

Aim: Screening of Muscle Relaxant Activity Using Rota-Rod Apparatus

References:

1. Dunham, N. W., & Miya, T. S. (1957). A note on a simple apparatus for detecting neurological deficit in rats and mice. *Journal of the American Pharmaceutical Association*, 46(3), 208-209.
2. Vogel, H. G. (2008). *Drug Discovery and Evaluation: Pharmacological Assays*. Springer.

Introduction:

The Rota-Rod apparatus is used to evaluate the muscle relaxant activity of compounds by measuring the time an animal can remain on a rotating rod. Muscle relaxants typically reduce the time the animal can maintain its grip and balance on the rod.

Objective:

To determine the muscle relaxant activity of a test compound using the Rota-Rod apparatus in mice.

Materials and Reagents:

- Mice (20-25 g, either sex)
- Test compound
- Standard muscle relaxant (e.g., diazepam)
- Saline or vehicle (control)
- Rota-Rod apparatus
- Syringes and needles
- Stopwatch or timer
- Animal cages
- Disposable gloves
- Laboratory coat

Procedure:

Animal Preparation

1. Acclimatize the mice to the laboratory conditions for at least one week before the experiment.
2. Fast the mice overnight with free access to water prior to the experiment.

Experimental Groups:

Divide the mice into the following groups, with a minimum of six animals per group:

1. **Control group:** Receive saline or vehicle
2. **Standard group:** Receive a standard muscle relaxant (e.g., diazepam)
3. **Test groups:** Receive different doses of the test compound

Administration of Compounds:

1. Administer the test compound, standard muscle relaxant, or vehicle intraperitoneally (i.p.) according to the group designation.
2. Allow 30 minutes for absorption.

Training on the Rota-Rod:

1. Train the mice on the Rota-Rod apparatus at a constant speed (e.g., 10 rpm) for three consecutive trials of 2 minutes each, with a rest period of at least 15 minutes between trials.
2. Only mice that can remain on the rotating rod for at least 180 seconds during the training session should be included in the study.

Testing for Muscle Relaxant Activity:

1. Place each mouse on the rotating rod set at a constant speed (e.g., 10 rpm).
2. Record the time each mouse stays on the rotating rod (fall-off time) up to a maximum of 180 seconds.
3. Perform three trials per mouse with at least 15 minutes rest between trials.
4. Record the fall-off time for each trial.

Calculation of Muscle Relaxant Activity:

1. Calculate the mean fall-off time for each mouse.
2. Compare the mean fall-off times of the test and standard groups with the control group.
3. A significant reduction in fall-off time compared to the control group indicates muscle relaxant activity.

Results and Discussion:

1. Present the data in a table showing the fall-off time for each mouse in all groups.
2. Calculate and present the mean fall-off time for each group along with the standard deviation.
3. Perform statistical analysis to determine the significance of differences between groups.
4. Discuss the results, comparing the muscle relaxant activity of the test compound with the control and standard groups. A significant reduction in fall-off time compared to the control group indicates muscle relaxant activity.

Safety and Ethical Considerations:

1. Ensure all experimental procedures involving animals comply with institutional and national ethical guidelines for the care and use of laboratory animals.
2. Handle all animals with care and minimize their distress.
3. Dispose of all biological waste according to safety guidelines.

Conclusion:

Summarize the findings, stating whether the test compound demonstrated significant muscle relaxant activity and how it compared to the standard muscle relaxant.

Sample Data Table

Group	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	Mean Fall-Off Time (seconds) \pm SD
Control	180	175	178	180	177	176	177.7 ± 1.86
Standard (Diazepam)	65	60	62	63	61	64	62.5 ± 1.87
Test (10 mg/kg)	140	135	138	136	137	139	137.5 ± 1.72
Test (20 mg/kg)	100	105	102	101	103	104	102.5 ± 1.72