VOL. 56 NO. 8, AUG. 2003 THE JOURNAL OF ANTIBIOTICS pp. 725 — 726

Inhibition of CTP: Phosphocholine Cytidylyltransferase Activity by 15-Deoxyspergualin

MANABU KAWADA and MASAAKI ISHIZUKA*

Institute for Chemotherapy, Microbial Chemistry Research Foundation,
18-24 Miyamoto, Numazu-shi, Shizuoka 410-0301, Japan

(Received for publication March 24, 2003)

15-Deoxyspergualin (DSG) is a potent synthetic analogue of spergualin isolated from a microbial cultured broth as an antitumor compound.[1~3] DSG inhibits growth of various tumor cell lines *in vitro* and *in vivo*.[4,5] Furthermore, DSG has a potent immunosuppressive effect and has been used clinically as an immunosuppressant.[6,7] Focusing on its antitumor effect, we have previously reported that DSG inhibits Akt kinase activation and phosphatidylcholine (PC) synthesis.[8] However, the mechanism for inhibition of PC synthesis by DSG is still unknown.

In this report, the effect of DSG on biosynthetic pathway of PC is described. PC is a main constituent of plasma membrane and plays an important role in various enzyme activities. PC is synthesized mainly through the CDP-choline pathway and the initial step of the pathway is catalyzed by choline kinase, which phosphorylates choline. Phosphocholine is then converted into CDP-choline by CTP : phosphocholine cytidylyltransferase (CCT), a rate-limiting enzyme in PC biosynthesis. The last step of the pathway is catalyzed by CDP-choline:1,2-diacylglycerol cholinephosphotransferase. To reveal which step is affected by DSG, we first examined the effect of DSG on choline metabolism.

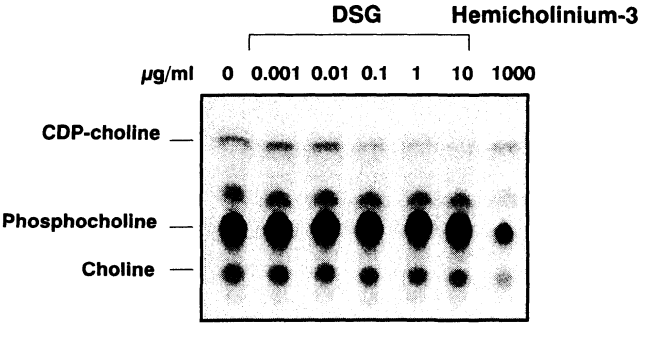
For analysis of choline metabolism, mouse EL-4 lymphoma cells (5×10^4) were cultured with or without DSG for 24 hours, and the cells were further incubated in choline-free minimum essential medium containing 10% dialyzed FBS and 0.5 μCi/ml [^{14}C]choline chloride for 3 hours at 37°C. The cells were washed twice with phosphate buffered saline and resuspended in 200 μl of 50% MeOH. Then, 200 μl of $CHCl_3$ was added to the cell suspension and phases were separated by centrifugation. The aqueous phase containing choline, phosphocholine and CDP-choline was separated by thin-layer chromatography on a potassium oxalate-pretreated silica gel in a solvent system of MeOH - 0.5% NaCl - 28% NH_4OH (100 : 100 : 2).[9] The radiolabeled spots on a TLC plate were analyzed by a Fujifilm FLA-5000 image analyzer.

As shown in Fig. 1, hemicholinium-3, an inhibitor of choline kinase,[10] decreased the intracellular amounts of phosphocholine, but DSG did not affect the amounts of choline and phosphocholine. It is indicated that DSG does not inhibit incorporation of choline into cells and choline kinase. On the other hand, DSG significantly decreased the amounts of CDP-choline. At the concentration of 0.1 μg/ml, DSG inhibited the CDP-choline synthesis to 50% compared to the control (Fig. 1). Thus, it is suggested that DSG inhibits CCT activity.

We then next examined the effect of DSG on CCT activity. EL-4 cells were cultured with DSG for 24 hours and the cells were sonicated. CCT activity was measured using [*methyl*-^{14}C]phosphocholine as a substrate according to the method of MAEDA *et al.*[9] As a result, DSG treatment apparently suppressed CCT activity, but hemicholinium-3 did not (Fig. 2). This result indicates that DSG inhibits PC synthesis through suppression of CCT activity, a rate-limiting enzyme of PC synthesis.

