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Effect of Pooled Human Intravenous Globulin (IVIG) on the Reversal of Cholinergic Inhibition of Smooth Muscle by Immunoglobulins (IgGs) from Patients with Scleroderma (SSc)

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**Backgrounds & Aims**

The gastrointestinal (GI) tract is the most common internal organ system affected in SSc. We and others have shown before that the SSc immunoglobulins (IgGs) cause selective blockade of muscarinic type-3 cholinergic (M₃-R) in the GI tract. Presently, there is no effective treatment for SSc although numerous cytotoxic and immunomodulatory agents have been employed with limited success and are marred with serious side effects. Present studies investigated the reversibility of SScIgGs-caused M₃-R blockade by the pooled Intravenous immunoglobulins (IVIG).

**Methods**

Effects of SScIgGs and IgGs from normal individuals (NilgGs) on M₃-R activation by carbachol (BeCh) were determined in human internal anal sphincter (IAS) smooth muscle cells (SMCs), before and after IVIG. M₃-R occupancy and binding by the SScIgGs was determined via immunofluorescence (IF), Western blotting, and ELISA, respectively. Functional displacement of M₃-R occupancy by the SScIgGs was determined employing different concentrations of the IgGs during the sustained phase of the BeCh-induced contraction of rat IAS smooth muscle strips.

**Results**

SScIgGs and IgGs from normal individuals (NilgGs) on M₃-R activation by carbachol (BeCh) were determined in human internal anal sphincter (IAS) smooth muscle cells (SMCs), before and after IVIG. M₃-R occupancy and binding by the SScIgGs was determined via immunofluorescence (IF), Western blotting, and ELISA, respectively. Functional displacement of M₃-R occupancy by the SScIgGs was determined employing different concentrations of the IgGs during the sustained phase of the BeCh-induced contraction of rat IAS smooth muscle strips.

**Figures**

**Figure 1.** Co-localization of SScIgGs with M₃-R thus reducing M₃-R Immunofluorescence. Reversed by Pooled Human Immunoglobulin (IVIG).

**Figure 2.** Immunocytochemical co-localization of different IgGs (ab,cat,d) (FITC- conjugated) and M₃-R (TRITC-conjugated) on IAS muscle strip. (A) Schematic of immune reactivity calculation

**Figure 3.** SScIgG cause Functional Displacement of M₃-R: Reversed by IVIG

**Figure 4.** IVIG Reverses the binding of SScIgG with second Loop of M₃-R (M₃-R,L2)

**Figure 5.** SScIgG significantly bind with M₃-R (* p < 0.05). IVIG reverses the binding (** p < 0.05).

**Figure 6.** Active IVIG (not inactivated) (* p < 0.05) the binding of SScIgG with M₃-R and HSIIF.

**Summary**

1. IgG from scleroderma patients (SScIgGs) inhibits muscarinic type-3 cholinergic (M₃-R) activation, as shown by the data in human IAS smooth muscle cells and rat smooth muscle strips.
2. SScIgGs inhibit M₃-R occupation as shown by immunocytochemistry and ELISA-binding studies.
3. Pooled Intravenous globulin (IVIG) reverses the M₃-R occupancy and activation primarily by neutralizing circulating the SScIgGs.

**Conclusions**

- IVIG along SScIgGs-mediated block of M₃-R by blocking the circulating SScIgGs.

- This mechanism may be partly responsible for the restoration of M₃-R-mediated cholinergic dysfunction in SSc-related GI motility.