Novochizol™- botulinum toxin formulations: fundamental characteristics and therapeutic benefits in selected medical conditions

What is Novochizol™?

Novochizol™ technology transforms single chitosan polymer molecules into biocompatible nanoparticles, using a patented intramolecular cross-linking reaction. Novochizol™ nanoparticles overcome three fundamental obstacles that limit large scale chitosan use in pharma: lack of solubility at physiological pH, biological and physicochemical instability and heterogeneity inherent to natural polymers.

Principal advantages of Novochizol™-based drug formulations

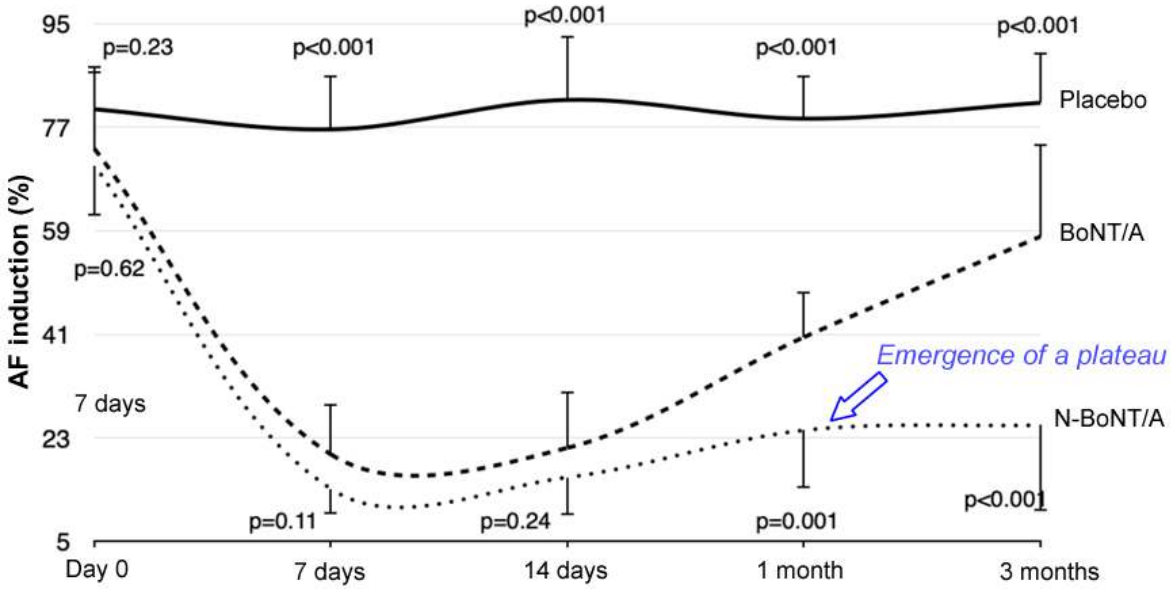
As carrier of active pharmaceutical ingredients (APIs) Novochizol™ presents the following advantages:

1. Formulation of all classes of APIs: small molecules (including recalcitrant, highly hydrophobic compounds), peptides, nucleic acids and large molecules, including proteins, such as botulinum toxin.
2. The Novochizol™ drug formulation process yields low viscosity suspensions, allowing subsequent creation of all galenic forms: tablets or capsules, liquid formulations (injections, drops), ointments, plasters, suppositories, and aerosols (sprays).
3. Novochizol™-formulated drugs strongly adhere to tissues as the site of their administration. This allows physical targeting of the API, avoiding systemic distribution.
4. Sustained, slow release of the API over extended periods of time (hours to months).
5. Intracellular delivery of all classes of APIs (including large proteins) to different cell types. As a result, new biological targets become accessible to the chosen API, enabling entirely new mechanisms of action (as demonstrated for botulinum toxin) and new medical uses for old drugs (repurposing)
6. In addition to full biocompatibility, Novochizol™ has anti-inflammatory properties, a favorable safety profile and other beneficial characteristics inherited from conventional chitosan.

Table 1. Comparative analysis of commercial botulinum toxin preparations (BoNT/A: Relatox ®, Xeomin ®) and equivalent Novochizol™-formulations (N-BoNT/A) in preclinical investigations of botulinum toxin effects on atrial fibrillation (AF).

