



Article

Hybrid Lipid Nanoformulations for Hepatoma Therapy: Sorafenib Loaded Nanoliposomes—A Preliminary Study

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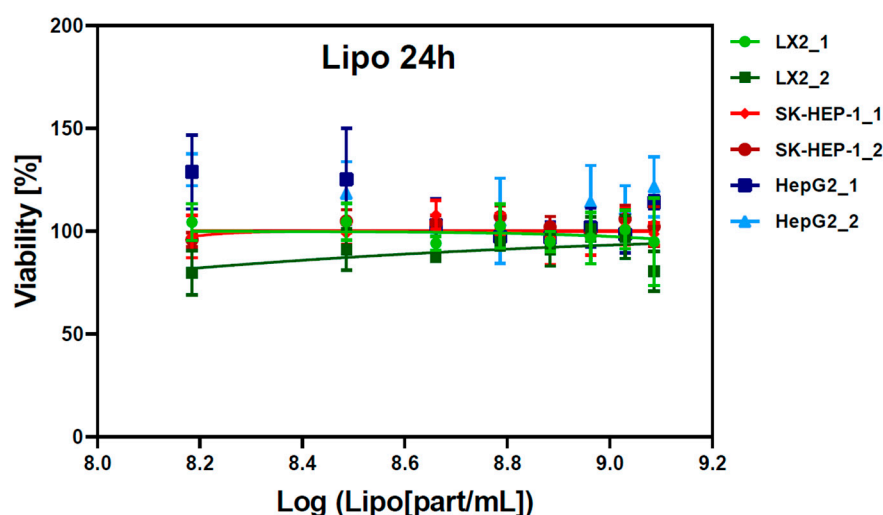


Figure S1. Evaluation of IC₅₀ doses of Lipo treatment at 24 h. The experiments were performed on duplicate for each cell line.

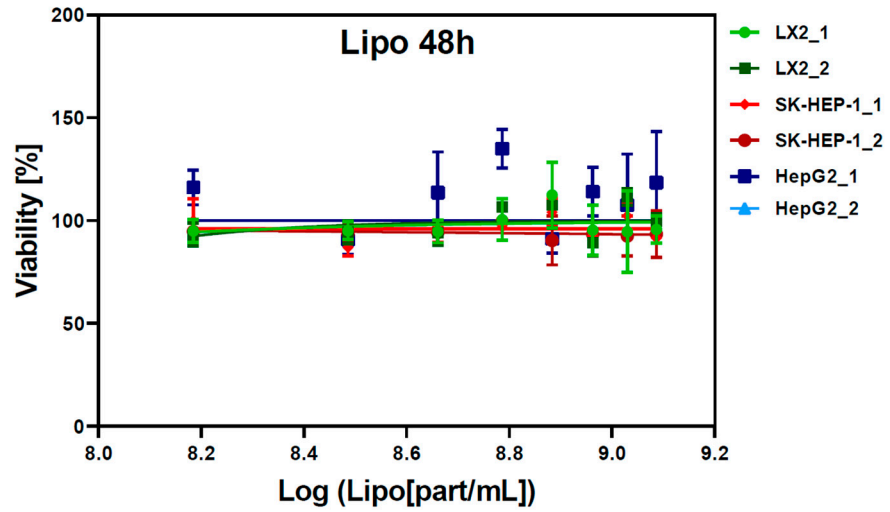


Figure S2. Evaluation of IC₅₀ doses of Lipo at 48 h. The experiments were performed on duplicate for each cell line.

