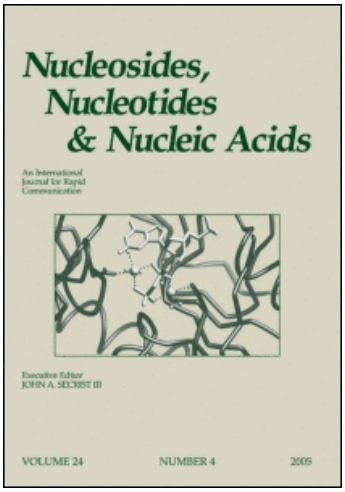

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Regulation of Equilibrative Nucleoside Uptake by Protein Kinase Inhibitors

Min Huang[a]; Yanhong Wang[a]; Beverly S. Mitchell[a]; Lee M. Graves[a]
[a] Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Online publication date: 27 October 2004

To cite this Article Huang, Min , Wang, Yanhong , Mitchell, Beverly S. and Graves, Lee M.(2004) 'Regulation of Equilibrative Nucleoside Uptake by Protein Kinase Inhibitors', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1445 — 1450
To link to this Article: DOI: 10.1081/NCN-200027667
URL: http://dx.doi.org/10.1081/NCN-200027667

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1445–1450, 2004

Regulation of Equilibrative Nucleoside Uptake by Protein Kinase Inhibitors

Min Huang,* Yanhong Wang, Beverly S. Mitchell, and Lee M. Graves

Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

ABSTRACT

The uptake of nucleosides and nucleoside analogs into human leukemia K562 cells is facilitated by the equilibrative transporters ENT1 and ENT2. Incubation of K562 cells with a variety of protein kinase inhibitors inhibited the transport of both uridine (ARA-C) and cytidine (CPEC) analogs. These inhibitory effects were observed for a large number of kinase inhibitors including those against p38 MAPK, the EGF receptor kinase, protein kinase C, TOR and others. Thus these results suggest that the nucleoside transporters are unexpected targets for kinase inhibitors and may influence the design and application of combinatorial approaches of nucleoside analogs and kinase inhibitors in clinical applications.

Key Words: Kinase inhibitors; Nucleoside transport; CTP; Leukemia cells.

INTRODUCTION

Nucleoside transporters are essential for the uptake of a large number of natural nucleosides and nucleoside analogs. These transporters can be classified into two structurally and functionally distinct groups, the equilibrative transporters (ENTs,

*Correspondence: Min Huang, Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

DOI: 10.1081/NCN-200027667
1525-7770 (Print); 1532-2335 (Online)

SLC29) which transport nucleosides in a sodium-independent manner and the concentrative transporters (CNTs, SLC28) which require sodium gradients and ATP for the transport of nucleosides (Fig. 1 and reviewed in Refs. [1,2]). Multiple equilibrative and concentrative transport activities have been observed and the recent cloning of the genes for these transporters has resulted in the identification of four ENTs (ENT1-4) and three CNT's (CNT1, CNT2 and CNT3).[1–5] The ENTs are broadly expressed in mammalian cells whereas the CNTs show limited expression in specialized cells. The ENT 1 and ENT2 can be further distinguished by their differential sensitivity to nitrobenzylthioinosine (NBMPR). ENT1 (es) is inhibited by nanomolar concentrations of this drug whereas ENT2 (ei) is only slightly inhibited by micromolar concentrations of NBMPR.[1–4] Both ENT1 and ENT2 are inhibited by cardioprotective agents (dilazep, draflazine and dipyridamole), although species differences of these effects have been observed.[6]

ENT1 and ENT2 are broadly selective for pyrimidine and purine nucleosides and transport adenosine, uridine, cytidine and thymidine as well as a large number of nucleoside analogs (e.g. cytosine arabinoside (AraC), gemcitabine, 2-chlorodeoxyadenosine (2-CdA), 3′-azido-3′-deoxythymidine (AZT)).[1,2,5–7] Despite the fact that both ENT1 and 2 are broadly selective, significant differences in their function may exist. ENT2 has been shown to specifically transport hypoxanthine and cyclic ADP ribose, suggesting selective functions for this transporter.[1,8,9] While investigating the influence of protein kinase inhibitors on cell proliferation, we obtained results that were consistent with inhibition of nucleoside uptake. In this article we summarize recent research demonstrating that equilibrative nucleoside transport is affected by a number of protein kinase inhibitors at concentrations typically used to inhibit protein kinases.

