Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 16, pp. 1967-1970, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00353-8

PENTAMIDINE CONGENERS. 4. DNA BINDING AFFINITY AND ANTI-*PNEUMOCYSTIS CARINII* ACTIVITY OF BUTAMIDINE ANALOGUES

Isaac O. Donkor,[a]* Sherry F. Queener,[b] and James T. Dalton[a]

[a]*Department of Pharmaceutical Sciences, College of Pharmacy, The University of Tennessee, Memphis, TN 38163 and* [b]*Department of Pharmacology and Toxicology, Indiana University School of Medicine, IN 46202.*

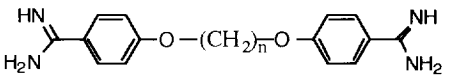
Abstract: A number of pentamidine-like compounds are known to bind to DNA. DNA could therefore be the molecular target for the anti-*P. carinii* activity of these compounds. We provide the first evidence demonstrating a correlation between in vitro anti-*P. carinii* activity and in vitro DNA binding affinity of pentamidine related aromatic dicationic compounds. Copyright © 1996 Elsevier Science Ltd

Pneumocystis carinii pneumonia (PCP) is one of the earliest clinical signs of progression from latent HIV infection to full-blown AIDS.[1] It is also a major cause of morbidity and mortality in AIDS patients.[2] Pentamidine (**1**), an aromatic diamidine, is one of the drugs of choice for treating PCP despite its toxicity.[3] Novel nontoxic compounds related to pentamidine could be developed based on the mechanism of action of the drug. However, the exact mechanism by which pentamidine achieves its anti-*P. carinii* activity is yet to be established. Results from X-ray crystallography,[4] molecular modeling,[5] NMR,[6] and footprinting[7] studies all converge to indicate that pentamidine and its analogues bind to the minor groove of double helix DNA and prefer AT rich sites. These studies suggest that DNA could be the molecular target for the anti-*P. carinii* activity of aromatic diamidines. However, in an attempt to establish the mechanism of action of pentamidine, we[8] and others[9] could not demonstrate any correlation between the in vivo anti-*P. carinii* activity of a series of pentamidine analogues and their in vitro DNA binding affinities. One possible explanation for this lack of correlation was that pharmacokinetics/metabolism of these compounds in the in vivo model could complicate evaluation of activity directly against the organism. One way of avoiding these complications was to use an in vitro model to assess activity of the compounds against *P. carinii*. Using compounds **3**-**6** (Chart 1) we present the first evidence demonstrating a correlation between in vitro DNA binding affinity and in vitro anti-*P. carinii* activity of aromatic dicationic compounds related to pentamidine.

Synthesis of compounds **3**-**6**, determination of DNA binding affinity, and evaluation as anti-PCP agents in a rat model of the disease were carried out as previously reported.[8] In vitro anti-*P. carinii* activity of the compounds were determined following reported procedures.[10] In the in vitro assay the compounds were tested at a concentration of 0.2 μM. DMSO (0.1%) and TMP/SMX (50/250 μg/mL) were used as negative and positive controls, respectively. Activity of each compound as a percentage of control was calculated using the following formula: 100 x (average number of organisms seen per 1,000 field in experimental wells on day 7/average number of organisms seen per 1,000 field in control wells on day 7). Thus, the smaller the value, the greater the anti-*P.*

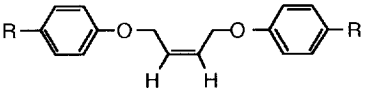
carinii action of the compound. For the in vivo studies a dose of 10 mg/kg/day of each test drug was administered by iv injection with saline and pentamidine serving as negative and positive controls respectively.[8] In this study,

Chart 1

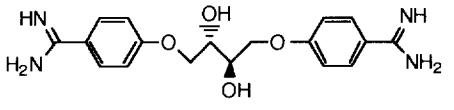


Pentamidine (**1**), n = 5

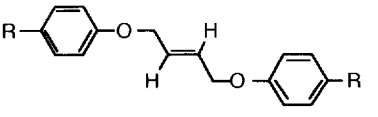
Butamidine (**2**), n = 4



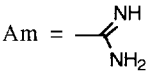
3, R = Am; **5**, R = Im

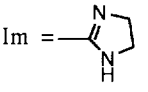


7



4, R = Am; **6**, R = Im





activity of each compound was computed as the number of cysts counted per gram of lung tissue expressed as a percentage of the number of cysts counted per gram of lung tissue of saline treated animals (saline control = 48.9 x 10^6 cysts per gram of lung tissue). Again, the smaller the value, the greater the anti-*P. carinii* action of the compound.