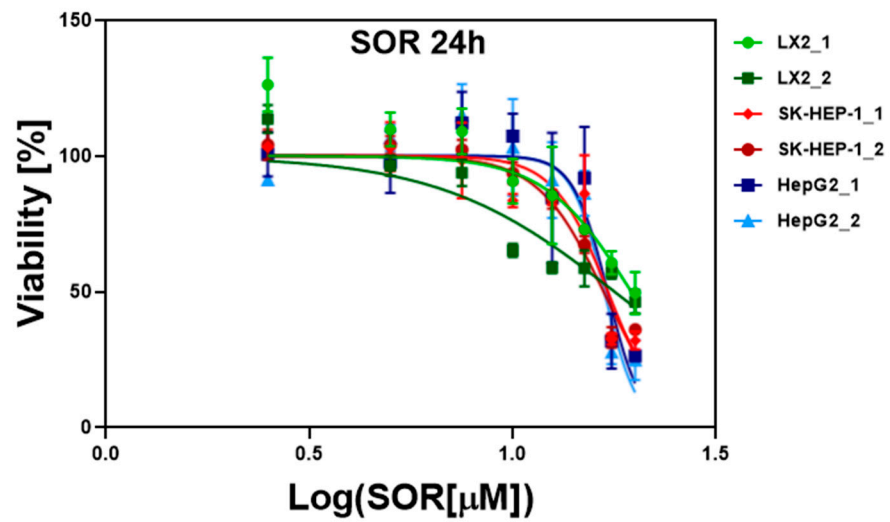


Figure S3. Evaluation of IC₅₀ doses of SOR treatment at 24 h. The experiments were performed on duplicate for each cell line.

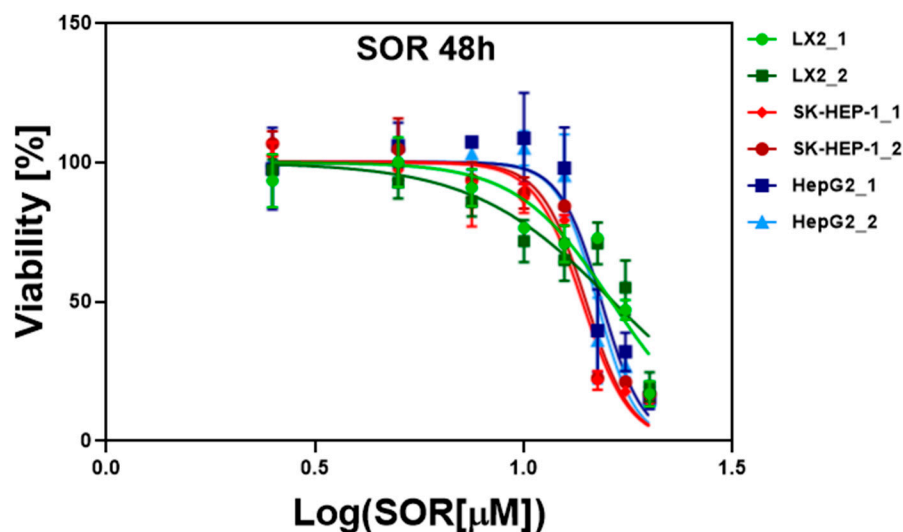


Figure S4. Evaluation of IC₅₀ doses of SOR treatment at 48 h. The experiments were performed on duplicate for each cell line.

Table S1. Statistical analysis in the case of blank liposomes treatments effect on 24 and 48 h.

