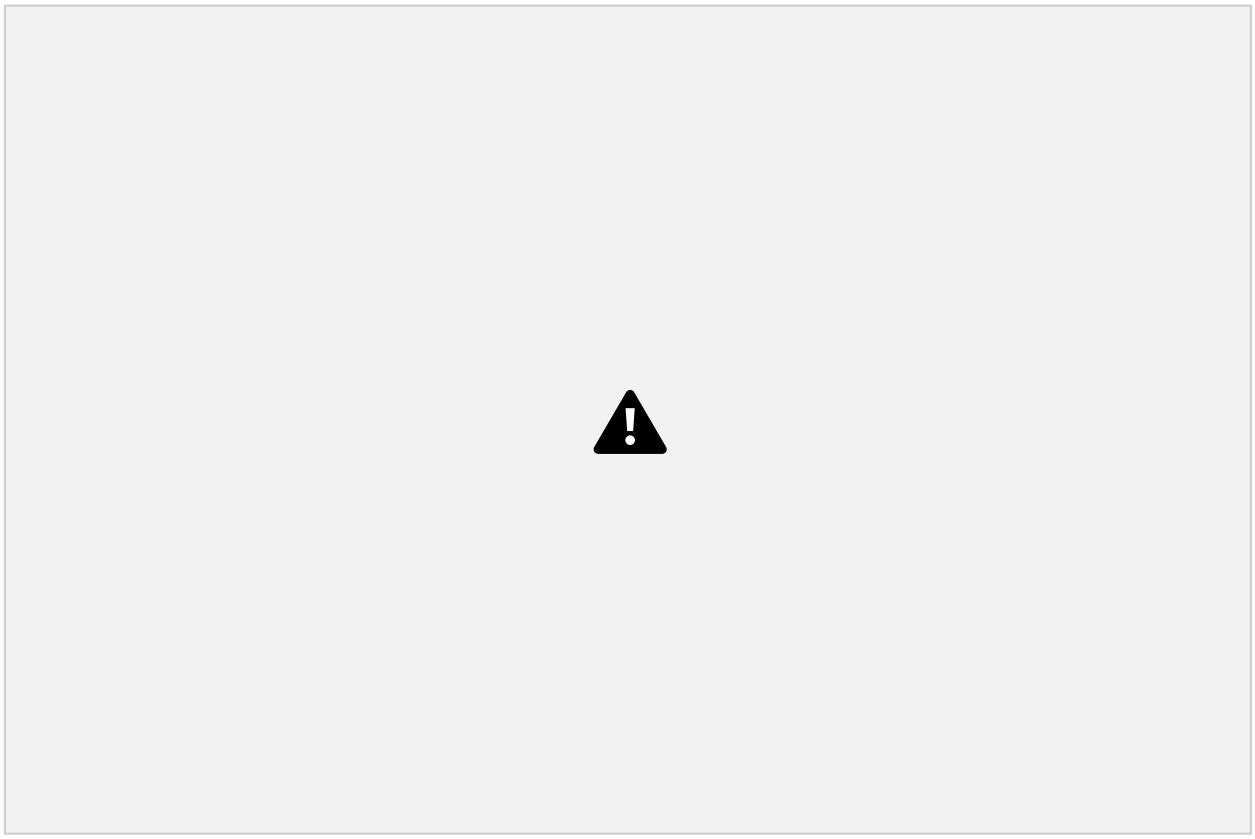
This is an amazing paper showing targeted parenteral delivery of rapamycin with an engineered formulation…a proof of concept new paradigm in human translation.

Comparative pharmacokinetics of RAD001 (Everolimus) in normal and tumor-bearing rodents

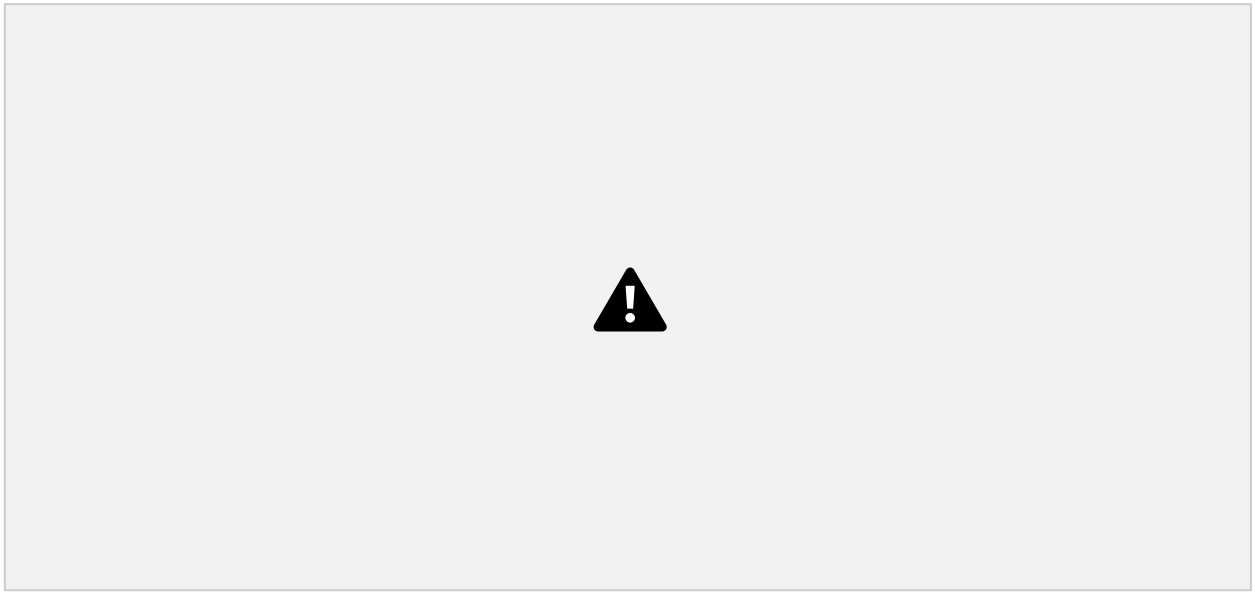
https://sci-hub.se/10.1007/s00280-009-1068-8



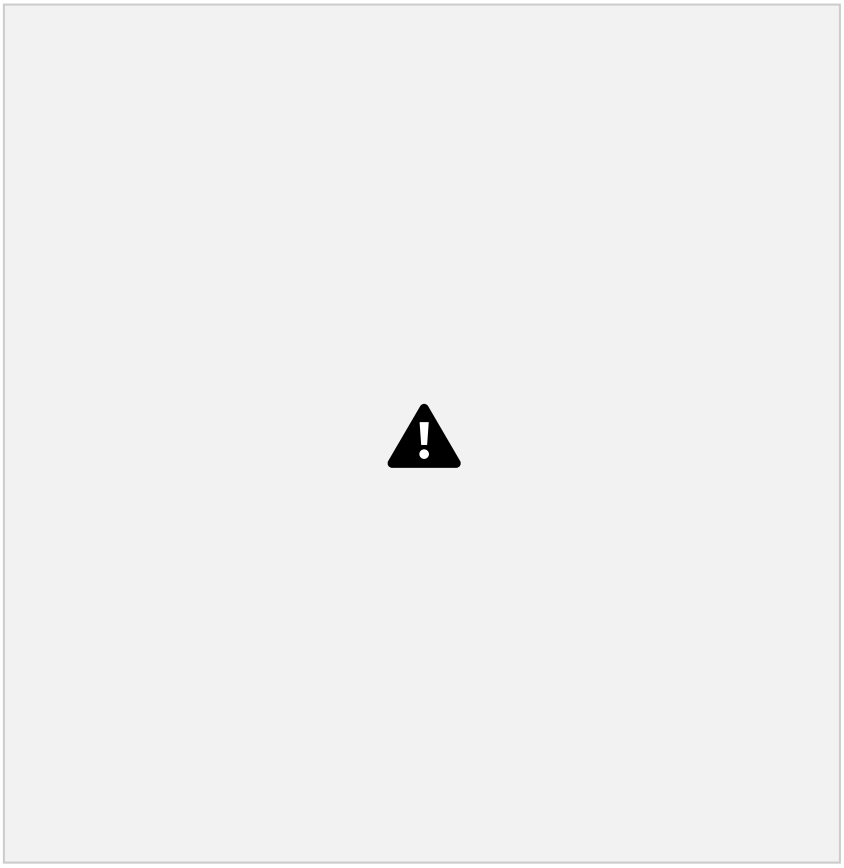
Notice in above graph/table the FAR HIGHER AUC of IV vs ORAL for same dose.

