C3 transferase

C3 Transferase is a bacterial enzyme that plays a role in modifying Rho GTPases, which are critical regulators of the cytoskeleton and various cellular functions. Specifically, **C3 exoenzyme** is produced by *Clostridium botulinum* and *Clostridium limosum*, and it ADP-ribosylates RhoA, RhoB, and RhoC at a conserved asparagine residue.

Key Features and Functions of C3 Transferase: - **Inactivation of Rho GTPases**: By ADP-ribosylating RhoA, RhoB, and RhoC, C3 Transferase prevents their normal activation, leading to **disruption of the actin cytoskeleton**. - **Cellular Effects**:

1. Loss of **stress fibers** and **focal adhesions**.
2. Decreased cell adhesion and increased motility.
3. Changes in **cell morphology**, often leading to rounded or elongated cells.

- **Research Applications**:

1. Used as a **biochemical tool** to study Rho-mediated signaling pathways.
2. Investigated for its potential in **cancer therapy** due to its effects on cell adhesion and migration.
3. Used in **neuroscience research** for its ability to promote axonal regeneration by inhibiting RhoA signaling.

Potential Therapeutic Applications: Given that RhoA inhibition can promote **neurite outgrowth and regeneration**, C3 Transferase has been explored in **spinal cord injury** and **neurodegenerative disease** models.

Lasko et al., describe a simple and fast fluorescence-based assay to evaluate the enzymatic activity of cell-permeable C3 proteins purified from Escherichia coli. The assay measures glycohydrolase (GH) activity of C3 that cleaves NAD+ into ADP-ribose and nicotinamide. Results from the GH activity correlate with other tests carried out in tissue culture cells such as neurite outgrowth or ADP ribosylation of RhoA. This method provides reliable measurements of the activity of permeable C3 proteins or other C3-related proteins [1)].

The bacterial enzyme C3-ADP ribosyltransferase (C3) selectively and irreversibly inhibits the activation of RhoA and stimulates axon outgrowth and regeneration. However, effective intracellular delivery of the C3 protein in vivo is limited by poor cell permeability and a short duration of action.

To address this, Gutekunst et al., have developed a gene therapy approach using viral vectors to introduce the C3 gene into neurons or neuronal progenitors. Our vectors deliver C3 in a cell-autonomous (endogenous) or a cell-nonautonomous (secretable/permeable) fashion and promote in vitro process outgrowth on inhibitory chondroitin sulfate proteoglycan substrate. Further conditional control of our vectors was achieved via the addition of a Tet-On system, which allows for transcriptional control with doxycycline administration. These vectors will be crucial tools for promoting continued Axon regeneration after CNS injuries or neurodegenerative diseases [2)].

Systematic reviews

A comprehensive search was conducted in Ovid MEDLINE, Embase, Scopus, and Web of Science Core Collection utilizing a combination of keywords. All in-vivo animal studies of acute or chronic spinal cord injury that evaluated the pharmacological effects of Rho/ROCK inhibitors in English literature were included in this study.

A total, of 2320 articles were identified, of which, 60 papers were included for further analysis. A total of 47 (78%) studies were conducted merely on rats, 9 (15%) on mice, 3 (5%) used both, and the remaining used other animals. Y-27632, Fasudil, C3 Transferase and its derivatives (C3-05/PEP-C3/CT04/C3bot154-182/C3bot26mer(156-181)), Ibuprofen, Electroacupuncture (EA), SiRhoA, miR-133b, miR-135-5p, miR-381, miR-30b, Statins, 17β-estradiol, β-elemene, Lentivirus-mediated PGC-1a, Repulsive guidance molecule (RGMa), Local profound hypothermia, Jisuikang (JSK), Hyperbaric oxygen (HBO), Lv-shRhoA (Notch-1 inhibitor), Anti-Ryk antibody, LINGO-antagonist, BA-210, p21Cip1/WAF1, ORL-1 antagonist, Epigallocatechin-3-gallate (EGCG), Tamsulosin, AAV.ULK1.DN, and Indomethacin were the 28 reported agents/procedures with anti-RhoA/ROCK effects. The pooled SMD for BBB scores was 0.41 ($p = 0.048$) in the first week, 0.85 ($p < 0.001$) in the second week, 1.22 ($p = 0.010$) in the third week, and 1.53 ($p = 0.001$) in the fourth week.

Of the 28 identified anti-RhoA/ROCK agents, all but two (C3bot and its derivatives and EGCG) demonstrated promising results. The results of the meta-analysis cautiously indicate a significant increase in BBB scores over time after SCI [3].

Khavandegar et al. (2025) provide a valuable systematic review of RhoA/ROCK inhibitors in Spinal cord injury outcomes, highlighting the potential of various agents in enhancing locomotor function. The study benefits from a comprehensive search strategy and a meta-analysis of functional outcomes. However, limitations such as heterogeneity in included studies, absence of quality assessment, and potential publication bias should be addressed in future research. Moving forward, standardized methodologies and rigorous risk-of-bias evaluations will be essential to strengthen the clinical relevance of these findings.

1)
Lasko D, McKerracher L. Fluorescent assay of cell-permeable C3 transferase activity. Methods Enzymol. 2006;406:512-20. PubMed PMID: 16472683.
2)
Gutekunst CA, Tung JK, McDougal ME, Gross RE. C3 transferase gene therapy for continuous conditional RhoA inhibition. Neuroscience. 2016 Oct 13. pii: S0306-4522(16)30531-0. doi: 10.1016/j.neuroscience.2016.10.022. [Epub ahead of print] PubMed PMID: 27746349.
3)
Khavandegar A, Ahmadi NS, Mousavi MA, Ramezani Z, Khodadoust E, Hasan Zadeh Tabatabaei MS, Hasanpour Segherlou Z, Zeinaddini-Meymand A, Nasehi F, Moafi M, RayatSanati K, Masoomi R, Hamidi S, Pourkhodadad S, Rahimi-Movaghar V. The potential role of RhoA/ROCK-inhibition on locomotor recovery after spinal cord injury: a systematic review of in-vivo studies. Spinal Cord. 2025 Feb 16. doi: 10.1038/s41393-025-01064-2. Epub ahead of print. PMID: 39956860.