

ORIGINAL RESEARCH

Novel Edaravone Formulation: Part II: *in-vivo* assessment for efficacy and safety in Alzheimer's disease

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Self-nanomicellizing solid dispersion of Edaravone: Part II: *in-vivo* assessment of efficacy against behavior deficits and safety in Alzheimer's disease model

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Table S1 Protocol for tissue processing in Leica ASP300

| Step | Reagent | Duration (min) | Temp |
|------|---------------------|----------------|------|
| 1 | Formalin | 5 | - |
| 2 | Ethanol 70% | 45 | - |
| 3 | Ethanol 90% | 60 | - |
| 4 | Ethanol Absolute | 60 | - |
| 5 | Ethanol Absolute | 60 | - |
| 6 | Ethanol Absolute | 90 | - |
| 7 | Xylene | 60 | - |
| 8 | Xylene | 60 | - |
| 9 | Xylene | 90 | - |
| 10 | Paraffin Wax | 60 | 62 |
| 11 | Paraffin Wax | 60 | 62 |
| 12 | Paraffin Wax | 60 | 62 |

Table S2 Statistical analysis for Figure 1A

| Group | P value |
|---|---------|
| Control vs. Cuso ₄ | < 0.01 |
| Control vs. EDR against Cuso ₄ | < 0.01 |
| Control vs. NEF against Cuso ₄ | < 0.01 |
| Cuso ₄ vs. EDR against Cuso ₄ | < 0.01 |
| Cuso ₄ vs. NEF against Cuso ₄ | < 0.01 |
| EDR against Cuso ₄ vs. NEF against Cuso ₄ | 0.8211 |

Table S3 Statistical analysis for Figure 1B

| Group | P value |
|---|---------|
| Control vs. H ₂ O ₂ | < 0.01 |
| Control vs. EDR against H ₂ O ₂ | < 0.01 |
| Control vs. NEF against H ₂ O ₂ | < 0.01 |
| H ₂ O ₂ vs. EDR against H ₂ O ₂ | < 0.01 |
| H ₂ O ₂ vs. NEF against H ₂ O ₂ | < 0.01 |
| EDR against H ₂ O ₂ vs. NEF against H ₂ O ₂ | 0.8768 |

Table S4 Statistical analysis for Figure 1C

| Group | P value |
|---|---------|
| Control vs. A β | < 0.01 |
| Control vs. EDR against A β | < 0.01 |
| Control vs. NEF against A β | < 0.01 |
| A β vs. EDR against A β | < 0.01 |
| A β vs. NEF against A β | < 0.01 |
| EDR against A β vs. NEF against A β | 0.7398 |

Table S5 Statistical analysis for Figure 1D

| Time | P value |
|-------|---------|
| 0.5 h | 0.1729 |
| 2 h | < 0.01 |

Table S6 Statistical analysis for Figure 2

| Group | P value | | | | | |
|---|-----------|-----------|-----------|-----------|-----------|-----------|
| | Figure 2A | Figure 2B | Figure 2C | Figure 2D | Figure 2E | Figure 2F |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Wt Ctrl (n=8) | < 0.01 | < 0.01 | < 0.01 | 0.8521 | 0.9842 | 0.6371 |
| 17 mo Tg Ctrl (n=12) vs. 14 mo Tg Ctrl (n=11) | 0.9955 | 0.8883 | 0.9994 | >0.9999 | 0.9952 | 0.9999 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg BC (n=10) | 0.9996 | 0.9998 | > 0.9999 | 0.9937 | 0.9997 | 0.9870 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF LD (n=11) | 0.0375 | < 0.01 | 0.0843 | 0.7672 | 0.9996 | 0.9969 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF MD (n=12) | 0.0126 | < 0.01 | 0.0272 | 0.3215 | 0.9973 | 0.9275 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF HD (n=9) | < 0.01 | < 0.01 | 0.0209 | 0.1994 | 0.9883 | 0.8907 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg DNP (n=9) | 0.1350 | 0.0252 | 0.9622 | 0.7453 | 0.9984 | 0.9978 |

Table S7 Statistical analysis for Figure 3A: A-B

| Group | P value | |
|------------------------|----------------|----------------|
| | Figure 3A: A-B | Figure 3B: A-B |
| 17 mo Wt Ctrl (n=8) | > 0.9999 | 0.0143 |
| 14 mo Tg Ctrl (n=11) | > 0.9999 | 0.9955 |
| 17 mo Tg Ctrl (n=12) | > 0.9999 | > 0.9999 |
| 17 mo Tg BC (n=10) | > 0.9999 | > 0.9999 |
| 17 mo Tg NEF LD (n=11) | > 0.9999 | 0.3645 |
| 17 mo Tg NEF MD (n=12) | > 0.9999 | 0.0538 |
| 17 mo Tg NEF HD (n=9) | 0.9993 | 0.0527 |
| 17 mo Tg DNP (n=9) | > 0.9999 | 0.7780 |

Table S8 Statistical analysis for Figure 3C

| Group | P value |
|---|---------|
| 17 mo Tg Ctrl (n=12) vs. 17 mo Wt Ctrl (n=8) | < 0.01 |
| 17 mo Tg Ctrl (n=12) vs. 14 mo Tg Ctrl (n=11) | 0.0139 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg BC (n=10) | 0.9485 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF LD (n=11) | < 0.01 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF MD (n=12) | < 0.01 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF HD (n=9) | < 0.01 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg DNP (n=9) | < 0.01 |