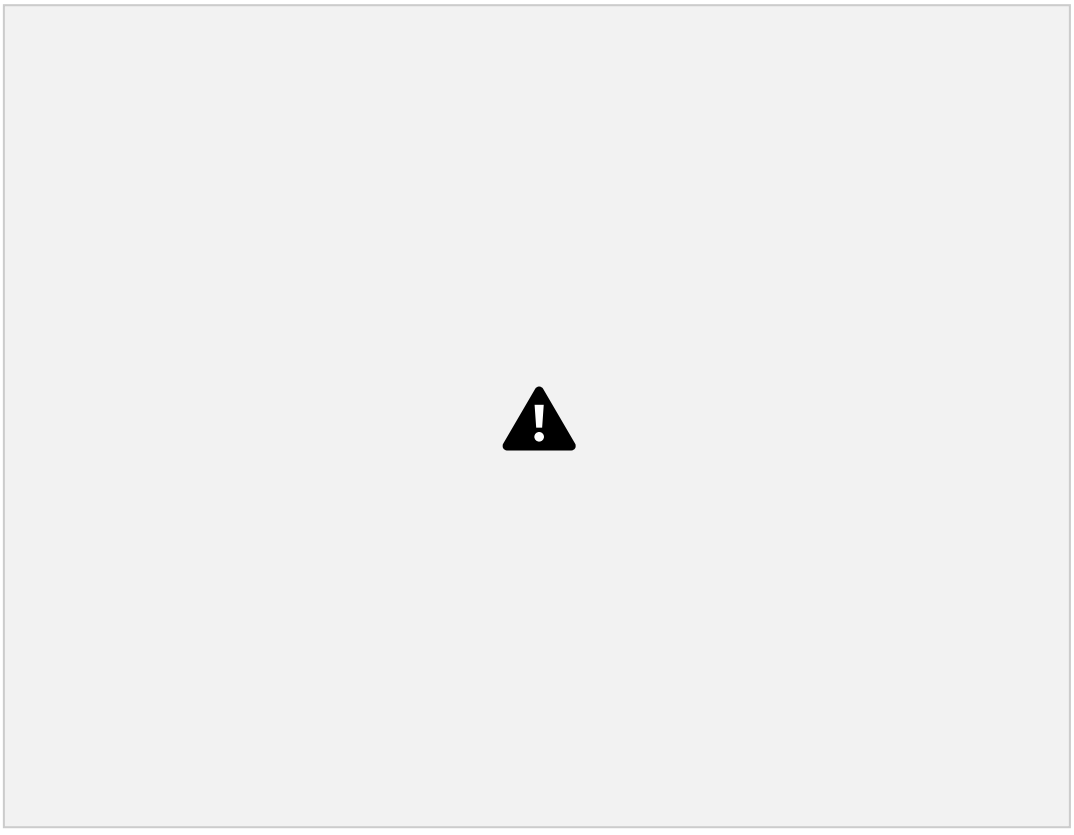


Notice in above table the FAR HIGHER AUC of 10mg/kg vs 15mg/kg PO (oral)!!



Notice in above table the FAR HIGHER tissue levels of Everolimus at higher doses.





Notice higher brain levels with higher doses, and rapid brain clearance (Everolimus)

I've been on biweekly TRT injections for years in my upper outer thigh using ½" long, 0.5mL volume, 27G bevelled syringes.

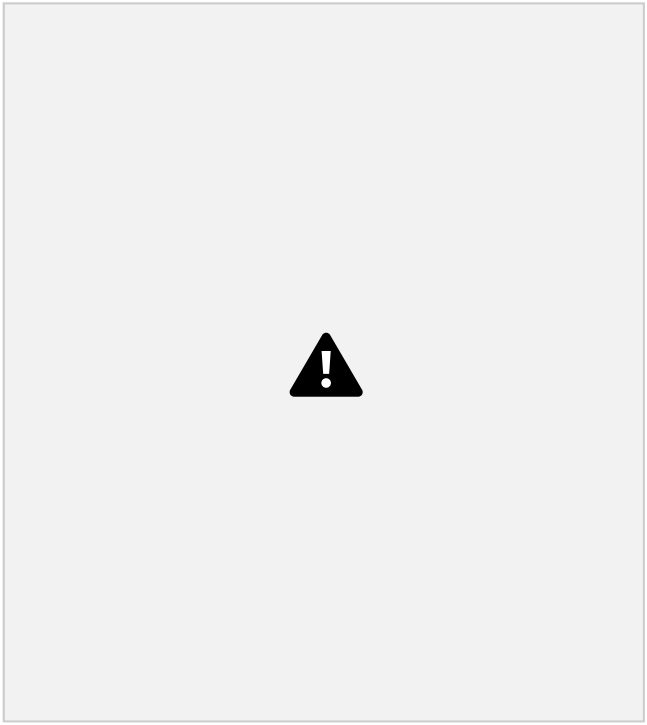
A reference on IM injection sites:

Anatomically safe sites for intramuscular injectons: a cross-sectonal study on young adults and cadavers with a focus on the thigh

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7012163/pdf/khvi-16-01-1646576.pdf

"The middle of the vastus lateralis is an appropriate site for intramuscular injections because of the low risk of vascular or nerve damage. The present results support good practices for site selection for intramuscular injections."

I actually do most of my injections at the upper section of the vastus lateralis, softer tissue, easier needle penetration.



ENGINEERING THE DSMO/RAPAMYCIN FORMULATION

Because the concept of IN & IM is to deliver the target dose at MINIMUM delivery volume (typically < 0.5mL for IM, and 100uL for IN), the target rapamycin dose in mg can ONLY BE ACHIEVED WITH HIGH QUANTITY RAW POWDER. The use of tablets is highly problematic, firstly the cost of a few hundred mg minimum IM + IN dosing batch production would be much higher than powder, and secondly, perhaps more fundamentally limiting, the use of fillers and other agents in tablets that one might not want to deliver to the brain or systemically without the intended gastro processing, filtering out, and elimination (as they were engineered by the pharma companies).

Fortuitously, my initial foray into rapamycin was via powder which enabled this quick pivot alternate delivery route experimentation. I had been experimenting with powder, the source being confirmed via 3rd party Sirolimus testing with a few forum individuals, and consistent rapamycin side effects. I've posted on my source and 3rd party lab testing:

https://www.rapamycin.news/t/sirolimus-powder-3rd-party-analysis/1333

My own n=1 experience over the past year was unremarkable with ever increasing powder doses (no discernible side effects, no Sirolimus blood level on one low dosing protocol), so clearly massive destruction of rapamycin powder via 1st pass metabolism. Because of the 0.1uL volume limit for intranasal, and 0.5mL volume limit for intramuscular, and thinking about initial dosing discovery starting point of approximately < 1mg for IN and 5-10mg IM, and balancing with the remaining amount of rapamycin powder at hand, I chose to produce stock solutions of 300mg rapamycin powder in 10ml DSMO, or 30 mg/mL concentration. From these stock solutions, I could titrate dosing to achieve a range for further exploration.

Initially there was some consideration of producing a super concentrated rapamycin/DSMO solution and then diluting down with water to perhaps deliver less DSMO per dose…but in experimenting with the concept, in a small amount, as soon as water is added to a DSMO/rapamycin mixture, the rapamycin IMMEDIATELY crystallizes out of solution, as DSMO dissociates with rapamycin and attaches itself to water. So DSMO/water was quickly dropped from further consideration. The use of water, would also require the use of FAR HIGHER amounts of rapamycin powder to overcome the diluent effect.