EXPERIMENTAL PROTOCOL

Human K562 cells were grown and incubated with protein kinase inhibitors as described earlier.[10,11] All protein kinase inhibitors were dissolved in DMSO and the

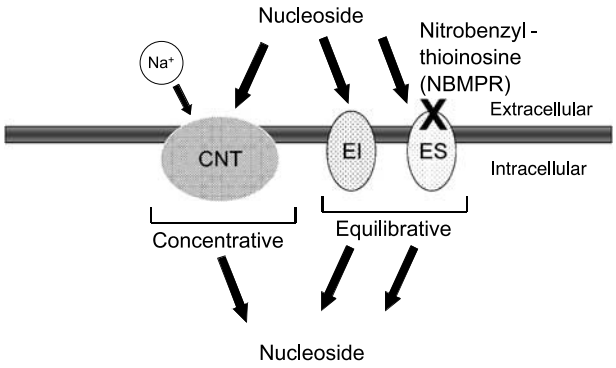

Figure 1. Classification of equilibrative and concentrative nucleoside transporters. A model depicting the two major classes of nucleoside transporters in mammalian cells is shown. CNT refers to the concentrative transporters whereas ES and EI refers to NBMPR-sensitive or insensitive equilibrative transporters, respectively.

uptake of ^{3}H-uridine and the determination of nucleotide triphosphate levels was measured as described previously.[10,12]

RESULTS AND DISCUSSION

Previously we demonstrated that inhibition of de novo pyrimidine synthesis with leflunomide inhibited the growth and increased the erythroid differentiation of human leukemia K562 cells in a CTP-dependent manner.[13] Incubation with cyclopentenyl cytosine (CPEC), a nucleoside analog and inhibitor of the de novo synthesis of cytidine nucleotides,[14] depleted cellular cytidine triphosphate CTP pools and increased the differentiation of these cells (Fig. 2 and data not shown). CPEC treatment also slightly increased UTP and ATP levels (~1.6 fold), but did not affect GTP levels (Fig. 2).

Because p38 MAP kinase has been implicated in regulating cell differentiation,[15,16] we tested whether a pyridinylimidizole inhibitor of p38 MAP kinase, SB203580, prevented the effects of CPEC on these cells. Incubation with SB203580 did not affect the levels of nucleotide triphosphates (Fig. 2) however, co-incubation of K562 cells with CPEC and SB203580 resulted in a significant reversal of the CPEC-induced CTP depletion and cell differentiation (Fig. 2 and data not shown). To examine the possibility that SB203580 was inhibiting the cellular uptake of CPEC or other nucleosides, K562 cells were incubated with increasing concentrations of SB203580 and the uptake of ^{3}H-uridine measured.[10] The results of these studies demonstrated that SB203580 inhibited the uptake of ^{3}H-uridine in a dose-dependent manner with an IC_{50} of approximately 0.69 uM.[10] Thus these results suggested that the effects of SB203580 on CPEC-induced differentiation, could in part, result from inhibition of CPEC uptake by the kinase inhibitor SB203580.

To further investigate the effects of kinase inhibitors on nucleoside transport, a number of additional kinase inhibitors (or inactive analogs) were evaluated for inhibition of ^{3}H-uridine uptake. The list of compounds included inhibitors of p38

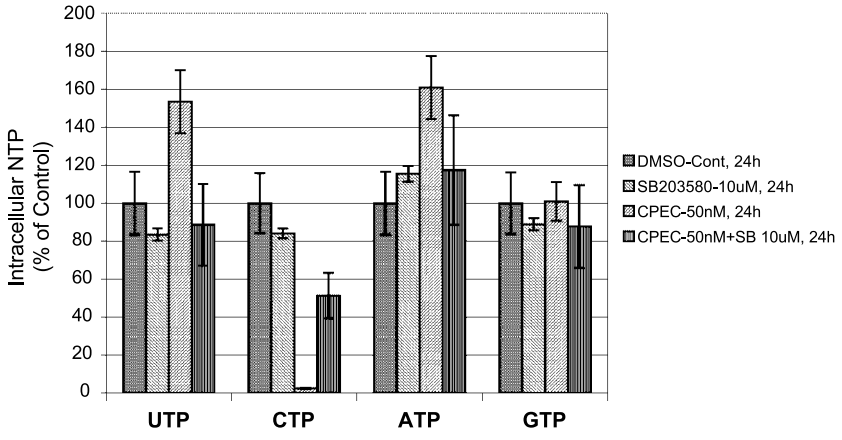

Figure 2. Effects of CPEC and SB203580 on nucleotide triphosphate pools in K562 cells. K562 cells were grown and treated with the concentration of drug indicated. Nucleotide triphosphates (NTP's) were extracted and determined as described earlier. (From Ref. [10].)