| Cell line | 1.22E+09 part/ml | 1.07E+09 part/ml | 9.17E+08 part/ml | 7.64E+08 part/ml | 6.11E+08 part/ml | 4.58E+08 part/ml | 3.06E+08 part/ml | 1.53E+08 part/ml |
|-----------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Duplicate 1 24h | | | | | | | | |
| LX2 | 0.769328 | 0.947867 | 0.817012 | 0.678590 | 0.849069 | 0.624289 | 0.723553 | 0.735599 |
| SKHEP | 0.772832 | 0.730028 | 0.857338 | 0.107944 | 0.458241 | 0.179640 | 0.795779 | 0.702046 |
| HEPG2 | 0.045177 | 0.848654 | 0.810619 | 0.648596 | 0.588334 | 0.761715 | 0.167473 | 0.063622 |
| Duplicate 2 24h | | | | | | | | |
| LX2 | 0.087038 | 0.765906 | 0.898290 | 0.308474 | 0.419783 | 0.126129 | 0.365206 | 0.088683 |
| SKHEP | 0.757732 | 0.328753 | 0.715451 | 0.695232 | 0.219940 | 0.411216 | 0.367465 | 0.375057 |
| HEPG2 | 0.172483 | 0.422020 | 0.380560 | 0.833452 | 0.755598 | 0.860321 | 0.235949 | 0.050281 |
| Duplicate 1 48h | | | | | | | | |
| LX2 | 0.438823 | 0.677189 | 0.584409 | 0.268253 | 0.946938 | 0.310058 | 0.293018 | 0.321055 |
| SKHEP | 0.024743 ↑* | 0.621539 | 0.043293 ↑* | 0.417953 | 0.272675 | 0.094648 | 0.018794 ↑* | 0.990909 |
| HEPG2 | 0.256268 | 0.294356 | 0.710103 | 0.971164 | 0.009092 ↑** | 0.947129 | 0.564198 | 0.023731 ↑* |
| Duplicate 2 48h | | | | | | | | |
| LX2 | 0.087038 | 0.765906 | 0.898290 | 0.308474 | 0.419783 | 0.126129 | 0.365206 | 0.088683 |
| SKHEP | 0.388417 | 0.276780 | 0.054595 | 0.244687 | 0.852371 | 0.053357 | 0.111586 | 0.048279 ↑* |
| HEPG2 | 0.172483 | 0.422020 | 0.380560 | 0.833452 | 0.755598 | 0.860321 | 0.235949 | 0.050281 |

↑ - increased viability; ↓ - decreased viability; P-value <0.05 (*), P-value <0.01 (**) and P-value <0.001 (**).

Discovery determined using the Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%.

Each row was analyzed individually, without assuming a consistent SD. Number of t tests: 3.

Table S2. Statistical analysis in the case of Sorafenib treatments effect on 24 and 48 h.

| Cell line | 20 μ M | 17.5 μ M | 15 μ M | 12.5 μ M | 10 μ M | 7.5 μ M | 5 μ M | 2.5 μ M |
|-----------------|----------------------|------------------|------------------|------------------|-----------------|-------------|-----------|----------------|
| Duplicate 1 24h | | | | | | | | |
| LX2 | 0.001208 ↓*** | 0.001287 ↓*** | 0.003413 ↓*** | 0.263599 | 0.218253 | 0.229341 | 0.151472 | 0.021292 ↓* |
| SKHEP | 0.000308 ↓*** | 0.000279 ↓*** | 0.233513 | 0.050174 | 0.044889 ↓* | 0.874839 | 0.675029 | 0.580075 |
| HEPG2 | 0.000591 ↓*** | 0.001958 ↓*** | 0.570509 | 0.381779 | 0.452142 | 0.288447 | 0.827872 | 0.943004 |
| Duplicate 2 24h | | | | | | | | |
| LX2 | 0.000302 ↓*** | 0.000493 ↓*** | 0.001641 ↓** | 0.000520 ↓** | 0.001062 ↓** | 0.263530 | 0.459452 | 0.048789 ↓* |
| SKHEP | 0.000005 ↓*** | 0.000017 ↓*** | 0.000171 ↓*** | 0.003403 ↓** | 0.089980 | 0.423007 | 0.171031 | 0.309791 |
| HEPG2 | 0.000413 ↓*** | 0.000283 ↓*** | 0.130263 | 0.416992 | 0.799905 | 0.159974 | 0.339079 | 0.193787 |
| Duplicate 1 48h | | | | | | | | |
| LX2 | 0.000064 ↓*** | 0.000289 ↓*** | 0.002609 ↓** | 0.006094 ↓** | 0.004276 ↓** | 0.176328 | 0.991945 | 0.390000 |
| SKHEP | 0.000017 ↓*** | 0.000021 ↓*** | 0.000038 ↓*** | 0.004603 ↓** | 0.048843 ↓* | 0.057974 | 0.472784 | 0.634548 |
| HEPG2 | 0.000108 ↓** * | 0.000454 ↓*** | 0.003706 ↓** | 0.840712 | 0.466975 | 0.221270 | 0.437520 | 0.830644 |
| Duplicate 2 48h | | | | | | | | |
| LX2 | 0.000302 ↓*** | 0.000493 ↓*** | 0.001641 ↓** | 0.000520 ↓*** | 0.001062 ↓** | 0.263530 | 0.459452 | 0.048789 |
| SKHEP | 0.000005 ↓*** | 0.000017 ↓*** | 0.000171 ↓*** | 0.003403 ↓** | 0.089980 | 0.423007 | 0.171031 | 0.309791 |
| HEPG2 | 0.000413 ↓*** | 0.000283 ↓*** | 0.130263 | 0.416992 | 0.799905 | 0.159974 | 0.339079 | 0.193787 |