DSMO turns from liquid to solid at about 19C, so keeping the solutions in the fridge/freezer would solidify them, and require a quick dethawing before dosing. It appears from other studies, stock solutions of rapamycin kept at 4C or lower are quite stable. Since rapamycin itself is stable at - 20C, I simply keep the stock solutions in the freezer to ensure long term stability of a batch that it intended to last many months+. Again, a simple but important clinical translation requirement.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2882366/pdf/pone.0011013.pdf

“Stability studies consisted of HPLC analysis at time zero (t = 0) and after 1-month storage at different temperatures. Stability was maintained at 4 C for 1 month. A portion of the remaining volume was held at 4uC for 6-month stability studies, again showing stability of this initial (COTC003) formulation at 4 C. Although some precipitation was observed due to low solubility, it was overcome with sonication”

Rapamycin powder previously discussed, was measured on a very accurate mg scale, the DSMO was purchased on Amazon (odour free, pharma grade), the nasal sprayer over the counter, and syringes repurposed same as those for used for TRT injections. The 300 mg rapamycin easily dissolved in the 10mL DSMO (even at 300mg/mL solubility, it would take 3,000mg to reach solubility threshold). I did notice some very fine residual particles even after hand shaking, and perhaps sonification might further dissolve those particles, or those could be remnants analogs from original powder manufacture.

The rapamycin powder/DSMO was produced and stored in a small glass vial (DSMO will dissolve many types of plastics, so container selection is CRITICAL). The small glass vial also allowed for easy drawing of dose for IM given the ½” needle length. The glass vial was boiled and further rinsed with 70% Isopropyl alcohol prior to production of the powder/DSMO batch. For the nasal sprayer, I confirmed the entire assembly was made of polypropylene, inert to DSMO. Instead of boiling and risk of altering the mechanical properties of the sprayer, I simply rinsed thoroughly with Isopropyl alcohol prior to producing the 10mL of 30mg/mL rapamycin solution. The glass vial in the embedded image shows the residual DSMO shows the frozen DSMO, as it the vial was lying on its side in the freezer.

Intranasal Delivery to the Central Nervous System: Mechanisms and Experimental Considerations

https://sci-hub.se/10.1002/jps.21924

On day of dosing, I simply take the sprayer and glass vial out of the freezer, and either warm under hot water or simply allow 10-15 mins to defrost, followed by 10-20 seconds of hand shaking. For the intranasal, after a prime pump, one full pump per nozzle, with head normal position, and then switching to the “praying to mecca” position for 2 minutes to try and optimize drug delivery to the olfactory nerve area. Because DSMO solubilizes mucosal layers, there can be some “runniness” felt after dosing, and some of this flow can be felt draining down the nasal cavity, and is either lost to degradation or absorbed systemically. For IM, I simply place the syringe tip into the glass vial, and draw out the liquid contents making sure to avoid any air bubbles.

INTRANASAL SPRAYER CALIBRATION		

10 PUMPS CLARITIN SPRAYER		4 grams
Density		1 gm/cm3
Volume per SINGLE pump	0.094	
Volume per SINGLE pump		4 uL

INTRANASAL FORMULATION/DOSING @ 30 MG/ML					
INTRANSAL RAPA MOUSE DOSE		5 mg/kg		STUDY	
HUMAN EQUIVALENT DOSE	0.29			MOUSE mg/kg / 12 x 70kg	
VOLUME DOSE	0.00967			@30 MG/ML	
VOLUME DOSE	9.7				
VOLUME FACTOR MICE > HUMAN		0 < 10% HUMAN OLFACTORY UPTAKE			
HUMAN VOLUME DOSE		7 uL			
# OF SPRAY PUMPS	1.0				
HUMAN DOSE/PUMP		9 mg			

50

INTRAMUSCULAR FORMULATION/DOSING @ 30 MG/ML	
VOLUME OF DOSE (ML)	MG RAPAMYCIN
0.1	3
0.17	5
0.2	6

0.25	7.5
0.3	9
0.33	10
0.4	12
0.5	15

6 WEEK DOSING SCHEDULE

Time: ZERO 7.5mg IM 5.8mg IN

Time: 1 WEEK 10mg IM

5.8mg IN

Time: 2 WEEKS 15mg IM

5.8mg IN

Time: 3 WEEKS 15mg IM

5.8mg IN

Time: 4 WEEKS SIROLIMUS TROUGH + SIROLIMUS CMAX 1, 2 3 HR

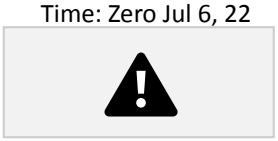
IM

5.8mg IN

Time: 6 WEEKS 15mg

Week 6:

Time: Zero Jul 6, 22



CMAX 1, 2, 3 HRS 15MG 5.8mg IN

Aug 17, 22

Time: 5 WEEKS SKIP DOSING

NOTES ON DOSING/OVERT SIDE EFFECTS

- The time zero 1st IM injection was a full 0.25mL injection volume. Immediate observation that DSMO/rapamycin injections are quite stinging vs no reaction with the TRT formulation
- The time zero 0.25mL created quite a large bump on my thigh, which was quite painful (impacted my gait) and actually lasted several weeks before completely dissipating. • Removing the syringe with the DSMO/rapamycin injections, often blood would flow out of injection site: this never happens with the TRT injections.
- The intranasal spray dosing stings for perhaps 10-30 seconds, then quickly dissipates. There is no lingering irritation or inflammation in the nasal area after the 5 doses of weekly intranasal dosing.
- I noticed immediately changes to my bowel movements and stool (smaller, softer) during

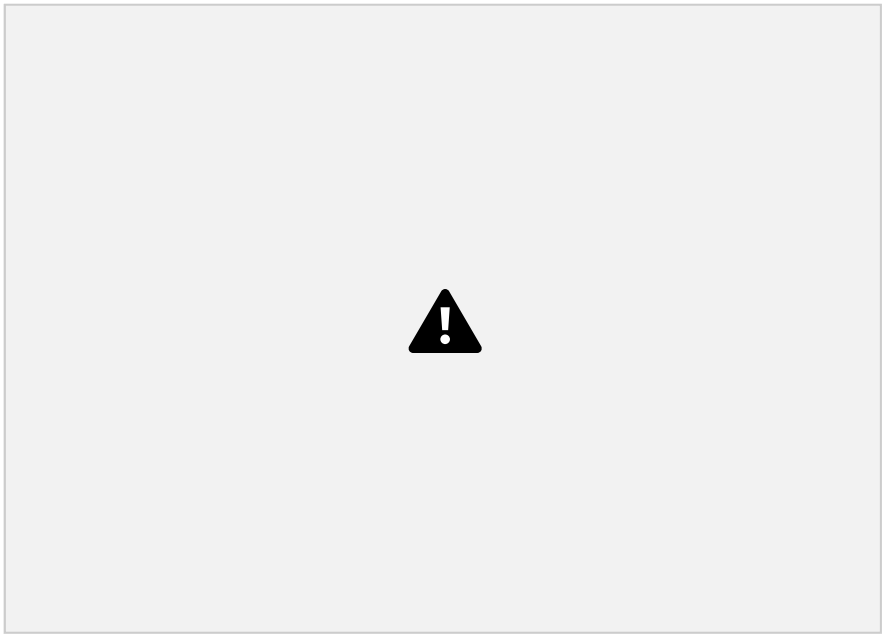
the 1st week of dosing. No other side effects.

- On week 1, I upped dose to 10mg, but split the 0.33mL into 3 x 0.1mL to reduce the volume of DSMO delivered per injection, splitting into 2 one left thigh, one right thigh. Stinging was still observed at injection site for perhaps 90 minutes, but after 24 hrs, had dissipated. Further ongoing changes to bowel movements/stool, definitely smaller, lighter color, and softer stool.

Transient rapamycin treatment can increase lifespan and healthspan in middle-aged mice

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4996648/pdf/elife-16351.pdf

We know from rapamycin/mice studies, there appears to be a gut microbiome remodelling, resulting in fundamentally altered fecal profile.