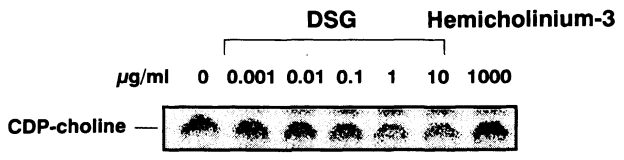
When DSG was directly added into the reaction mixture of CCT assay, it did not affect the CCT activity even at 100 μg/ml (data not shown). Therefore, DSG would suppress the CCT activity indirectly in the cells. The CCT activity is reported to be regulated by reversible translocation between cytosol and cellular membrane. It is considered that CCT in membrane fractions is active and that in cytosol is inactive. Protein kinase A phosphorylates

Fig. 1. Effect of DSG on choline metabolites in EL-4 cells.



* Corresponding author: imcic@shizuokanet.ne.jp

Fig. 2. Effect of DSG on CCT activity in EL-4 cells.



CCT and sustains the CCT in cytosol fraction as an inactive form.[11] There is a possibility that DSG might activate protein kinase A activity. Although our preliminary result showed that DSG partially activated protein kinase A, but the effect was not significant. Although the precise mechanism of DSG on CCT is still unclear, inhibition of CCT activity might be a target for discovering a new type antitumor drug.

Acknowledgements

We thank Dr. M. NISHIJIMA (National Institute of Infectious Diseases, Japan) for helpful advice. This work was supported by a grant-in-aid from the Japanese Ministry of Science, Education and Art.

References

1) TAKEUCHI, T.; H. IINUMA, S. KUNIMOTO, T. MASUDA, M. ISHIZUKA, M. TAKEUCHI, M. HAMADA, H. NAGANAWA, S. KONDO & H. UMEZAWA: A new antitumor antibiotic, spergualin: Isolation and antitumor activity. J. Antibiotics 34: 1619~1621, 1981
2) UMEDA, Y.; M. MORIGUCHI, H. KURODA, T. NAKAMURA, H. IINUMA, T. TAKEUCHI & H. UMEZAWA: Synthesis and antitumor activity of spergualin analogues. 1. Chemical modification of 7-guanidino-3-hydroxyacy moiety. J. Antibiotics 38: 886~898, 1985
3) IWASAWA, H.; S. KONDO, D. IKEDA, T. TAKEUCHI & H. UMEZAWA: Synthesis of (−)-15-deoxyspergualin and (−)-spergualin-15-phosphate. J. Antibiotics 35: 1655~1669, 1982
4) PLOWMAN, J.; D. STEADMAN, J. HARRISON, M. W. TRADER, P. DANIEL, J. GRISWOLD, M. CHADWICK, M. F. MCCOMISH, D. M. SILVEIRA & D. ZAHARKO: Preclinical antitumor activity and pharmacological properties of deoxyspergualin. Cancer Res. 47: 685~689, 1987
5) NISHIKAWA, K.; C. SHIBASAKI, M. HIRATSUKA, M. ARAKAWA, K. TAKAHASHI & T. TAKEUCHI: Antitumor spectrum of deoxyspergualin and its lack of cross-resistance to other antitumor agents. J. Antibiotics 44: 1101~1109, 1991
6) NEMOTO, K.; M. HAYASHI, F. ABE, T. NAKAMURA, M. ISHIZUKA & H. UMEZAWA: Immunosuppressive activities of 15-deoxyspergualin in animals. J. Antibiotics 40: 561~562, 1987
7) MASUDA, T.; S. MIZUTANI, M. IIJIMA, H. ODAI, H. SUDA, M. ISHIZUKA, T. TAKEUCHI & H. UMEZAWA: Immunosuppressive activity of 15-deoxyspergualin and its effect on skin allografts in rats. J. Antibiotics 40: 1612~1618, 1987
8) KAWADA, M.; T. MASUDA, M. ISHIZUKA & T. TAKEUCHI: 15-Deoxyspergualin inhibits Akt kinase activation and phosphatidylcholine synthesis. J. Biol. Chem. 277: 27765~27771, 2002
9) MAEDA, M.; M. NISHIJIMA, Y. AKAMATSU & Y. SAKAKIBARA: Alteration in the characters of CDP-choline synthetase and phospholipid-choline exchange enzyme upon choline starvation in Chinese hamster ovary cells. J. Biol. Chem. 260: 5925~5930, 1985
10) HERNANDEZ-ALCOCEBA, R.; L. SANIGER, J. CAMPOS, M. NUNEZ, F. KHALESS, M. GALLO, A. ESPINOSA & J. LACAL: Choline kinase inhibitors as a novel approach for antiproliferative drug design. Oncogene 15: 2289~2301, 1997
11) SANGHERA, J. & D. VANCE: CTP : phosphocholine cytidylyltransferase is a substrate for cAMP-dependent protein kinase *in vitro*. J. Biol. Chem. 264: 1215~1223, 1989