

トランスフェリン-マイトマイシンC結合体の HepG2細胞並びに初代培養ラット肝細胞における 受容体介在型エンドサイトーシスと細胞毒性

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Receptor Mediated Endocytosis and Cytotoxicity of Transferrin-Mitomycin C Conjugate in the HepG2 Cell and Primary Cultured Rat Hepatocyte

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ABSTRACT: Intracellular disposition and cytotoxicity of macromolecular conjugate of mitomycin C (MMC) with transferrin (TF) were examined in the human hepatoma cell line HepG2 cell and normal cultured rat hepatocyte. The conjugate (TF-MMC) was specifically bound to the HepG2 cell as well as TF. The number of the binding site and the association constant of TF-MMC in the HepG2 cell were $396,000 \pm 31,000$ molecules/cell and $3.24 \times 10^7 \pm 0.58 \times 10^7 \text{ M}^{-1}$, respectively. No difference in the binding parameters of TF-MMC and TF can be detected in the HepG2 cell. The association constant for the TF receptor was almost identical between HepG2 cell and hepatocyte, however, the numbers of the binding site of TF-MMC and TF in the HepG2 cell were from 40-times to 50-times greater than those in the hepatocyte. Furthermore, TF-MMC was internalized into the HepG2 cell and the hepatocyte as well as TF. The rates of internalization of TF-MMC and TF into the HepG2 cell were nearly identical to those into the hepatocyte. However, the levels of the internalization into the HepG2 cell were remarkably higher than those into the hepatocyte because the number of receptors in the HepG2 cell was larger than that in the hepatocyte, and the rate of release from the HepG2 cell was slower than that from the hepatocyte. TF-MMC inhibited the growth of the HepG2 cells. The 50 % growth inhibition (GI_{50}) of TF-MMC against the HepG2 cell was $0.9 \mu\text{g MMC/ml}$, which was a little higher than that of MMC ($GI_{50} = 0.5 \mu\text{g/ml}$). These results indicated that the TF-MMC might be useful for delivery of MMC to the HepG2 cell

抄録 ヒト肝癌由来のHepG2細胞と正常ラット培養肝細胞におけるマイトマイシンCとトランスフェリンとの結合体の細胞内動態と細胞毒性を検討した。結合体はトランスフェリンと同様HepG2細胞へ特異的に結合した。HepG2細胞における結合体の最大結合

部位数と会合定数はそれぞれ 396000 ± 31000 molecules/cellと $3.24 \times 10^7 \pm 0.58 \times 10^7$ M^{-1} で、結合体とトランスフェリンでは結合パラメーターに差がなかった。トランスフェリン受容体に関する会合定数はHepG2細胞と肝細胞でほとんど同じ値であったが、結合体とトランスフェリンのHepG2細胞での結合部位数は肝細胞の40倍から50倍であった。さらに、結合体はトランスフェリンと同様、HepG2細胞と肝細胞に内在化された。HepG2細胞への結合体並びにトランスフェリンの内在化速度は、それぞれの肝細胞への内在化速度と近い値であった。しかしながら、HepG2細胞への内在化のレベルは肝細胞に比べて著しく高く、HepG2細胞からの遊離速度は肝細胞からの遊離速度に比べて遅かった。さらに、結合体はHepG2細胞の増加を阻害した。結合体のHepG2細胞に対する50%増殖阻害濃度は $0.9 \mu\text{gMMC/ml}$ であった。これらの結果は、トランスフェリン-マイトマイシンC結合体がHepG2細胞へのマイトマイシンCの送達系として有用であることを示唆している。