- On week 2, I upped the dose to 15mg or 0.5mL. I split the dose into 5 x 0.1mL injection sites, 3 one thigh, 2 other. Again, stinging at injection site, but after few hours, had dissipated. I observed a few scalp pimples this week, no other outwardly signs, including NO canker sores (in fact, at time of writing, not a single canker sore, and pimples have dissipated)
- On week 3, repeat of week 2
- On week 4, booked a full blood 9 AM panel for my regular doc consult, and included sirolimus trough, and CMAX exploration. For CMAX exploration, gave myself 15mg IM + 5.8mg IN at the lab office, and waited for separate Sirolimus blood draws taken at 1, 2, 3 hrs.
- At approximately dinner time on week 4 (same day as panel, post 5th effective dose), I felt some strong gastro discomfort after dinner, and some intense dysuria. I did not feel that

in the AM post dose.

• This gastro completely dissipated, and so did the dysuria the following day. • The trough/Cmax was done on Aug 3, 22. The Cmax results came back quickly as shown below, but I had to wait approx 10 days for the trough level to come back (trough was bundled with the larger long time tests). In the interim, I SKIPPED the normal weekly dosing set for Aug 10, 22 (Week 5) until I received the trough level. Unfortunately, this particular lab does not report results over 40 ng/mL. Next draw, will try to ensure I get true absolute values from perhaps alternate lab.

• The trough and Cmax results shown in table below. ***Clearly with a trough of 11.1 ng/mL, I had easily achieved cancer/transplant therapeutic levels otherwise unachievable at similar weekly intermittent ORAL DOSING levels.***

BLOOD DRAW	SIROLIMUS (ng/mL)
TROUGH	11.1
1 HR	36.6
2 HR	>40
3 HR	>40

• I WAS/AM FEELING NO SIDE EFFECTS, SO I RESUMED WITH 15mg IM + 5.8mg IN on Aug 17, 22 (Week 6)

• The Aug 17, 22 IM injections were very strangely, only stinging at the point of needle insertion, but I felt NO lingering pain at the injection site like past injections?

AUC CALCULATIONS IM + IN vs ORAL

The true therapeutic dosing exposure is measured by AUC, a key parameter used by clinicians, which for oral rapamycin, is approximately linear with trough level, thus trough is used as proxy for AUC after such a long and repeatable history with the drug. But since IM+IN is a different delivery route, with different pharmacokinetics, calculating AUC is crucial to assessing and benchmarking this new paradigm vs oral.

https://en.wikipedia.org/wiki/Area_under_the_curve_%28pharmacokinetics%2

9 https://www.thebody.com/article/ab-cs-pharmacokinetics

https://www.icp.org.nz/oral-availability/auc

Sirolimus and everolimus in kidney transplantation

https://sci-hub.se/https://doi.org/10.1016/j.drudis.2015.05.006

Phase I studies of sirolimus alone or in combination with pharmacokinetic modulators in advanced cancer patients

https://pubmed.ncbi.nlm.nih.gov/22872575/

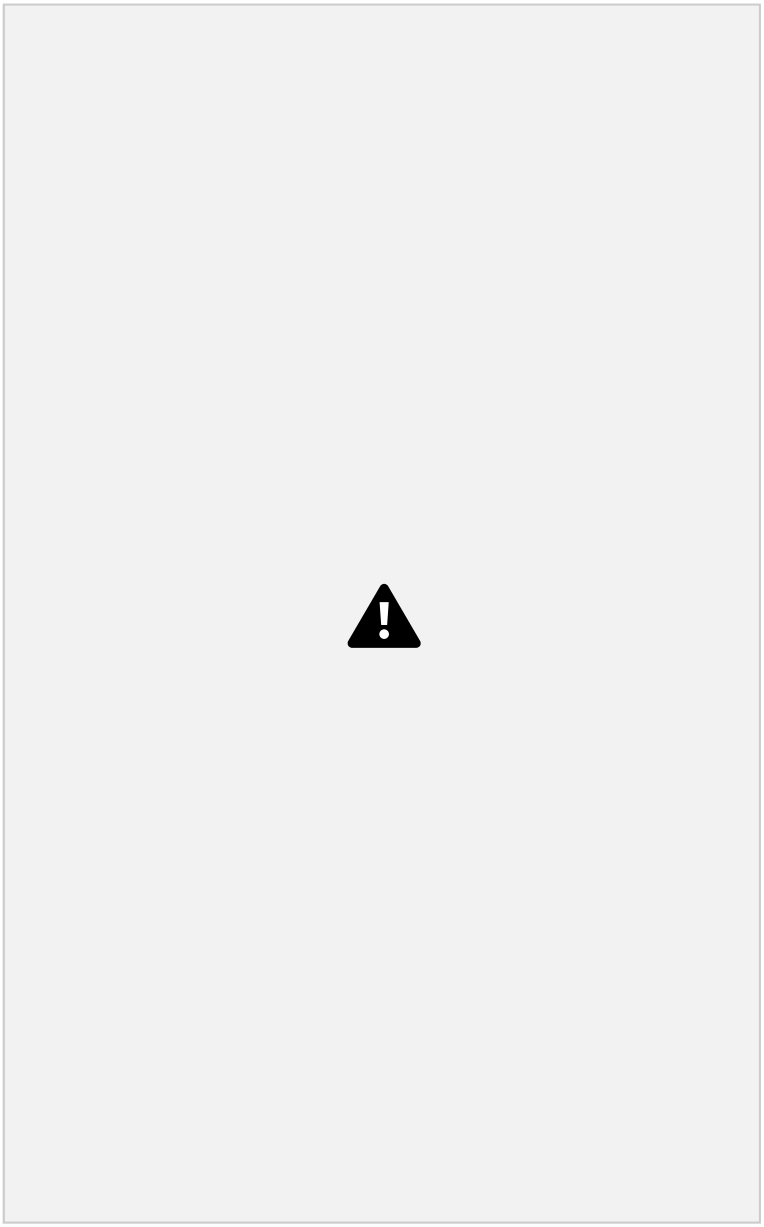
"The target sirolimus area under the concentration curve (AUC) of 3,810 ng-h/mL"

3,810 ng-h/mL was the target for this existing patients CANCER study.
Since I only had 0, 1, 2, 3 and trough levels, I needed to somehow approximate a complete 1 week rapamycin concentration vs time graph vs a similar dose of oral rapamycin to generate AUC comparison of IM+IN vs oral.

For oral rapamycin, I took the 15mg oral dose shown in image below.

The Effect of a High-Fat Meal on the Oral Bioavailability of the Immunosuppressant Sirolimus (Rapamycin)

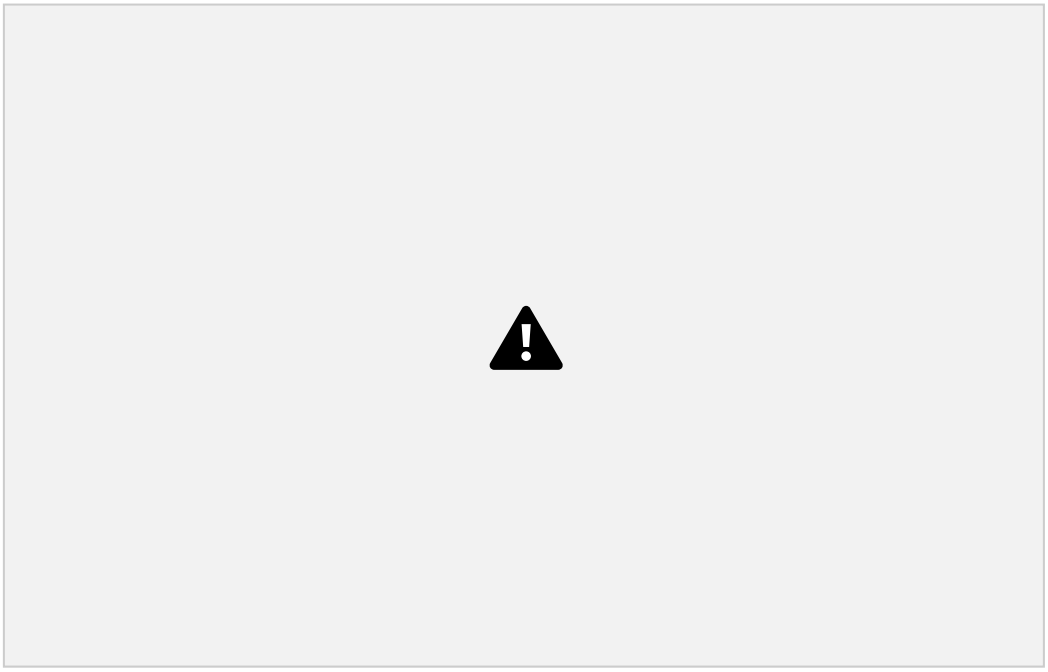
https://sci-hub.se/https://doi.org/10.1177/009127009903901107