Table S9 Statistical analysis for Figure 4

| Group | P value | | | | |
|---|------------|-----------------|-----------------|------------|-----------------|
| | Figure 4A | Figure 4B | Figure 4C | Figure 4D | Figure 4E |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Wt Ctrl (n=8) | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| 17 mo Tg Ctrl (n=12) vs. 14 mo Tg Ctrl (n=11) | 0.998 3 | 0.987 5 | 0.895 5 | 0.013 9 | 0.995 1 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg BC (n=10) | 0.999 9 | > 0.999 9 | > 0.999 9 | 0.948 5 | > 0.999 9 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF LD (n=11) | 0.032 5 | 0.027 9 | 0.019 6 | < 0.01 | 0.033 4 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF MD (n=12) | < 0.01 | 0.013 1 | < 0.01 | < 0.01 | < 0.01 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF HD (n=9) | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg DNP (n=9) | 0.043 4 | 0.048 7 | 0.036 7 | < 0.01 | 0.042 6 |

Table S10 Statistical analysis for Figure 5A

| Group | P value |
|---|----------|
| 17 mo Tg Ctrl (n=12) vs. 17 mo Wt Ctrl (n=8) | > 0.9999 |
| 17 mo Tg Ctrl (n=12) vs. 14 mo Tg Ctrl (n=11) | 0.9127 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg BC (n=10) | > 0.9999 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF LD (n=11) | 0.9878 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF MD (n=12) | 0.8894 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF HD (n=9) | 0.8530 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg DNP (n=9) | > 0.9999 |

Table S11 Statistical analysis for Figure 5B

| Group | P value | | | | |
|---|-----------------|-----------------|-----------------|-----------------|-----------------|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Wt Ctrl (n=8) | > 0.999 9 | 0.999 2 | > 0.999 9 | > 0.999 9 | 0.999 8 |
| 17 mo Tg Ctrl (n=12) vs. 14 mo Tg Ctrl (n=11) | 0.982 4 | 0.999 7 | 0.999 3 | 0.916 9 | 0.997 3 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg BC (n=10) | > 0.999 9 | > 0.999 9 | > 0.999 9 | > 0.999 9 | > 0.999 9 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF LD (n=11) | > 0.999 9 | > 0.999 9 | > 0.999 9 | > 0.999 9 | > 0.999 9 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF MD (n=12) | > 0.999 9 | > 0.999 9 | > 0.999 9 | > 0.999 9 | > 0.999 9 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF HD (n=9) | > 0.999 9 | > 0.999 9 | > 0.999 9 | > 0.999 9 | > 0.999 9 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg DNP (n=9) | > 0.999 9 | > 0.999 9 | > 0.999 9 | > 0.999 9 | > 0.999 9 |

Table S12 Statistical analysis for Figure 5C

| Group | P value | | | |
|---|-------------|-------------|-------------|-------------|
| | Day 1 | Day 2 | Day 3 | Day 4 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Wt Ctrl (n=8) | > 0.9999 | 0.9475 | 0.3034 | 0.0002 |
| 17 mo Tg Ctrl (n=12) vs. 14 mo Tg Ctrl (n=11) | > 0.9999 | > 0.9999 | > 0.9999 | > 0.9999 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg BC (n=10) | > 0.9999 | > 0.9999 | > 0.9999 | > 0.9999 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF LD (n=11) | > 0.9999 | > 0.9999 | 0.9992 | 0.0173 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF MD (n=12) | > 0.9999 | > 0.9999 | 0.9039 | < 0.01 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF HD (n=9) | > 0.9999 | > 0.9999 | 0.6710 | < 0.01 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg DNP (n=9) | > 0.9999 | > 0.9999 | > 0.9999 | 0.0402 |

Table S13 Statistical analysis for Figure 5D

| Group | P value |
|---|----------|
| 17 mo Tg Ctrl (n=12) vs. 17 mo Wt Ctrl (n=8) | < 0.01 |
| 17 mo Tg Ctrl (n=12) vs. 14 mo Tg Ctrl (n=11) | > 0.9999 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg BC (n=10) | > 0.9999 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF LD (n=11) | 0.0245 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF MD (n=12) | < 0.01 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg NEF HD (n=9) | < 0.01 |
| 17 mo Tg Ctrl (n=12) vs. 17 mo Tg DNP (n=9) | 0.0490 |

Table S14 Statistical analysis for Figure 5E

| Group | P value |
|------------------------|---------|
| 17 mo Wt Ctrl (n=8) | 0.3625 |
| 14 mo Tg Ctrl (n=11) | 0.9795 |
| 17 mo Tg Ctrl (n=12) | 0.3805 |
| 17 mo Tg BC (n=10) | 0.5369 |
| 17 mo Tg NEF LD (n=11) | 0.8341 |
| 17 mo Tg NEF MD (n=12) | 0.6241 |
| 17 mo Tg NEF HD (n=9) | 0.5368 |
| 17 mo Tg DNP (n=9) | 0.9520 |