	Indication or parameter	Descripton	BoNT/A	N-BoNT/A	Comments/conclusions
1	Proportion of induced AF episodes = number of observed AF episodes/number of AF attempts through electrostimulation (in percent)	AF induction (%) measured in dogs in response to high frequency electrostimulation at three months post-injection of the toxin preparations (critical timepoint for the onset of AF) Unpublished data (Fig. 1)	59%	23%	The Novochizol™- botulinum toxin formulation is 2.5 times more effective in inhibiting induced FP. The appearance of a plateau in the inhibition with Novochizol™- (Fig. 1. month 1,2,3) is strongly suggestive of an even longer-lasting effect. References: Guidelines for conducting preclinical studies of drugs. Part I / Edited by A.N. Mironov. Finet et al.. Information learned from animal models of atrial fibrillation. Cardiol Clin. 2009 Feb;27(1):45-54, viii. https://doi.org/10.1016/j.ccl.2008.09.005

Figure 1: Atrial fibrillation induction (%) in response to high-frequency electrical stimulation after injection of BoNT/A and N BoNT/A formulations.



	Indication or parameter	Descripton	BoNT/A	N-BoNT/A	Comments/conclusions
2	Effective refractory period after atrial stimulation	Difference in average effective atrial refractory period in dogs before and after high-frequency electotrostimulation at three months post-injection of the toxin preparations (critical timepoint for the onset of AF)	21.4±7.3 ms	12.29±3.7 ms	The Novochizol™- botulinum toxin formulation displays a stronger stabilizing effect on the effective atrial refractory periods, indicative of higher resistance towards AF. References: S.Naimi et al. Dispersion of effective refractory period during abrupt reperfusion of ischemic myocardium in dogs. The American Journal of Cardiology, Volume 39, Issue 3, 1977, Pages 407-412, ISSN 0002-9149, https://doi.org/10.1016/S0002-9149(77)80097-0. Guidelines for conducting preclinical studies of drugs. Part I / Edited by A.N. Mironov. Finet et al.. Information learned from animal models of atrial fibrillation. Cardiol Clin. 2009 Feb;27(1):45-54, viii. https://doi.org/10.1016/j.ccl.2008.09.005
3	Proportion of induced AF episodes = number of observed AF episodes/number of AF attempts through adrenaline administration (in percent)	Frequency of arrhythmia in the adrenaline test. Novochizol™-Relatox ® was 20% more protective than Relatox ® alone (40% vs 50% of animals presented arrhythmia). Highest protection was observed with Novochizol™-Xeomin® (30% of animals presented arrhythmia).	50%	30% (Xeomin®) 40% (Relatox ®)	Hihger efficacy obtained with Novochizol™ formulations is significant, taking into account the small number of experimental animals (10 rats per group). The relative advantage of Xeomin® may be explained by the absence of hemagglutinins in the preparation, which probably interfere with the effectiveness of the toxin in combination with Novochizol™. It is important to emphasize that in the manufacturing process, Relatox ® can be purified from hemagglutinins by chromatography before forming a complex with Novochizol™. Novochizol™ not only improves the antiarrhythmic efficacy of botulinum toxin, it also helps to stabilize the purified protein immediately after production.
4	Ventricular fibrillation (VF)	The antiarrhythmic effect (measured as the delay before the onset of ventricular fibrillation after intravenous injection of calcium chloride) is apparent in rats as early as 15 minutes after the injection of Novochizol™-Relatox ® (similar findings for the other tested drugs) The clinical benefit was quasi-immediate, as opposed to developing			Novochizol™ formulations led to a much more rapid protective antiarrythmic effect (within 24 h) in comparison with radiofrequency ablation (after 14 days, with confirmation of benefit only after 6 months).

		the next day (pertaining to muscle denervation in rats, not antiarrhythmic effects)			
5	Ventricular fibrillation (VF)	In ventricular electrostimulation tests, Novochizol™ formulations (10 units/kg) increased the FV threshold by 25%. Novochizol™-Relatox ® (5 U/kg) increased the threshold by about 13%. Relatox ® on its own (10 U/kg) increased the threshold by about 5%, as did a lower dose of Novochizol™-Relatox ® (U/kg).	+25% (Relatox ®)	+5% (Relatox ®)	There is a significant Novochizol™-specific increase in the antiarrhythmic effect of Relatox ®, countering ventricular fibrillation. **This finding is strongly predictive of a reduced VF-related mortality in in clinics**.
6	Median lethal dose	LD_{50} (intramuscular Xeomin® inlections)	46.27 ± 5.68 U/kg	64.23 ± 7.55 U/kg	The 40% increase in botulinum toxin LD_{50} conferred by Novochizol™ significantly extends the therapeutic range of the drug in the clinical setting, offering a safe higher dose to patients that do not respond to lower doses. This advantage of the Novochizol™-formulation may be explained by the absence of any systemic distribution. Reference: Sergeevichev DS et al. . Globular chitosan prolongs the effective duration time and decreases the acute toxicity of botulinum neurotoxin after intramuscular injection in rats. Toxicon. 2018 Mar 1;143:90-95. doi: https://doi.org/10.1016/j.toxicon.2018.01.013