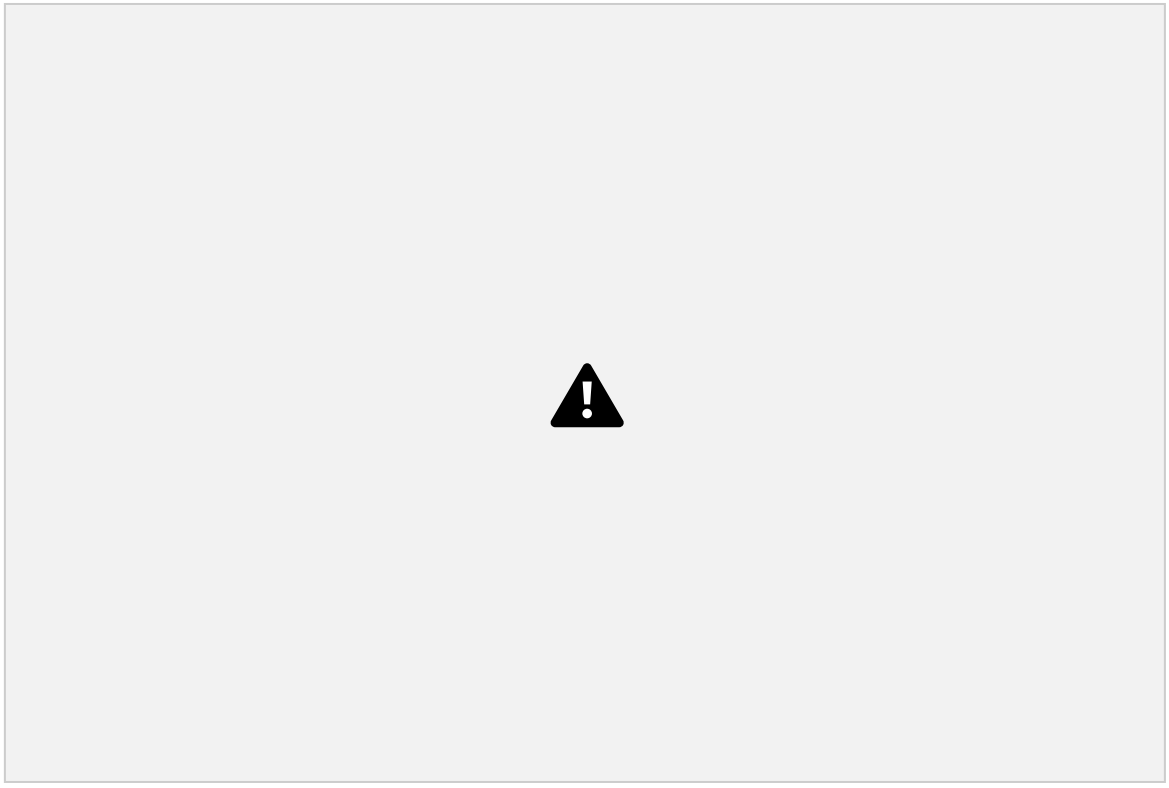
Since it is well known that the IM route results in a shift in Cmax as well as a slower decay curve vs oral, assumed similar approximate half life, I drew some reference from the IM rapamycin paper on dogs to help fill in the data points up to 168 hrs (1 week)

Rapamycin Pharmacokinetic and Pharmacodynamic Relationships in Osteosarcoma: A Comparative Oncology Study in Dogs (2010)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2882366/pdf/pone.0011013.pdf



The end result is the AUC graph summary shown below. To generate AUC, I used some simple high school physics, and cut out and accurately weighed the paper for the entire graphical max ranges rectangle to calculate a reference AUC. For each curve AUC, I then simply cut out carefully with scissor each curve to capture it's AUC graphically. I then weighed each area, and prorated to the reference area to calculate respective AUC.



As you can see, IM+IN was able to generate MASSIVE trough and AUC* compared to similar dose oral. The AUC of IM+IN of 6600 ng*h/mL is far higher than the rapamycin/cancer/GFJ trial AUC target (3810) so clearly FULL ON therapeutic+. In fact, comparing the AUC of IM+IN to the rapamycin/cancer/GFJ trial, no dosing protocol produced as high an AUC as this study (see below)

I am of course implicitly discounting contribution from IN, assuming 100% IM rapamycin systemic only. A future washout with IN only dosing protocol will have to be done to evaluate IN contribution to systemic rapamycin, without any way of knowing brain rapamycin level, unfortunately.

Remarkably, and purely coincidentally, the AUC of IM+IN vs ORAL calculated as approximately 10:1 factor, consistent with the scaling of trough levels, 11.1 ng/mL IM+IN and 1.1 ng/mL ORAL, respectively.

And this scaling is further and equally supported by with the fundamental relative bioavailability of IM+IN (near 100% for IM) vs approx. 10% for oral, so once again, a 10:1 factor.

*As already described above, with few rapamycin vs time concentration points, a best estimate of the complete concentration/curve was generated.

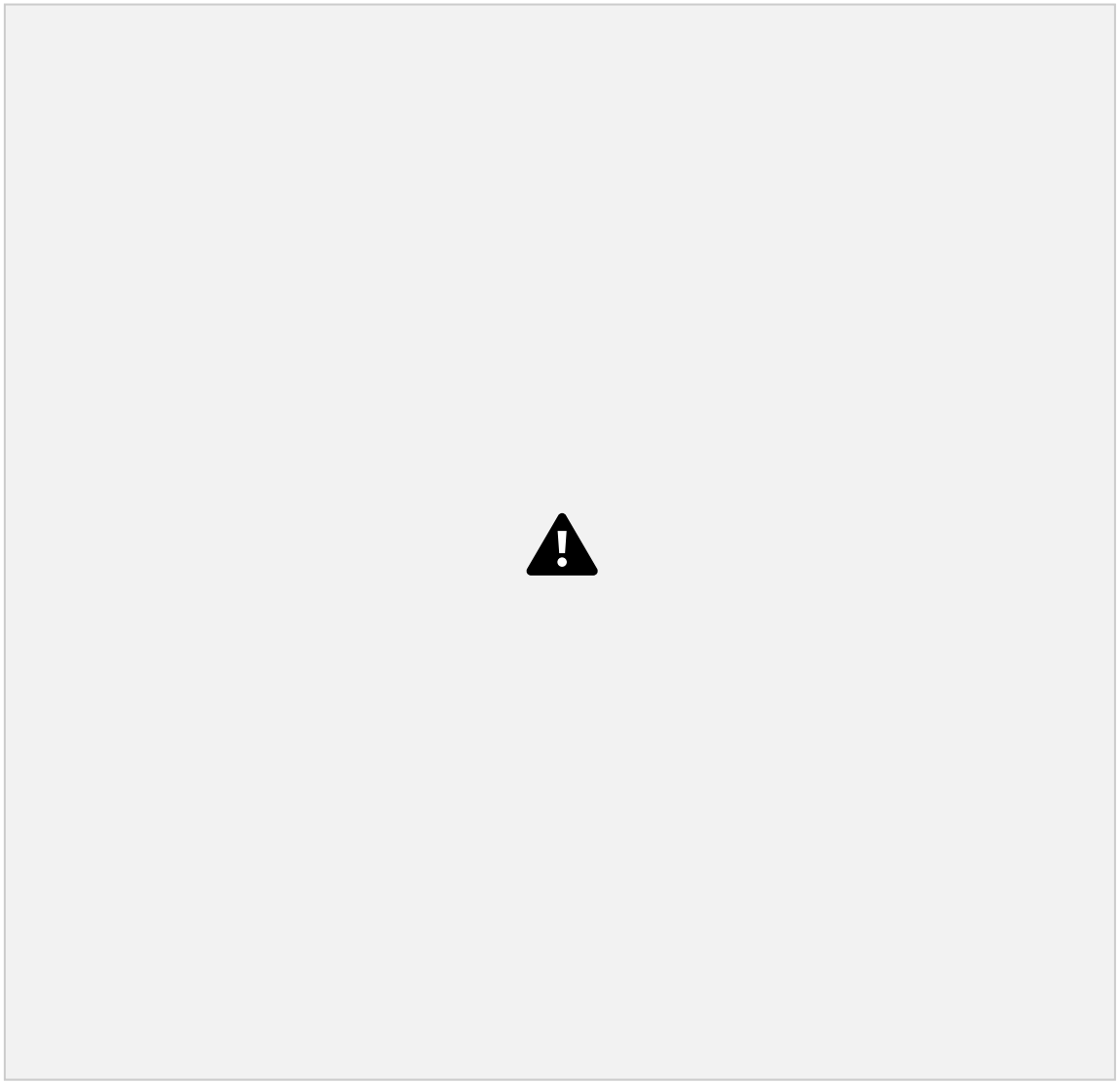
Let's compare current study to a clinical oral rapamycin study.

