

Evaluation of Repeat-Dose Toxicity with the Alginate Oligomer, OligoG

R. MYRVOLD¹, E. ONSØYEN¹, D. STEWART²

¹AlgiPharma AS, Industriveien 33, N-1337 Sandvika, Norway; ²Charles River Laboratories, Tranent, Edinburgh, EH33 2NE, United Kingdom

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Background. The alginate oligomer, OligoG disrupts bacterial biofilms and enhances the activity of antimicrobials and advocated for use in CF patients. This study was to investigate the toxicity of the OligoG in rats after once daily administration by the inhalation for 7-days, 14-days to 28-days.

Methods. Animals were dosed once daily using a snout only inhalation exposure technique using a modular (3 tier) stainless steel flow past systems. A total of 62 male and 62 female Sprague-Dawley rats were used for inhalation with 6% OligoG. Body weight, food consumption, mortality, detailed clinical observations, respiratory rate, tidal volume, respiratory minute volume were noted during the study. Blood and urine samples were obtained from 28-day dosing group for haematology and clinical chemistry screening.

Results. Organ weights did not show OligoG related changes apart from lung where there was a dose dependant increase in weight. The only OligoG related necropsy finding was bronchial lymph node enlargement in 8 animals treated with OligoG for 240 min per day, including 2/5 males and 2/5 females dosed for 14 days, 1/5 single dose males, 1/5 females dosed for 7 days, 1/5 males dosed for 28 days and 1/5 females dosed for 28 days followed by a 2-week off-test recovery period. There was alveolar foamy macrophage accumulation in the lung of all animals from the 28-day groups but these values ranged from minimal to mild and considered a normal clearance mechanism of the rat lung - this is not considered to be a toxicological effect of administration of 6% OligoG. All other histology was deemed to be normal. Blood and urine analysis displayed normal parameters.

Conclusion. Once daily administration of a 6% nebulised OligoG for up to 28 days produced no adverse clinical signs or test item-related changes in body weight, food consumption, respiratory measurement parameters, haematology, clinical chemistry or urinalysis.

INTRODUCTION

The alginate oligomer, OligoG disrupts bacterial biofilms and enhances the activity of antimicrobials and advocated for use in CF patients. This study was to investigate the toxicity of the OligoG in rats after once daily administration by inhalation for 7-days, 14-days to 28-days.

MATERIALS & METHODS

This study was performed in accordance with the OECD Principles of Good Laboratory Practice as incorporated into the United Kingdom Statutory Instrument for GLP. The test item or drug substance, OligoG, was formulated in sterile water at a concentration of 6 % (w/w), sterile filtered (0.22 µm) and stored at 2 - 8 °C. The mass aerosol concentration of the OligoG formulation or Vehicle in the animal's breathing zone via the reference port was measured gravimetrically for all groups during each exposure period. From the concentration samplings the achieved dose levels for each group were calculated based on the following criteria:

$$\text{Dose (mg/kg/day)} = \frac{C \times RMV \times T}{\text{Body weight (kg)}}$$

where C is the aerosol concentration, RMV the Respiratory Minute Volume (L/min) and T the duration of exposure (min).

MATERIALS & METHODS cont...

Animals were dosed once daily using a snout only inhalation exposure technique. Exposures to the OligoG aerosols and the control vehicle were performed using a modular (3 tier) stainless steel flow past systems. The system allowed a continuous supply of test aerosol to be delivered to each animal; the biased flow ensured no re-breathing of the test atmosphere. Separate exposure chambers were used for the Vehicle control and the OligoG groups.

