Procedure-Related Findings Associated with Subcutaneous Injection in Non-Human Primates Utilized in Preclinical Toxicity Studies

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Introduction

Recognition and differentiation of procedure-related findings versus test-article effects at the injection site is a critical component of determining potential toxicity in preclinical subcutaneous (SQ) injection studies. Description of procedure-related injection site findings in non-human primates (NHPs) is limited in recent literature, particularly with respect to the subcutaneous route. A retrospective review of historical control data was conducted to identify and describe procedural findings observed with subcutaneous injections in NHPs.

Methods

Microscopic data were compiled from 44 control Cynomolgus macaques (*Macaca fascicularis*) across six good laboratory practice (GLP) toxicology studies finalized over four years (2022-2025) including 106 SQ injection sites. Studies utilized 3-4 control animals/sex. Study main phase duration ranged from 28 to 275 days with repeat dosing ranging from weekly to monthly. Site of administration was the dorsum with either single or paired injections. Sites from studies in which administration rotated between two sites were documented as acute (1 day since final dose) or subacute/chronic (≥ 7 days since final dose). Time since final dose in subacute/chronic sites was 7-8 days in all but one study (36 days; 4 animals). Vehicle composition varied by study, including phosphate buffered saline, sterile water, L-histidine, propylene glycol, and/or normal saline. Microscopic terminology for the included studies was reviewed and aligned based on INHAND guidelines.

Results

Incidence and severity of injection site microscopic findings were generally comparable between males and females. Microscopic location was documented as subcutis/subcutaneous or was not otherwise specified. Figure 1 comprises representative photomicrographs of select findings, with frequency of these and remaining findings summarized in Table 1.

Acute

Common findings (≥ 20% incidence) in acute sites included fibroplasia (63.4%), macrophage infiltrate/inflammation (22.2%) and hemorrhage (20.4%). Infrequent findings (10-20%) comprised mixed cell infiltrate/inflammation (16.7%). Rare findings (< 10%) included eosinophilic extracellular material (9.3%), pseudocyst (7.4%), hair follicle atrophy (7.4%), muscle degeneration/necrosis (7.4%), mononuclear cell infiltrate (7.4%), macrophage pigment accumulation (3.7%), neutrophilic infiltrate (3.7%) and congestion (1.9%).

Subacute/Chronic

In subacute/chronic injection sites, fibroplasia (63.5%) was the only common finding. Infrequent findings comprised mixed cell infiltration/inflammation (11.5%) and hemorrhage (11.5%). Rare findings included macrophage infiltrate/inflammation (9.6%), mononuclear cell infiltrate (9.6%), muscle degeneration/necrosis (9.6%), crust/hyperkeratosis (3.8%), neutrophilic infiltrate (1.9%), and granuloma (1.9%).

Figure 1: SQ Injection Vehicle and Procedure-related Microscopic Findings

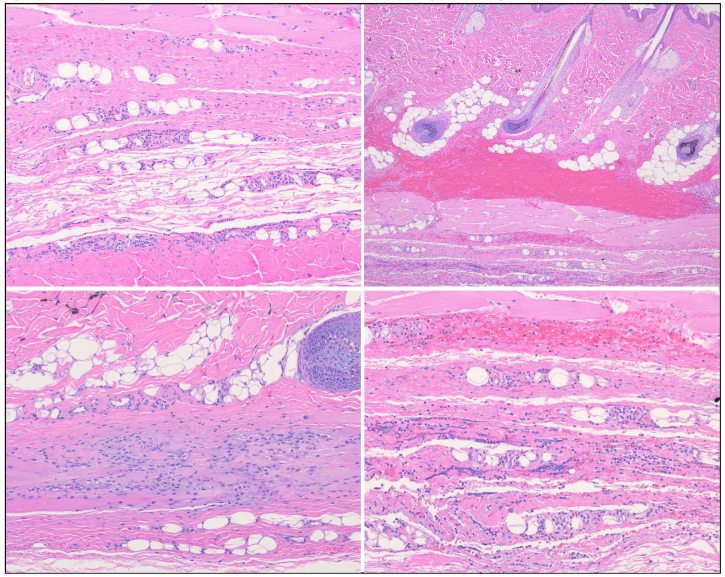

Figure 1: Vehicle and/or procedure-related findings at the subcutaneous injection site commonly included fibroplasia (A and C), hemorrhage (B and D); and mixed cell or macrophage infiltrate/inflammation (A, C, and D).