MAPK, EGF receptor tyrosine kinase (EGFR), BCR-ABL kinase, protein kinase C, the cyclin-dependent protein kinase (CDK's), the MAP kinase kinase (MEK), phosphatidylinositol-3 kinase and others. As described above, K562 cells were briefly incubated with these compounds (10 uM, 15 min) and the uptake of ^{3}H-uridine measured. The results of these studies[10,11] are summarized in Table 1. Similar to the results observed with SB203580, a number of kinase inhibitors were found to inhibit ^{3}H-uridine uptake. These included some of the pyridinylimidizole compounds, tyrphostins (AG1517, AG1478, AG825), PKC inhibitors (staurosporine, arcyriarubin, Ro31-6045, Ro31-8220, GF109203X) and the cyclin-dependent kinase inhibitors (roscovitine, olomoucine, indirubin). Similar results were obtained with the clinically important compounds STI-571 and rapamycin.[11]

By contrast, a number of kinase inhibitors had little or no effect on transport inhibition. This included inhibitors of MEK (PD98059, U0126) and inhibitors of PI-3 kinase (wortmannin, LY294002). Interestingly, our studies suggested that the effects of some of these compounds occurred independently of inhibition of the kinase itself. For instance, SB202474, an analog of SB203580 that does not inhibit p38 MAPK, also significantly inhibited nucleoside uptake in these cells (~65%) suggesting that the effects of these compounds occurred independently of p38 MAP kinase inhibition.[10,11] In addition, SB22025, a potent pyridinylimidazole inhibitor of p38 MAPK did not inhibit ^{3}H-uridine uptake, further demonstrating effects independent of p38 MAPK. Similar results were observed with R0 31-6045, a staurosporine analog that does not inhibit PKC.[11] Collectively these studies indicate a structure-dependent, kinase independent inhibition of nucleoside transport in these cells.

Table 1. Summary of protein kinase inhibitors and nucleoside transport.

Compound	% Inhibition	Compound	% Inhibition
SB203580	96	PD98059	7
SB203580-iodo	98	U0126	20
SB220025	7	RAF-1	82
SB202474	65	Wortmannin	0
AG1517	88	LY294002	1
AG1478	77	H89	45
AG825	94	KN93	19
AG18	40	Staurosporine	85
AG1879	10	GF109203X	79
AG490	29	Ro31-6045	92
WHI-P154	87	Ro32-0432	0
WHI-P180	66	Ro31-8220	86
WHI-P258	53	Arcyriarubin	60
WHI-P97	24	Indirubin	84
STI-571	70	Olomoucine	78
Genistein	40	Roscovitine	94
ZM336372	37	Rapamycin	81

Values compiled from previous studies.
(From Refs. [10,11].)

The profile of nucleoside transporter expression in K562 cells was previously shown to be predominantly ENT1 (~90%) with a smaller amount of ENT2 present (10%).[17] Nitrobenzylthioinosine (NBMPR) is a specific, high affinity inhibitor of ENT1-dependent nucleoside transport (es) (Ref. [2] and Fig. 1). To determine whether ENT was the target for the effects of these compounds, binding of ^{3}H-NBMPR was examined in the presence or absence of the pyridinylimidizole inhibitor SB203580-iodo. The results of these experiments demonstrated that binding of ^{3}H-NBMPR to K562 cells, or purified membrane proteins isolated from erythrocytes, was prevented by this compound.[10] Thus these results suggest that ENT1 (es) is a major target for the inhibitory effects of protein kinase inhibitors on nucleoside transport in K562 cells. However, recent characterization of the effects of Ro-31-6045 and SB203580-iodo on ENT2-dependent transport in Rat C6 glioma cells, a cell line predominantly expressing ENT2,[18] suggests that ENT2 is also inhibited by these compounds with an IC_{50} of, 0.52 μM and 0.77 μM respectively (Dr. Min Huang, unpublished observations).

