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TARGETING NITRIC OXIDE TO CANCER CELLS: CYTOTOXICITY STUDIES OF GLYCO-*S*-NITROSOTHIOLS

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Abstract: Glyco-*S*-nitrosothiols, fructose-2-SNAP and glucose-2-SNAP, were synthesized and found to be much more cytotoxic than SNAP in killing DU-145 human prostate cancer cells *in vitro*.

Nitric Oxide (NO) is involved in a wide variety of physiological and pathological processes.[1] NO has been found to play key roles in inhibiting tumor cell adhesion, arresting the growth of tumor cells, inducing genomic mutations in bacteria and assisting in the death of parasites.[2] Recently, several mechanisms have been proposed to account for the NO-mediated cytotoxicity.[2c] NO is known to degrade certain iron-containing prosthetic groups resulting in inhibition of the mitochondrial respiratory chain, DNA synthesis and aconitase activity.[3] NO can also react with superoxide produced from activated macrophages to form peroxynitrite ($^-$OONO) in which the protonated form (HOONO) acts as a potent chemical oxidant,[3,4] resulting in modification of protein functions and causing DNA damage.[5,6] It was also shown that the inhibition of A375 melanoma cells was directly correlated to the rate and extent of NO release from a diazeniumdiolate in solution.[2b] In addition, several antitumor drugs have demonstrated the ability to stimulate NO production.[7] Aside from the limited efforts in utilizing the cytotoxicity of NO donor compounds against cancer cells,[4a,8,9] therapeutic strategies to explore activities of NO to kill tumor cells are currently problematic due to the fact that the available NO delivery systems release or donate NO indiscriminately.[10,11] Therefore, the development for targeting NO donor compounds is of current interest. In our efforts to achieve specific targeting NO against tumor cells, new types of NO donor compounds, glyco-*S*-nitrosothiols or sugar-SNAPs, have been designed and synthesized.[12] In this paper, we report our preliminary cytotoxic studies of glyco-*S*-nitrosothiols against DU-145 human prostate cancer cells. Both glucose-2-SNAP and fructose-2-SNAP showed a much more effective cytotoxicity than SNAP, with fructose-2-SNAP even better than glucose-2-SNAP toward DU-145 human prostate cancers *in vitro*.

Design and synthesis of glyco-*S*-nitrosothiols. SNAP was prepared according to a method by Field *et al.*[13] Glucose-2-SNAP and fuctose-2-SNAP were synthesized according to our previous methods.[12] Our design and synthesis of glyco-*S*-nitrosothiols is based on the observations that the facilitated transport of monosacharrides in mammalian cells is accomplished by members of the GLUT family of transmembrane

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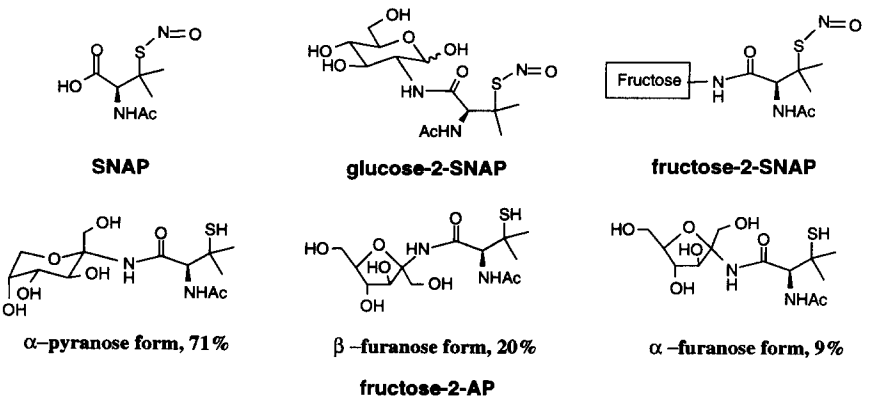
proteins.[14] Therefore, by conjugating sugar moieties with SNAP (*S*-nitroso-*N*-acetyl-D-penicillamine), a well known NO donor, we expected that the sugar-SNAPs would provide NO donor with enhanced water solubility, stability, cell penetration, and NO cytotoxicity.[15] These sugar-SNAPs showed a increased half-life and a prolonged NO releasing ability in comparison to SNAP.[12] The structures of SNAP and sugar-SNAPs are shown in Scheme 1. For monosaccharides such as fructose and fructose derivative, it is common to exist in a mixture of isomeric forms.[16] Based on the height of the anomeric carbon in ^{13}C NMR of fructose-2-AP, a stable precursor of fuctose-2-SNAP, we established that the mixture of fuctose-2-SNAP consists of α-pyranose form (71%), β-furonose form 20%, and α-furonose form 9%. The predominant formation of α-pyronose isomer (71%) is consistent with the isomeric compositions of other fructose derivatives.[16]



Scheme 1. Chemical structures of SNAP and glyco-*S*-nitrosothiols.