Discussion

Common acute findings comprised fibroplasia, hemorrhage, and inflammation/infiltration (macrophage or mixed cell). Fibroplasia, muscle degeneration/necrosis and/or mononuclear or neutrophilic infiltration occurred with similar incidence in the subacute/chronic sites while the incidences of other findings were generally reduced, reflecting inflammatory progression over time. Repeated dosing of injection sites (not controlled for in this review) may contribute to increased incidence of fibroplasia in acute sites or similar incidence of hemorrhage in acute and subacute/chronic sites.

Subcutaneous injection site pathology in control NHPs often reflected the initial inflammatory and tissue healing response following the localized mechanical trauma of the injection procedure (needle penetration, injection volume, etc), as seen in intramuscular or subcutaneous injections in other species. There is, however, significant overlap between these procedure-related microscopic findings and potential test article effects.

Familiarity with typical microscopic findings associated with the SQ dosing procedure is important in of potential differences between control/vehicle-dosed and test article-dosed animals and determination of toxicity and tolerability. Further data set expansion, characterization and comparison of SQ injection site findings in other species, and comparison with other routes of injectable administration will be valuable.

Table 1: SQ Injection Procedure-related Microscopic Findings

Chronicity	Acute		Subacute/Chronic	
Sex	Males	Females	Males	Females
Number of Sites	27	27	26	26
Fibroplasia[a]	**63%**		**64%**	
Minimal	10	14	12	16
Mild	6	1	3	2
Moderate	2	3	0	0
Infiltrate/Inflammation, Macrophage[a]	**22%**		**10%**	
Minimal	3	8	2	2
Mild	1	0	1	0
Hemorrhage[b]	**20%**		**12%**	
Minimal	1	2	2	1
Mild	5	3	0	1
Moderate	0	0	0	2
Infiltrate/Inflammation, Mixed Cell[c]	**17%**		**12%**	
Minimal	3	2	1	3
Mild	2	2	1	1
Infiltrate, Mononuclear Cell[a]	**7.4%**		**9.6%**	
Minimal	1	3	2	3
Degeneration/Necrosis[d]	**7.4%**		**9.6%**	
Minimal	0	3	3	1
Mild	0	1	0	1
Pseudocyst[c]	**7.4%**		-	
Present	3	1	0	0
Extracellular Material; Eosinophilic[c]	**9.3%**		-	
Present	3	2	0	0
Atrophy; Follicular	**7.4%**		-	
Minimal	1	2	0	0
Mild	1	0	0	0
Infiltrate, Neutrophil[f]	**3.7%**		**1.9%**	
Minimal	1	1	0	1
Pigment; Cytoplasm; Macrophage	**3.7%**		-	
Minimal	0	2	0	0
Crust/Hyperkeratosis[e]	-		**3.8%**	
Minimal	0	0	2	0
Granuloma[f]	-			
Minimal	0	0	0	1
Congestion[c]	**1.9%**		-	
Minimal	1	0	0	0

Findings ordered by incidence. Acute = 1 day since final dose; Subacute/Chronic ≥ 7 days
[a] Includes "Inflammation, Granulomatous"; subcutaneous, subcutis, or not otherwise specified
[b] Subcutaneous or focal
[c] Subcutaneous
[d] Includes "Degeneration/Regeneration; muscle or myofiber
[e] Focal or not otherwise specified
[f] Subcutis

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