水溶性グルタチオン—デキストラン結合体の合成法とその性質

金尾義治，藤原由美恵，田中哲郎，小川享子，藤田佳平衛，井口定男

International Journal of Pharmaceutics, 57 (3), 263-272 (1989).

Preparation and Characterization of a Soluble Glutathione-Dextran Conjugate

Yoshiharu KANEO, Yumie FUJIHARA, Tetsuro TANAKA, Kyoko OGAWA, Kahee FUJITA and Sadao IGUCHI

ABSTRACT Glutathione (GSH), a naturally occurring antidote, cannot permecate into the liver when given extracellularly and has a very short half-life in the body. A dextran conjugate of GSH was synthesized by coupling GSH covalently to dextran (T-40, M_w =43,900) by the CNBr activation method in order to improve these disadvantages. We have demonstrared that GSH is delivered effectively into the hepatic cells by the conjugate which protects mice from the acetaminophen hepatotoxicity (Kaneo,Y. et al., *Int. J. Pharm.*, 44 (1988) 265-267). The conjugate was a water-soluble white powder containing 10% w/w of GSH. The molecular weight of the conjugate was distributed more widely than the original dextran and that of the peak estimated by size exclusion chromatography was 2.5×10^5. The isoelectric point of the conjugate was estimated to be 2.5 by the cellulose acetate paper electrophoresis. Kinetics of GSH regeneration from the conjugate was examined at various pH values. The conjugate significantly stabilized GSH and liberated it gradually at physiological conditions ($t_{1/2}$ =99 min). Tripeptide GSH has one sulfhydryl, one amino, and two carboxyl groups in the molecule. It was found that the sulfhydryl groups participate in chemical bonding between GSH and CNBr-activated dextran and appear gradually by the cleavage of the bonding according to the determination of sulfhydryl groups by the method of Ellman. It was confirmed that the content of free amino groups of the conjugate is very high by the measurement of amino groups with 2,4,6-trinitrobenzenesulfonic acid (TNBS). These results indicated that at least 80% of the conjugated GSH are attached to dextran via sulfhydryl groups. This may contribute to chemical stability against autoxidation of the thiol group and then to the advantageous

features of the conjugate as a macromolecular prodrug.

抄録　天然に存在する解毒剤であるグルタチオン（GSH）は投与後肝細胞内に透過することなくすみやかに消失する。この欠点を改善する目的で，GSHとデキストランの結合体を合成した。結合体は10％ w/wのGSHを含有し，分子量2.5×10^5，等電点2.5の水溶性白色粉末であった。結合体はGSHを顕著に安定化し，生理的条件下で除々にGSHを遊離した。SH基とアミノ基の測定から，結合したGSHの80％はSH基を介してCNBr活性化デキストランと結合していることが示された。これが，SH基の自動酸化に対する化学的な安定化に寄与し，高分子プロドラッグとして優れた特徴を付与している。