Oral gavage offers an alternative route for the administration of tamoxifen

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Abstract

The advent of Tamoxifen (TAM)-inducible Cre recombinase has revolutionised mouse models of disease allowing temporal control of genetic modifications.

TAM dissolved in oil can be dosed through several routes, of which intraperitoneal (IP) is the most common. However there are other routes of administration considered less invasive which may be considered a refinement, were they to deliver TAM as efficiently. Furthermore there have been complications when TAM is incorrectly stored and dosed IP which may be avoided by other routes. Oral gavage (OG) is one such route but a direct comparison has not been carried out.

To test this, TAM was administered either IP or via OG to Granzyme B Cre-ERT2 mice during Influenza A infection. Cells expressing the cytokine Granzyme B will be labelled in the presence of TAM. This system allows the selective and irreversible labelling of immune cells that were producing Granzyme B during active influenza infection. A secondary dose of flu labelled with GFP was given in the hope that labelled CD8 cells from the first flu administration would localise around the infected site. We found that mice receiving OG lost more weight and showed poorer body conditioning as compared with mice dosed IP – although these differences were visibly noticeable. There was no difference between these groups in the effectiveness of TAM. This suggests that OG and IP administration of TAM are comparable in efficacy and show minimal differences in adverse effects. Given the higher rate of complications because of IP administration, OG could represent a reasonable refinement.

Method

Preparation and storage:

IP TAM can be prepared in advanced and stored in a -20°C freezer. It can be defrosted 3 times and disposed of on the third occasion. If water droplets or opaque appearance of TAM is observed it must be disposed of immediately.

TAM dosed OG was made and delivered fresh each day. Bead-beating is required to ensure solution stability.

Administration of substances:

Tamoxifen was dosed to all 12 Granzyme B Cre-ERT2 mice. Six of which were dosed via IP (15mg/ml) and six by OG (50mg/ml). Tamoxifen was administered over various days as shown on Figure 1 timeline. HkX31 10000PFU (flu A) was given to six animals (three from each administration route) and PR8-ZsGreen 100000PFU (flu B) was given to 7 animals across both groups. Both flus were administered Intranasal under gaseous anaesthetics (isoflurane).

Monitoring:

The biological research unit (BRU) staff performed daily health checks. Staff trained in the flu models performed enhance monitoring, recording body scores and weights for the first eleven days from day -1 and three days post flu B infection (Figure 1).

Schedule 1 was performed on day 40 via IP overdose of anaesthetic. Lungs, lymph nodes and blood samples were taken for imaging and flow cytometry.

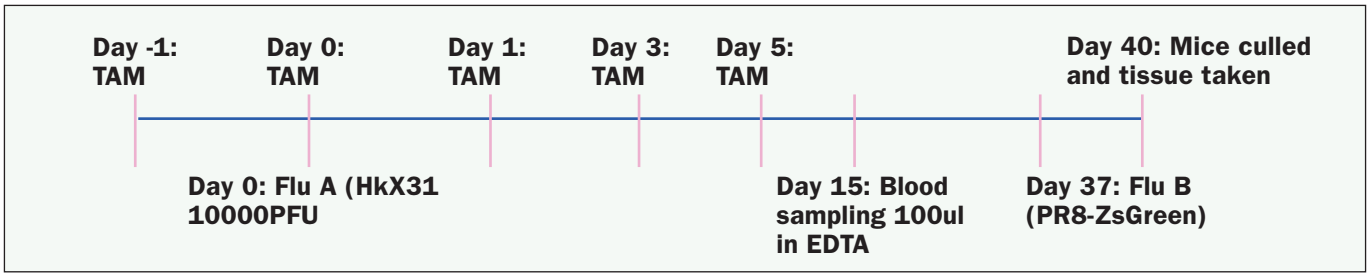


Figure 1. Experimental timeline.

Results

Health scoring and bodyweight were recorded from day -1 to 10 and then again from day 38 to 40. This can be seen in Tables 1, 3 and on Graph 1.