Phase of study	Dose group/ Treatment	Daily exposure duration (min)	Animal number	
			Males	Females
A - Single dose	1 - 6% OligoG	60	5	5
	2 - 6% OligoG	120	5	5
	3 - 6% OligoG	240	5	5
B - 7 days dosing	4 - 6% OligoG	60	5	5
	5 - 6% OligoG	240	5	5
C - 14 days dosing	6 - 6% OligoG	60	5	5
	7 - 6% OligoG	240	5	5
D - 28 days dosing	8 - Vehicle only	240	10	10
	9 - 6% OligoG	120	5	5
	10 - 6% OligoG	240	10	10

Table 1. The rats were allocated to 10 groups and treated as described above

Phase of study	Dose group/ Treatment	Daily exposure duration (min)	OligoG formulation in mg/kg/day	Non-aqueous component in mg/kg/day
A - Single dose	1 - 6% OligoG	60	500.0	75.6
	2 - 6% OligoG	120	997.5	150.8
	3 - 6% OligoG	240	1999.8	301.9
B - 7 days dosing	4 - 6% OligoG	60	511.4	71.0
	5 - 6% OligoG	240	2034.4	282.4
C - 14 days dosing	6 - 6% OligoG	60	498.5	71.6
	7 - 6% OligoG	240	1984.3	284.8
D - 28 days dosing	8 - Vehicle only	240	1916.7	0
	9 - 6% OligoG	60	494.4	71.6
	10 - 6% OligoG	240	1988.2	288.0

Table 2. Achieved dose levels for the dose groups

RESULTS

Observations. There were no adverse reactions to treatment with 6% OligoG formulation noted for any of the treatment regimens.

Laboratory investigations/Clinical Pathology. A comprehensive haematology and clinical chemistry screen was performed. There were no treatment related changes in haematology and clinical chemistry parameters. There were no treatment related changes in urinary parameters.

Respiratory Monitoring. Respiratory parameters (respiratory rate, tidal volume and respiratory minute volume) were recorded for 5 rats/sex for groups 8 and 10. There were no treatment related changes in the respiratory parameters.

Terminal studies. Necropsies were performed one day after the last exposure for the repeat dose groups 4 - 10. Organ weights did not show treatment related changes, with the exception of the lung weights. A dose/exposure duration dependant increase in lung weight was related to 6 % OligoG formulation treatment.

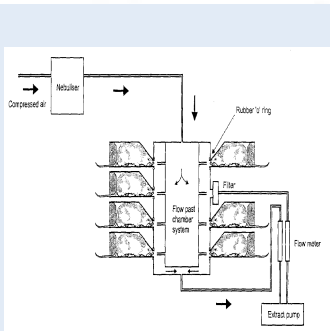


Fig 1. Schematic diagram of dosing apparatus

RESULTS cont...

Necropsies. The only treatment related finding was bronchial lymph node enlargement in 8 animals treated with the test item for 240 min per day, including 2/5 males and 2/5 females dosed for 14 days (Phase C), 1/5 single dose males (Phase A), 1/5 females dosed for 7 days (Phase B), 1/5 males dosed for 28 days and 1/5 females dosed for 28 days followed by a 2-week off-test recovery period (Phase D).

Histopathological investigations. Covered the respiratory system (plus any abnormalities) and were performed on all study animals for 28-days exposure. There was alveolar foamy macrophage accumulation in the lung of all animals from groups 9 and 10. The severity was graded minimal or mild in group 9 (60 min exposure), and minimal to moderate in group 10 (240 min exposure). Alveolar macrophage accumulation is a normal adaptive response to the presence of inhaled material in the lung. It is considered that in the absence of an inflammatory response, the accumulation of foamy alveolar macrophages is due to the normal clearance mechanism of the lung. This is not considered to be a toxicological effect of administration of 6 % OligoG formulation.

The lung tissue stained positively with Alcian Blue stain for the presence of OligoG in some animals treated for 60 minutes (group 9) graded as minimal and in all animals treated for 240 minutes (group 10) graded as minimal to moderate.

CONCLUSIONS

- Once daily administration of a nebulised aerosol of 6% OligoG formulation for up to 28 days produced no adverse clinical signs or test item-related changes in body weight, food consumption, respiratory measurement parameters, haematology, clinical chemistry or urinalysis.

- A dose dependent increase in lung weight was observed in all phases of the study affecting only the male population. For female animals the lung weight was unaffected by the inhalation of the OligoG formulation.

- Alveolar macrophage accumulation is a normal adaptive response to the presence of inhaled material in the lung and not considered to be a toxicological effect of administration of 6% OligoG formulation.