The table below is reprinted from:

Phase I studies of sirolimus alone or in combination with pharmacokinetic modulators in advanced cancer patients

https://pubmed.ncbi.nlm.nih.gov/22872575/

As you can see, no sirolimus dose, with or without ketoconazole or GFJ, produced as high an AUC as current study 15mg IM + 5.8mg IN.



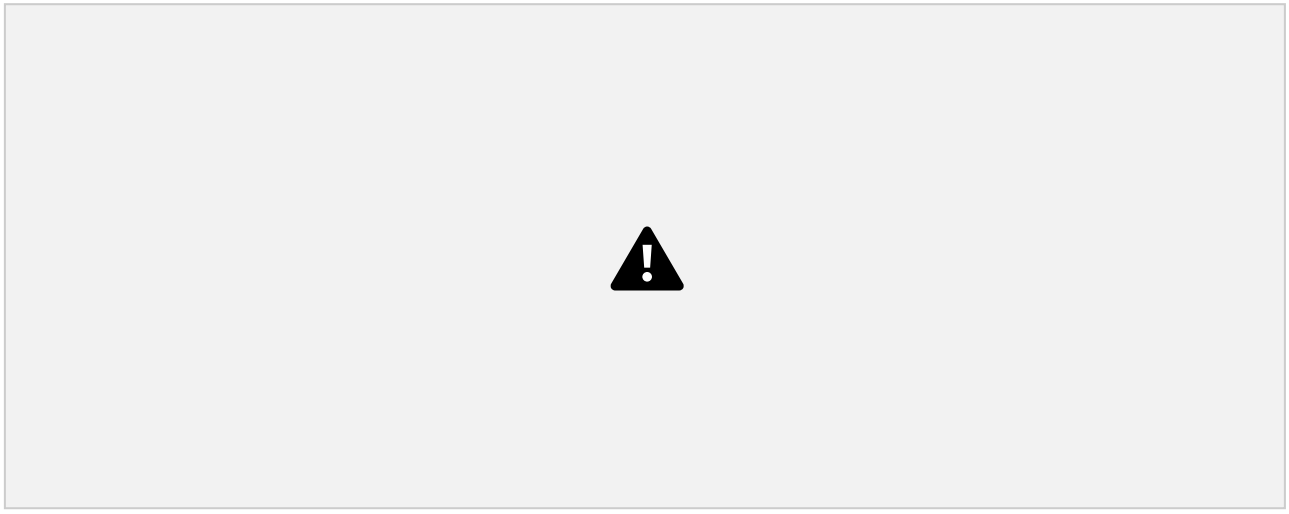
As far as comparing current study to the above clinical re timing of blood draws.

"***Sirolimus was administered once weekly*** as a 1 mg/ml oral solution (sirolimus alone and sirolimus plus grapefruit juice studies) or as a 1 mg tablet (sirolimus plus ketoconazole study). In the sirolimus alone study, blood was collected on day 1 (baseline, then 2 and 4 hours post dose), day 2 (prior to dosing then 0.4 and 3 hours post dose), day 4 (48 hours post second dose), day 8 (prior to dosing), and day 29 (prior to dosing). In the sirolimus plus ketoconazole and grapefruit juice studies, blood was collected in week 1 prior to administration of sirolimus alone and then at 0.25, 0.5, 1, 2, 4, 8, 24, and 48 hours; in week 2 prior to administration of sirolimus and either ketoconazole or grapefruit juice and then at 0.25, 0.5, 1, 2, 4, 8, 24, and 48 hours; and in week 3 prior to sirolimus administration."

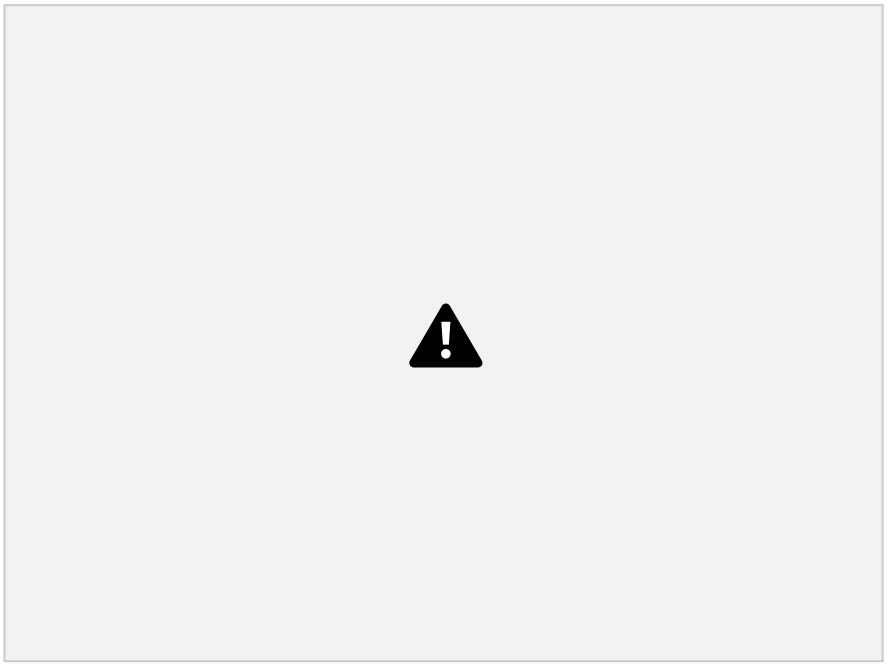
Let's put current study short term results in the context of the mainstream rapamycin biohacking community.