↑ - increased viability; ↓ - decreased viability; P-value <0.05 (*), P-value <0.01 (**) and P-value <0.001 (***).
Discovery determined using the Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%.
Each row was analyzed individually, without assuming a consistent SD. Number of t tests: 3.

Table S3. Statistical analysis in the case of Lipo_SOR treatments effect on 24 and 48 h.

| Cell line | 15.08 μ M | 13.17 μ M | 11.28 μ M | 9.4 μ M | 7.51 μ M | 5.64 μ M | 3.76 μ M | 1.88 μ M |
|-----------------|------------------|------------------|------------------|------------------|------------------|-----------------|------------------|------------------|
| Duplicate 1 24h | | | | | | | | |
| LX2 | 0.004120 ↓*** | 0.000160 ↓*** | 0.672048 | 0.612151 | 0.984074 | 0.714449 | 0.000332 ↑*** | 0.000898 ↑*** |
| SKHEP | 0.000004 ↓*** | 0.000006 ↓*** | 0.002297 ↓** | 0.018093 ↓* | 0.093159 | 0.038076 ↓* | 0.903695 | 0.011109 ↑* |
| HEPG2 | 0.005756 ↓** | 0.003551 ↓** | 0.145049 | 0.127007 | 0.076598 | 0.057279 | 0.901877 | 0.361602 |
| Duplicate 2 24h | | | | | | | | |
| LX2 | 0.007062 ↓** | 0.001828 ↓** | 0.562223 | 0.182374 | 0.177440 | 0.610755 | 0.003986 ↑** | 0.034356 ↑* |
| SKHEP | 0.000048 ↓*** | 0.000018 ↓*** | 0.001027 ↓** | 0.000934 ↓*** | 0.021367 ↓* | 0.002733 ↓** | 0.554501 | 0.066765 |
| HEPG2 | 0.002364 ↓** | 0.000267 ↓*** | 0.146928 | 0.010949 ↓* | 0.348438 | 0.101571 | 0.026474 ↑* | 0.021574 ↑* |
| Duplicate 1 48h | | | | | | | | |
| LX2 | 0.001312 ↓*** | 0.020901 ↓* | 0.010244 ↓* | 0.112281 | 0.146689 | 0.874352 | 0.008119 ↑** | 0.000603 ↑*** |
| SKHEP | 0.000036 ↓*** | 0.000048 ↓*** | 0.000050 ↓*** | 0.000347 ↓*** | 0.000917 ↓*** | 0.408659 | 0.464314 | 0.393643 |
| HEPG2 | 0.000127 ↓*** | 0.000143 ↓*** | 0.000176 ↓*** | 0.006473 ↓** | 0.036219 ↓* | 0.685431 | 0.704974 | 0.930465 |
| Duplicate 2 48h | | | | | | | | |
| LX2 | 0.013747 ↓* | 0.062985 | 0.295600 | 0.428195 | 0.835431 | 0.569662 | 0.505756 | 0.012286 ↑* |
| SKHEP | 0.000028 ↓*** | 0.000015 ↓*** | 0.000010 ↓*** | 0.000220 ↓*** | 0.000260 ↓*** | 0.548100 | 0.167830 | 0.342118 |
| HEPG2 | 0.000096 ↓*** | 0.000087 ↓*** | 0.000082 ↓*** | 0.018249 ↓* | 0.097682 | 0.912583 | 0.502474 | 0.586861 |

↑ - increased viability; ↓ - decreased viability; P-value <0.05 (*), P-value <0.01 (**) and P-value <0.001 (***).

Discovery determined using the Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%.

Each row was analyzed individually, without assuming a consistent SD. Number of t tests: 3.