	D-1	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Total Health score (IP Only)	0	0	0	0	0	1	0	0	0	0	0	0
Total Health score (IP + Flu)	0	2	0	0	0	2	0	0	9	0	0	0
Total Health score (OG Only)	0	0	0	0	0	0	0	0	0	0	0	0
Total Health score (OG + Flu)	0	0	0	0	0	0	0	0	14	11	0	0

Table 1. Cumulative health score for each TAM and Flu A group for the categories listen in Table 2.

Score	HEALTH SCORING CHART (HSC)
	APPEARANCE
1	General lack of grooming
2	Rough haircoat/slightly hunched
3^	Piloerection, hunched up
	CLINICAL SIGNS
2	Slight changes: e.g. diminished activity, pale ears, eyes & feet, semi closed eyes
4	Moderate changes: e.g. diarrhoea, intermittent abnormal breathing
6*	Severe changes: e.g. immobility, abnormal gait, paresis/paralysis, eyes sunken, laboured respiration
	NATURAL BEHAVIOUR
1	Subdued but responsive. Animal shows normal behaviour patterns and interacts with peers.
2	Subdued. Animals shows subdued behaviour with little peer interaction with some response.
3^	Generally dull, possibly isolated from others.
	PROVOKED BEHAVIOUR
1	Slight depression or exaggeration. Subdued but responsive. May be agitated when disturbed.
2	Moderate change in expected behaviour. Subdued with some response. May be agitated when disturbed.
3*	Very weak/depressed, generally unresponsive.

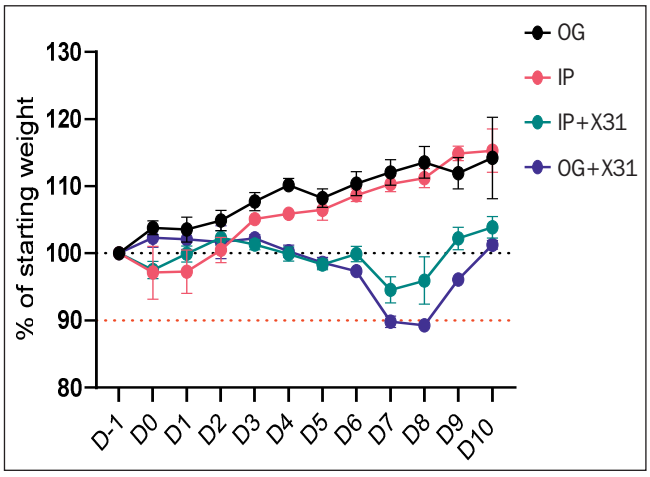
Table 2. Health scoring chart. Health score of 10 or more per animal must be euthanised immediately.

	D38	D39	D40
Total Health score (IP + FLU B)	0	4	4
Total Health score (IP + FLU A+B)	3	5	5
Total Health score (OG + FLU B)	0	4	4
Total Health score (OG + FLU A+ B)	2	3	3

Table 3. Cumulative health score for Flu A and Flu A+B.

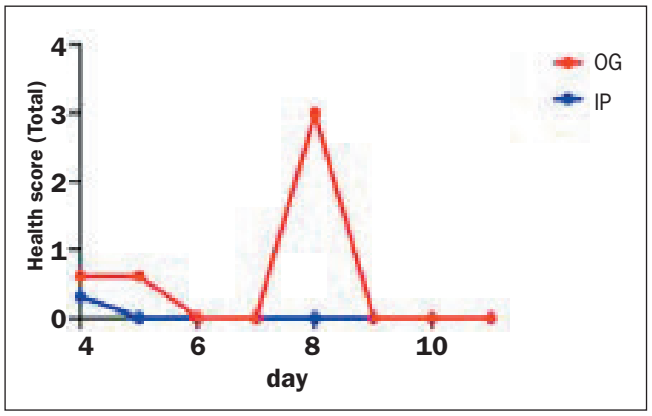
It was generally observed that the overall health score was higher at days 7 and 8 however this was expected as the flu infection is at its peak. By day 10 all of the mice had fully recovered with health scores of 0 and weight back to basal as shown Graph 1.

The combination of OG and flu A showed a more marked decline in physical appearance than IP and flu A. By comparing both tables, you can see that the addition of secondary flu B, the IP administration route showed a higher health score. Table 2 shows the health scoring system used for this study (approved by Glasgow university).