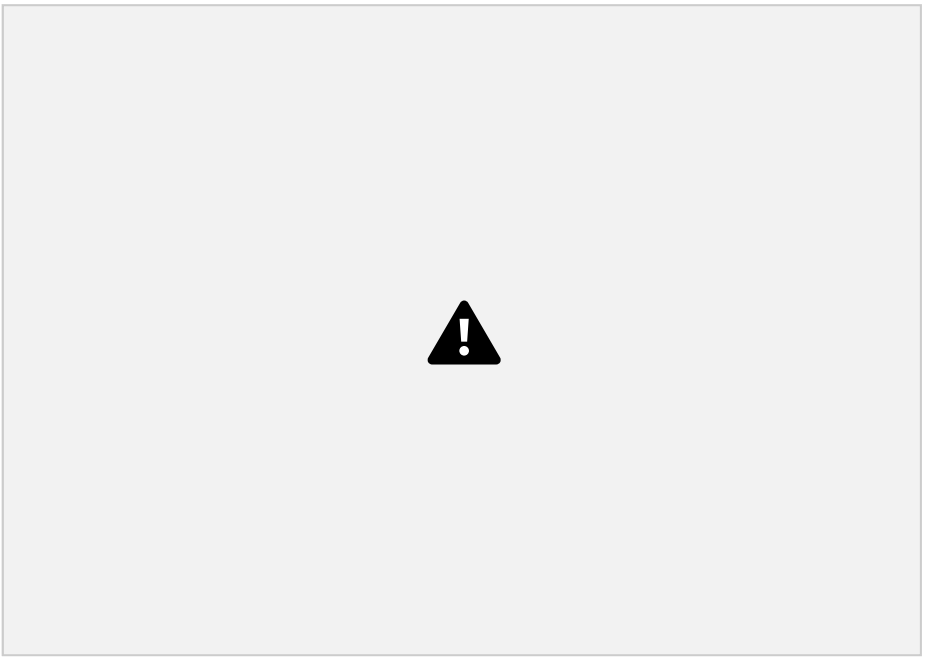
A large percentage of users are taking 6 mg/week (Dr Alan Green reference perhaps). One member of a forum posted that his 1 week trough level after taking 6mg with GFJ was 2.2 ng/L. Considering that GFJ typically raises AUC by 350%, and trough is linear with AUC for oral, then someone taking 6mg/week WITHOUT any CYP3A4 enzyme blockage, would have a trough level of 2.2/3.5, or approx. 0.62 ng/L, VERY LOW. ***Thus, 15mg IM+IN with a trough level of 11.1 is effectively 17.6X higher than 6mg/week oral!!*** Many people taking 6mg/week (without GFJ), depending on their gastro/enzyme genetics, probably have 1 week trough levels <1 . ***And anyone taking say < 5 mg/week, most certainly has 1 week trough levels at zero. There are insufficient mTOR inhibition levels. Most any "cancer suppression" study targets a min trough level of 5 ng/mL.***

BIOMARKERS COMPARISON POST IM+IN DOSING

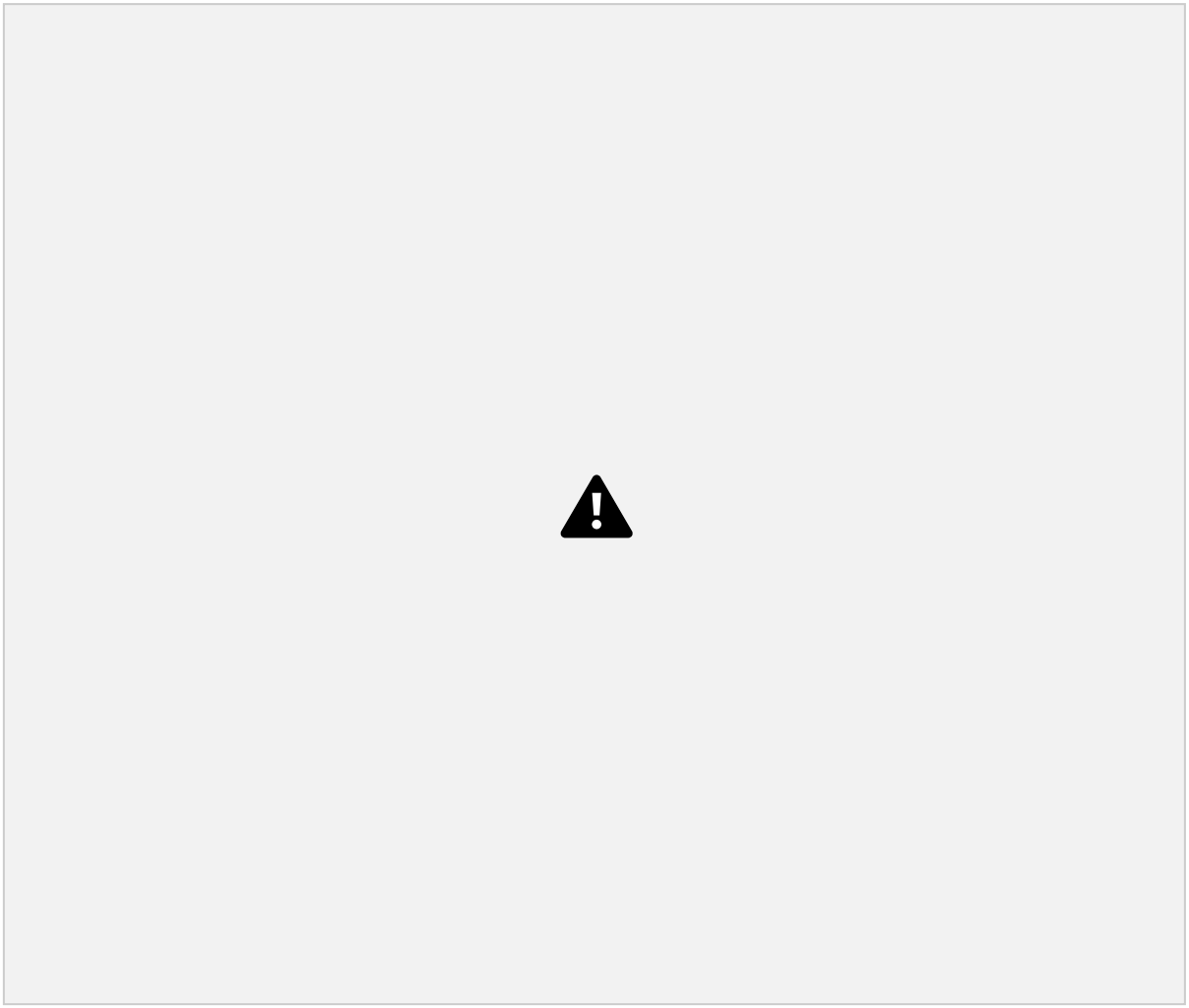


Not unexpected, hemoglobin and hematocrit tipping below lab range. A combination of very low iron stores due to regular 8 week phlebotomy to start with (I had donated on July 15, 22, during this intervention) and likely tipped over by rapamycin. The rest of the CBC is within lab range. MCH and WBC lymphocytes, based on trending, only two markers that "appear" to be significantly different, although it is still early into this experimentation.





Lipids/glucose and other metabolic markers



Above lipids/glucose/iron biomarker summary is consistent with high dose rapamycin: elevated TG, elevated fasting glucose/hbA1c, anemia.

The lipids and glucose rise are QUITE MUTED considering the massive AUC dose. They are no too concerning to me at time of writing, given my superb physical health, ketogenic diet, very high VO2max and low CAC score (my CAC is from chronic endurance running, quite normal)

The full blown clinical iron deficient anemia, completely unsurprising. I was borderline after years of 8 week regular phlebotomy (iron dumping a major biohack).

Iron Deficiency: ferritin< 12, transferrin saturation < 20%
Iron deficiency anemia: ferritin < 12, transferrin saturation < 15, Hb < 14

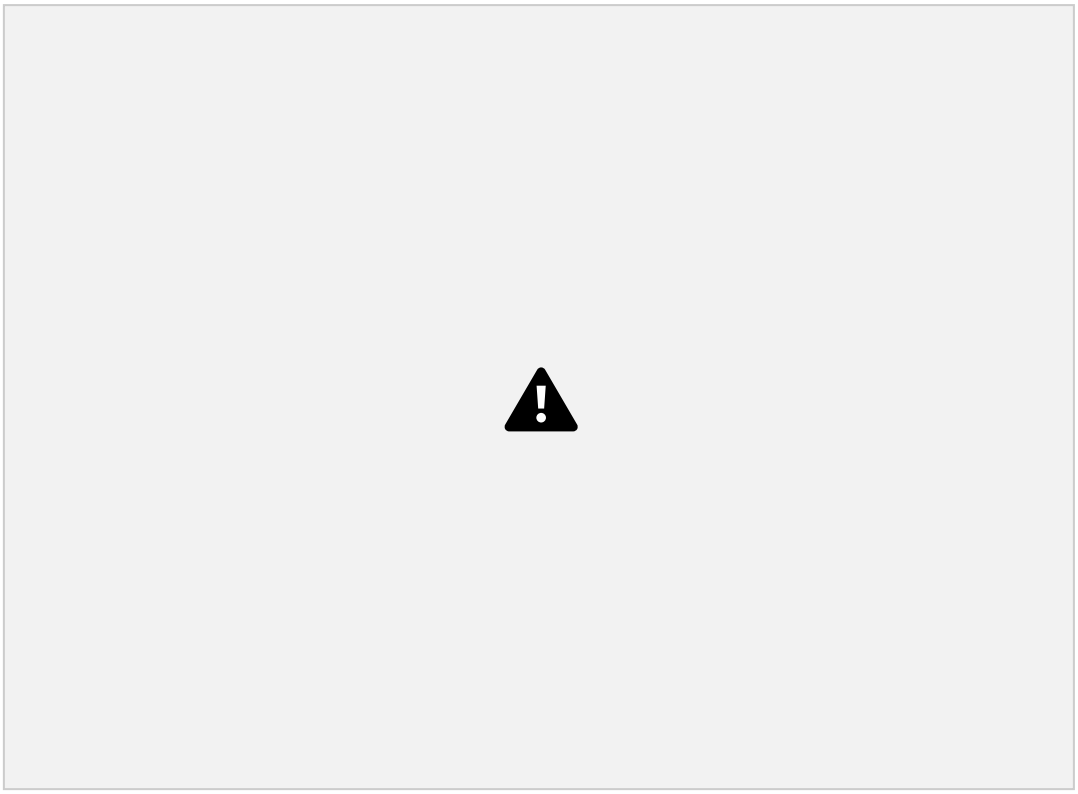
The rise in hsCRP and PSA (might explain the flare of dysuria post Week 4 dose) was surprising. IL-6, TNF-alpha, considered significant inflammatory biomarkers (no prior tests) were both very low, even though IL-6 is typically correlated with hsCRP?