Table 2. New medical indications and modes of action: comparative analysis of commercial botulinum toxin preparations (BoNT/A: Relatox ®, Xeomin®) and equivalent Novochizol™-formulations (N-BoNT/A)

	Medical indication(s) or parameter	Descripton	BoNT/A	N-BoNT/A	Comments/conclusions
7	Hypertension	In *in vivo* swine experiments, Novochizol™- botulinum toxin injections into renal arteries led to long term inhibition of hypertension caused by electrical stimulation of the ganglionic plexuses located at the mouths of the renal arteries. In *in vitro* experiments with juxtaglomerular cells, Novochizol™-botulinum toxin treatment (but not botulinum toxin alone) inhibited renin release into the culture medium and increased intracellular renin accumulation.			Novochizol™ enables a prolonged antihypertensive effects of botulinum toxin thanks to at least two distinct mechanisms of action: 1. inhibition of renin release through inhibition of acetylcholine release via SNAP25 in renal neurons 2. direct inhibition of renin release through SNAP23 in renal juxtaglomerular cells **A radically new mechanism of action of botulinum toxin has been demonstrated: inhibition of vesicular renin transport. This new mechanism is only possible because of Novochizol™-enabled intracellular transport of botulinum toxin into non-neuronal cells.** **Similar inhibition of vesicular transport is expected in other cases (such as the release of immune-inhibitory exosomes by cancer cells), opening up broad applications of botulinum toxin for unexpected medical uses.** Reference : Role of the SNARE protein SNAP23 on cAMP-stimulated renin release in mouse juxtaglomerular cells. Mendez M and Gaisano H Y. 2013. Am J Physiol Renal Physiol. 304(5): F498–F504. https://dx.doi.org/10.1152%2Fajprenal.00556.2012
8	All medical indications in which botulinum toxin may have therapeutic potential	Dose-dependence of the increase in the electrostimulation threshold in rat muscles. Data not published (internal report, Integration project of the Russian Academy of Sciences)	Emergence of a plateau (loss of dose-dependence at higher concentrations of botulinum toxin and manifestations of toxic effects)	Maintenance of dose-dependence, without a plateau, and absence of toxicity	Novochizol™ delivers botulinum toxin inside cells, to reach intracellular targets, and it simultaneously prevents systemic toxic effects. **Experimental results confirm the extended therapeutic range of the drug.**

9	Target cells (all medical indications)	Intracellular accumulation of botulinum toxin or fluorescein after treatment with Novochizol™ formulation in a variety of cell types. Data not published (included in the Patent to be published in August 2021)	Motor neurons	Corneal cells, Myocytes, fibroblasts, leucocytes.	**Novochizol™ is expected to promote intracellular delivery of almost any API to different cell types, enabling new mechanisms of action and opening up new possibilities for existing drugs (repurposing).**
10	All medical indications in which inhibition of vesicular transport may offer therapeutic potential.	Receptors mediating intracellular uptake of botulinum toxin (receptor-mediated endocytosis)	synaptic vesicular protein SV2, specifically expressed in presynaptic membranes of activated neurons in neuromuscular nodes	clathrin and caveolin-dependent endocytosis (CLDE and CADE), macropinocytosis, phagocytosis (similar to chitosan)	**All data support the hypothesis of a universal uptake mechanism for all APIs formulated with Novochizol™, mediated by receptors that bind chitosan**. The presence of such receptors on a variety of cells could allow the use of botulinum toxin and other APIs for a wide variety of medical applications. References: Jahn R. Neuroscience. A neuronal receptor for botulinum toxin. Science. 2006 Apr 28;312(5773):540-1. https://doi.org/10.1126/science.1127236 Zubareva, A.A., Svirshchevskaya, E.V. Interactions of chitosan and its derivatives with cells (review). *Appl Biochem Microbiol* **52,** 465–470 (2016). https://doi.org/10.1134/S0003683816050185
11	Hyperhidrosis, blepharospasm, migraine, movement disorders and other conditions in which botulinum toxin may have therapeutic potential	In all cases, Novochizol™-botulinum formulations are expected to provide a faster onset and prolong the duration of the biological activity of botulinum toxin with an extended therapeutic range. **It is possible that these characteristics will eventually provide curative treatments for pathologies that are currently incurable.**			