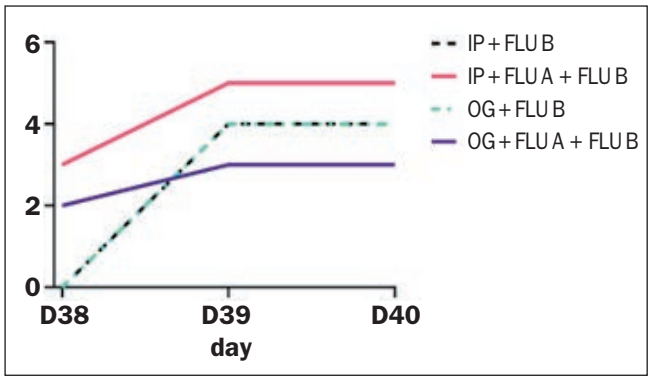
Graph 1. Bodyweights.

Although health score appears high, when averaged across the TAM administration groups the max health score was 3 as reflected in Graph 2.



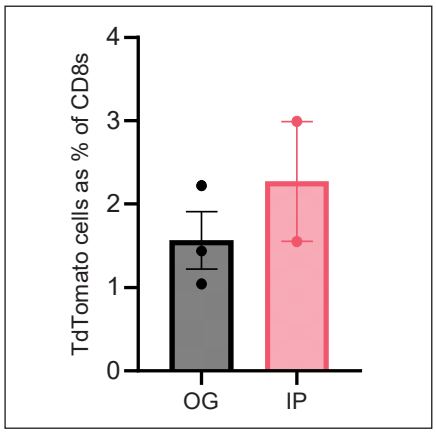
Graph 2. Average health score for each administration route.

At first glance the trends are similar across flu groups however mice which had tamoxifen administered IP and flu are seen to be higher, as shown in Graph 3.

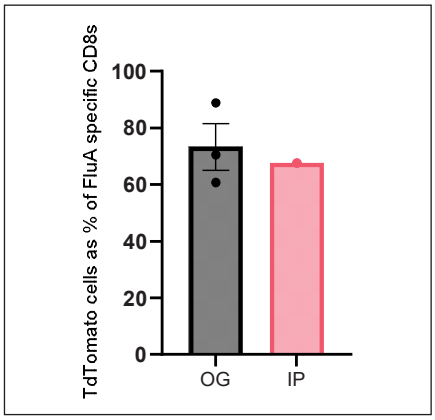


Graph 3. Average health score for different flu groups.

Looking at Graphs 4 and 5 there is a statistically significant difference between the CD8 labelling in naive and infected mice. However on closer inspection there is no significant difference between administration routes.

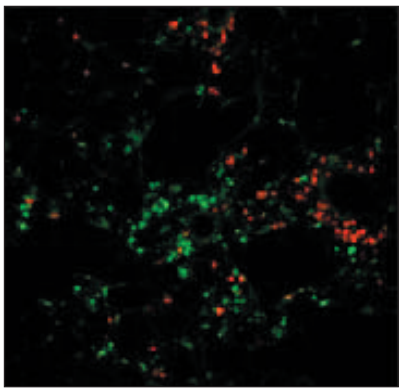


Graph 4. Labelling of CD8 cells using TdTom in Tamoxifen only animals.



Graph 5. Flu specific CD8 cells using TdTom in TAM and flu A animals.

As expected, labelled cells from secondary infection were found to localise around CD8 cells from the primary flu infection which can be seen in picture 1.



Picture 1. FImmunofluorescent image of mouse lung Red = TdTom cells: Green = Infected cells.

Conclusion

The results are comparable looking at both IP and OG routes for the administration of TAM.[1] When choosing administration routes several factors will need to be considered including volume, frequency, concentration, preparation/storage and adverse effects. From an operator perspective skill set and experience are very important.

Acknowledgements

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Reference

[1] **Donocoff, R.S., Teteloshvii, N., Chun, H. *et al*.** Optimization of tamoxifen-induced cre ctivity and its effect on immune cell populations. Naturesearch. (2020) 10:15244 https://www.nature.com/articles/s41598-020-72179-0