Cytotoxic effects of glyco-*S*-nitrosothiols. The cytotoxicity of glucose-2-SNAP and fructose-2-SNAP was evaluated and compared to SNAP using DU-145 human prostate cancer cells.

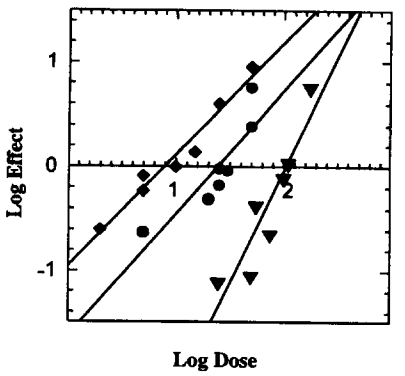


Figure 1. Cytotoxicity of glucose-2-SNAP (▼), fructose-2-SNAP (◆), and SNAP (●) in DU-145 human prostate cancer cells.

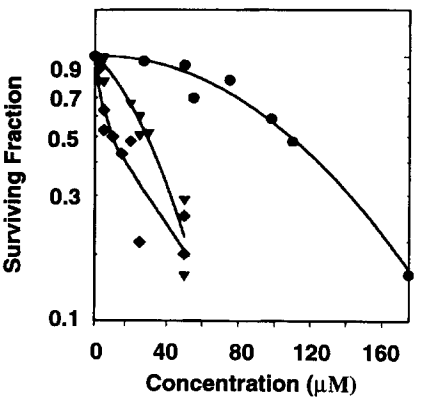


Figure 2. Surviving fraction vs. concentration. glucose-2-SNAP (▼), fructose-2-SNAP (◆), and SNAP (●) (semi-log format).

Figure 1 shows a median effect dose (MED) plot for the cytotoxicity of glucose-2-SNAP, fructose-2-SNAP, and SNAP toward human prostate cancer cells *in vitro*. The data suggests that toward DU145 human prostate cancer cells, the MED is approximately 110 μM, 32μM, and 8.5 μM for SNAP, glucose-2-SNAP, and fructose-2-SNAP, respectively. Figure 2 is the same data plotted in a semi-log format which indicates that glucose-2-SNAP and frucose-2-SNAP are approximately 4- to 13- fold more cytotoxic than SNAP. It has been observed that human cancer cells have overexpressed one or more of these sugar transporters. Therefore, the uptake of glucose or fructose by these cancer cells is facilitated.[17] Consistent with these findings, experimental results confirmed our expectation on an increased uptake and a greater cytotoxicity of sugar-SNAPs. In addition, it was found that the expression of fructose transporter GLUT5 is sugar-dependent in which fructose feeding yielded a three fold higher abundance of GLUT5.[18] Thus, the increased cytotoxicity of these sugar-SNAPs could also be explained by a stimulated generation of sugar transporters by the sugar moiety.

In conclusion, glyco-*S*-nitrosothiols (glucose-2-SNAP and fructose-2-SNAP) were synthesized and found to be very effective targeting cancer cells *in vitro*. Both glucose-2-SNAP and fructose-2-SNAP were shown to be much more cytotoxic than SNAP, with fructose-2-SNAP even better than glucose-2-SNAP in cytotoxicity toward DU-145 human prostate cancer cells *in vitro*.

Experimental

Biological testing. The cytotoxicity of glucose-2-SNAP and fructose-2-SNAP was evaluated and compared to SNAP using DU-145 human prostate cancer cells. Cancer cells were plated in T25 flasks at a density of 2×10^4 cell/cm^2. After 4 hrs, the cells were washed twice and 4 mL of fresh serum free media was added. NO donor solutions were prepared immediately before each experiment by dissolving glucose-2-SNAP, fructose-2-SNAP and SNAP in sterile saline, filtered through a 0.2 micron filter, diluted, and added to the cells. Cancer cells were exposed to NO donors for 30 min at 37 ° C. The cells were then washed twice with serum free media or phosphate buffered saline, trypsinized from the flask, washed, counted, diluted, and then plated out at several dilutions in fresh tissue culture media containing 10% serum. The cultures were then incubated for 10-14 days at which time the colonies were fixed, stained (methylene blue) and counted.

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References and Notes

1. (a) Moncada, S.; Palmer, R. M. J.; Higgs, E. A. *Pharmacol. Rev.* **1991**, 43, 109. (b) Beckman, J. S. In *The Physiological and Pathological Chemistry of Nitric Oxide, Nitric Oxide, Principles and Actions*, Lancaster, J. Jr., Ed.; Academic; New York; 1996; pp 1-69.
2. (a) Kong, L.; Dunn, G. D.; Keefer, L. K.; Korthuis, R. *J. Clin. Exp. Metastasis* **1996**, *14*, 335. (b) Maragos, C.; Wang, J. M.; Hrabie, J. A.; Oppenheim, J. J.; Keefer, L. K. *Cancer. Res.* **1993**, *53*, 564. (c) Laval, F.; Wink, D. A. *Carcinogenesis* **1994**, *15*, 443. (d) Rajan, T. V.; Porte, P.; Yates, J. A.;

Keefer, L.; Shultz, L. D. *Infect. Immun.* **1996**, *64*, 3351. (e) Fouad Ahmed, S.; Oswald, I. P.; Caspar, P.; Hieny, S.; Keefer, L.; Sher, A.; James, S. L. *Infect. Immun.* **1997**, *65*, 219.