CONCLUSIONS

The results of these studies demonstrate potent inhibition of nucleoside transport by a number of structurally distinct protein kinase inhibitors. These studies, combined with our earlier observations that inactive analogs of both p38 MAPK inhibitors (SB202474[10]) and PKC (RO 31-6045[11]) also prevented nucleoside uptake, suggest that these effects are independent of kinase inhibition and dependent on the compounds themselves. Research is currently being directed at determining the specificity of these compounds and the effects of protein kinase inhibitors on the pharmacological availability of clinically important nucleoside analogs such as ARA-C and other compounds.

REFERENCES

1. Baldwin, S.A.; Beal, P.R.; Yao, Y.M.S.; King, A.E.; Cass, C.E.; Young, J.D. Pflugers Arch.-Eur. J. Physiol. **2003**, epub ahead of print.
2. Gray, J.H.; Owen, R.P.; Giacomini, K.M. Pflugers Arch.-Eur. J. Physiol. **2003**, epub ahead of print.
3. Cass, C.E.; Young, J.D.; Baldwin, S.A. Biochem. Cell. Biol. **1998**, *76*, 761–770.
4. Cass, C.E.; Young, J.D.; Baldwin, S.A.; Cabrita, M.A.; Graham, K.A.; Griffiths, M.; Jennings, L.L.; Mackey, J.R.; Ng, A.M.; Ritzel, M.W.; Vickers, M.F.; Yao, S.Y. Pharm. Biotechnol. **1999**, *12*, 313–352.
5. Crawford, C.R.; Patel, D.H.; Naeve, C.; Belt, J.A. J. Biol. Chem. **1998**, *273*, 5288–5293.
6. Burke, T.; Lee, S.; Ferguson, P.J.; Hammond, J.R. J. Pharmacol. Exp. Ther. **1998**, *286*, 1333–1340.
7. Yao, S.Y.; Ng, A.M.; Sundaram, M.; Cass, C.E.; Baldwin, S.A.; Young, J.D. Mol. Membr. Biol. **2001**, *18*, 161–167.
8. Yao, S.Y.; Ng, A.M.; Vickers, M.F.; Sundaram, M.; Cass, C.E.; Baldwin, S.A.; Young, J.D. J. Biol. Chem. **2002**, *277*, 24938–24948.

9. Guida, L.; Bruzzone, S.; Sturla, L.; Franco, L.; Zocchi, E.; De Flora, A. J. Biol. Chem. **2002**, *277*, 47097–47105.
10. Huang, M.; Wang, Y.; Collins, M.; Gu, J.J.; Mitchell, B.S.; Graves, L.M. J. Biol. Chem. **2002**, *277*, 28364–28367.
11. Huang, M.; Wang, Y.; Cogut, S.B.; Mitchell, B.S.; Graves, L.M. J. Pharmacol. Exp. Ther. **2003**, *304*, 753–760.
12. Huang, M.; Kozlowski, P.; Collins, M.; Wang, Y.; Haystead, T.A.; Graves, L.M. Mol. Pharmacol. **2002**, *61*, 569–577.
13. Huang, M.; Wang, Y.; Collins, M.; Mitchell, B.S.; Graves, L.M. Mol. Pharmacol. **2002**, *62*, 463–472.
14. Gharehbaghi, K.; Zhen, W.; Fritzer-Szekeres, M.; Szekeres, T.; Jayaram, H.N. Life Sci. **1999**, *64*, 103–112.
15. Park, J.I.; Choi, H.S.; Jeong, J.S.; Han, J.Y.; Kim, I.H. Cell Growth Differ. **2001**, *12*, 481–486.
16. Witt, O.; Sand, K.; Pekrun, A. Blood **2000**, *95*, 2391–2396.
17. Boleti, H.; Coe, I.R.; Baldwin, S.A.; Young, J.D.; Cass, C.E. Neuropharmacology **1997**, *36*, 1167–1179.
18. Sinclair, C.J.; LaRiviere, C.G.; Young, J.D.; Cass, C.E.; Baldwin, S.A.; Parkinson, F.E. J. Neurochem. **2000**, *75*, 1528–1538.