At time of writing, I am feeling COMPLETELY NORMAL/AMAZING…as if I'm not taking a massive dose of rapamycin! As for weight loss, there appears to be perhaps 1-1.5kg weight loss, but cannot confirm this signal yet. No fatigue, continuing intense daily exercise same as pre dosing intervention. My weight lifting reps and recovery, zero impact. As for endurance, I have been working on increasing my already very high VO2max by doing 10 minute treadmill ramping speed/incline sortees to reach and hold a very high heart rate (same max heart rate as my recent V02max test) for the last 2 minutes. All the while I am full on clinically iron deficient anemic?? NO EFFECTS.

Might some of these markers be transient and/or still ramping? We have evidence from long term sirolimus trials that many of the lipid markers RETURN TO NORMAL over several months.

Tolerability and steady-state pharmacokinetics of everolimus in maintenance renal transplant patients

https://sci-hub.se/10.1093/ndt/gfh322

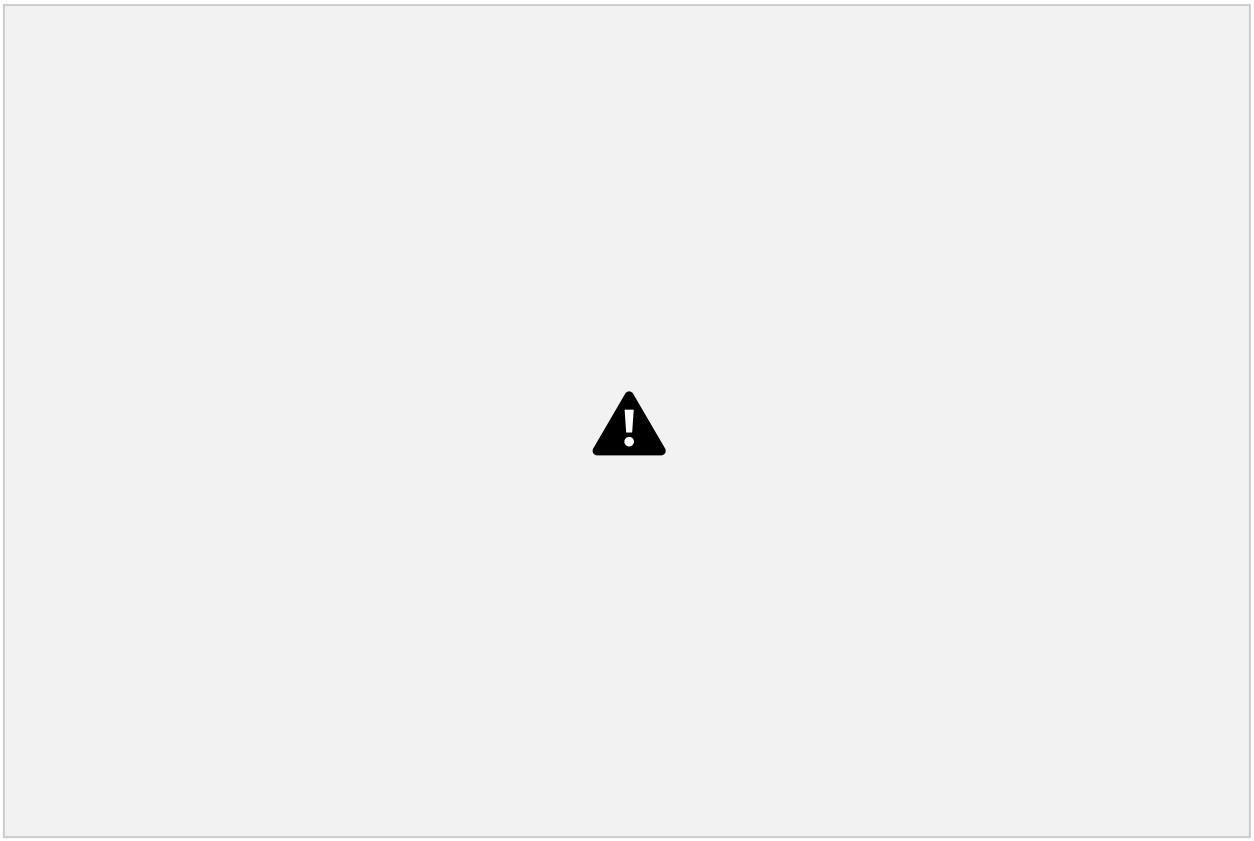


The above figure, albeit with everolimus, shows an immediate short term drop in platelets and WBC, but slowly recover with time (42 days capture). It also shows TC rising and then dipping, and same for TG.

Although my n=1 data on current IM+IN 6 week dosing protocol is quite a different dynamic. My TC stayed constant, TG much less of a rise, and NO massive short term drop in platelets and

WBC?? ***Are we looking at a fundamentally different pharmacodynamic paradigm??***

Let's put my short term n=1 IM+IN experimentation and biomarkers and side effects in context. I have reformatted the cancer/rapamycin/GFJ study of side effects across the various dosing protocols, and overlayed my 6 week IM+IN intervention (see chart below). Other than anemia, which is very much explained by my chronic phlebotomy regiment, I have NONE of the side effects of the cohort (shaded yellow to capture highest DLT ranges), at arguably FAR HIGHER AUC, although, I am in superb physical health vs likely ill health cancer patients.



QUESTIONS/FUTURE THOUGHTS

- How is this massive rapamycin AUC dose not resulting in massive side effects? Is this all due to fundamentally bypassing 1st pass metabolism?

- Is my superb health, low glucose, high insulin sensitivity, TRT phenotype resistant to greater dysregulation by rapamycin vs typical clinical trial patient who is metabolically in ill health?

- Are we just looking at how a superior health phenotype can withstand higher rapamycin AUC? But many healthy biohackers taking FAR lower oral doses are reporting side effects?

• How will these biomarkers and side effects manifest with continued longer-term dosing? •

The bump in fasting glucose and hbA1c, indicative of some level of mTOR2 inhibition?

• Is my CVD risk elevated with elevated TG, although the level of lipid dysregulation vs dose is quite muted vs clinical trial data, especially lipids dysregulation.

• Is IN delivering rapamycin to brain and/or contributing to systemic levels measured? A washout with just IN dosing should be done to elucidate if any systemic contribution.

CONCLUSION

Quite conclusively, it has been shown in an n=1 short term study that the use of rather straightforward and common parenteral routes of drug delivery, a low cost long term safe for human use excipient low DSMO intake, and rapamycin powder can be leveraged to produce an order of magnitude therapeutic bioavailability vs similar dose oral with apparent few side effects.

I hope this short term study sparks greater experimentation beyond out dated oral rapamycin delivery to truly deliver a quantum leap in mTOR inhibition translation for human lifespan extension.

MAC
Rapamycin.news