3. Hibbs, J. B.; Taintor, R. R.; Varin, Z.; Rachlin, E. M. *Biochem. Biophys. Res. Commun.* **1988**, *157*, 87.
4. (a) Farias-Eisner, R.; Chaudhuri, G.; Aeberhard, E.; Fukuto, J. M. *J. Bio. Chem.* **1996**, *271*, 6144. (b) Stuehr, D. J.; Nathan, C. F. *J. Exp. Med.* **1989**, *169*, 1543. (c) Sherman, M. P.; Aeberhard, E. E.; Wong, V. Z.; Griscavage, J. M.; Ignarro, L. J. *Biochem. Biophys. Res. Commun.* **1993**, *191*, 1301. (d) Koppenol, W. H.; Moreno, J. J.; Pryor, W. A.; Ischiropolous, H. Beckman, J. S. *Chem. Res. Toxicol.* **1992**, *5*, 834.
5. Bechman, J.S.; Beckman, T. W.; Chen, J.; Marshall, P. A.; Freeman, B. A. *Proc. Natl. Acad, Sci. U.S.A.* **1990**, *87*, 1620.
6. Beckman, J. S; Crow, J. P. *Biochem. Soc. Trans.* **1993**, *21*, 330.
7. Lind, D. S.; Kontaridis, M. I.; Edwards, P. D.; Josephs, M. D.; Moldawer, L. L.; Copeland, E. M. *J. Surg. Res.* **1997**, *69*, 283.
8. Sumitani, K.; Kamijo, R.; Naqumo, M. *Anticancer Res.* **1997**, *17*, 865.
9. Cook, J. A.; Krishna, M. C.; Pacelli, R.; DeGraff, W.; Liebmann, J.; Mitchell, J. B.; Russo, A.; Wink, D. A. *Br. J. Cancer* **1997**, *76*, 325.
10. Markings, L. R.; Tsien, R. Y. *J. Bio. Chem.* **1994**, 269 *(9)*, 6282.
11. Garfield, R. E.; Balaban, A. T.; Seitz, W. A.; Klein, D. J. Lesko, M. PCT WO 96/36326.
12. (a) Ramirez, J.; Yu, L.; Li, J.; Braunschweiger, P. G.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **1996**, *6 (21)*, 2575. (b) Hou, Y.; Wang, J.-Q.; Ramirez, J.; Wang, P. G. *Methods in Enzymology* **1999**, *301*, 242.
13. Field, L.; Dilts, R. V.; Ravichandran, R.; Lenhert, P. G.; Carnahan, G. *J. Chem. Soc., Chem. Commun.* **1978**, 249.
14. (a) Bell, G. I.; Burant, C. F.; Takeka, J.; Gould, G. W. *J. Bio. Chem.* **1993**, *268*, 19161. (b) Mellanen, P.; Minn, H.; Greman, R.; Harkonen, P. *Int. J. Cancer*, **1994**, *56*, 622.
15. Lefer, A. M.; Lefer, D. J. *Ann. Rev. Pharmacol. Toxicol.* **1993**, *33*, 71.
16. (a) Gómez-Sánchez, A.; Martin, M. G. G. *Carbohydrate Res.* **1986**, *149*, 329. (b) Mossine, V. V.; Glinsky, G. V.; Feather, M. S. *Carbohydrate Res.* **1994**, *262*, 257.
17. (a) Yamamoto, T.; Seino, Y.; Fukumoto, H.; Koh, G.; Yano, H.; Inagaki, N.; Yamada, Y.; Inoue, K.; Manabe, T.; Imura, H. *Biochem. Biophys. Res. Commun.* **1990**, *170*, 223. (b) Zamora, L. S. P.; Golde, D. W.; Concha, I. I.; Rivas, C. I.; Delgado, L. F.; Baslega, J.; Nualart, F.; Vera, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1847. (c) Brown, R. S.; Wahl, R. L. *Cancer* **1993**, *72*, 2979. (d) Nishioka, T.; Oda, Y.; Seino, Y.; Yamamoto, T.; Inagaki, N.; Yano, H.; Imura, H.; Shigemoto, R.; Kikuchi, H. *Cancer Res.* **1992**, *52*, 3972. (e) Gould, G. W.; Bell, G. I. *Trends Biochem. Sci.* **1990**, *15*, 18.
18. Mesonero, J.; Matosin, M.; Cambier, D.; Rodriguez-Yoldi, M. J.; Brot-Laroche, E. *Biochem. J.* **1995**, *312*, 757.