The cell culture studies (Table 1) revealed that pentamidine is more active than its alkyl bridge contracted homologue, butamidine. The in vitro anti-*P. carinii* activity of these two drugs correlated with their DNA binding affinities. However, pentamidine was significantly less active than butamidine in vivo. Additionally, a reverse correlation was observed for comparison of in vivo activity with DNA binding affinity for pentamidine and butamidine. Pentamidine has been reported to undergo extensive hepatic metabolism in vivo.[11] This could account for the lack of correlation between the in vitro and in vivo activity of the drug.

Introduction of a double bond at position two of the flexible butyl bridge of butamidine gave geometric isomers (**3** and **4**) that were more active than the parent drug. Generally the *cis*-isomers (**3** and **5**) were more potent anti-*P. carinii* agents and better binders to DNA than their *trans* counterparts (**4** and **6**), respectively. Additionally, amidines (**3** and **4**) were more potent and better binders to DNA than their respective imidazoline derivatives (**5** and **6**). Thus, the in vitro anti-*P. carinii* activity of these pentamidine related aromatic dicationic compounds correlated with their in vitro DNA binding affinities and followed the order: *cis*-amidine (**3**) > *trans*-amidine (**4**) and *cis*-imidazoline (**5**) > *trans*-imidazoline (**6**). This trend between DNA binding affinity and anti-*P. carinii* activity was not observed when the compounds were studied in vivo. In the rat model of PCP the geometric isomers were almost equipotent despite the observed differences in their DNA binding affinities and in vitro anti-*P. carinii* activities. We hypothesized that the equipotency of the geometric isomers in vivo could be due in part to their metabolism to a common bioactive metabolite, presumably the *trans* diol **7**. To test this hypothesis we performed in vitro hepatic metabolism studies of compounds **3** and **4** following reported procedures.[12] The

Table 1. In Vitro and In Vivo Activity of Semi-Rigid Congeners of Butamidine.

	Anti-*P. carinii* Activity		ΔT_m	
Compound	In vitro	In vivo[a]	CT [b]	AT [b]
Control	100 ± 0.62	100 ± 9.83	ND[c]	ND
TMP/SMX	17.0 ± 0.52	ND	ND	ND
Pentamidine (**1**)	27.0 ± 0.29	5.31 ± 1.48	10.0	22.9
Butamidine (**2**)	39.0 ± 0.69	0.73 ± 0.33	8.3	20.4
3	9.00 ± 0.22	1.10 ± 0.84	11.0	31.5
4	19.0 ± 0.29	0.67 ± 0.17	7.7	23.3
5	25.0 ± 0.26	1.22 ± 0.48	9.3	28.9
6	42.0 ± 0.97	1.22 ± 0.40	7.9	20.8
7	78.0 ± 0.68	ND	ND	ND

[a]The values were taken from reference 8. [b]CT is sonicated calf thymus DNA and AT is sonicated poly(dA).poly(dT) homopolymer. [c]ND = Not Determined.

compounds did not undergo any detectable metabolism using rat liver homogenates over a 4 h period. As control, identical liver homogenates metabolized 20 ± 5% of phenacetin to acetaminophen over the same 4 h period. Further, there was no nonenzymatic degradation of phenacetin by pre-boiled enzymes within the 4 h period, confirming the activity of the liver homogenates. These studies demonstrate that **3** and **4** do not undergo significant hepatic metabolism in the rat. We also synthesized *trans* diol **7** and studied its anti-*P. carinii* activity in vitro. Compound **7** was over eight times less active than **3** and four times less active than **4**. Clearly, **7** cannot be the bioactive metabolite of the geometric isomers. In the absences of metabolism of the geometric isomers to a common bioactive metabolite, we propose that stereoselective pharmacokinetics of the compounds could account for the observed differences in the in vitro and in vivo anti-*P. carinii* activity of the compounds. Studies are currently on going in our laboratory to address this issue. In summary, we have presented initial data demonstrating a correlation between DNA binding affinity and anti-*P. carinii* activity of aromatic dicationic compounds related to pentamidine. We are currently generating additional data to investigate if such a correlation is statistically significant and hence support the use of DNA as a template for designing novel anti-*P. carinii* agents for treating PCP in AIDS patients.

Acknowledgement

This work was supported in part by The University of Tennessee College of Pharmacy (IOD) and by local funding at Indiana University School of Medicine, Department of Pharmacology and Toxicology (SFQ) and the Department of Pathology and Laboratory Medicine where the in vitro tests were performed in the laboratory of Professor Marilyn Bartlett. The excellent technical assistance of Xiao-Tao Yao is also gratefully acknowledged.

References

1. Davey, R. T.; Jr., Masur, H. *Antimicrob. Agents Chemother* **1990**, *34*, 499.

2. Neidt, G. W.; Schinella, R. A. *Arch. Pathol. Lab. Med.* **1985**, *109*, 727.
3. Moskowitz, L.; Hensley, G. T.; Chan, J. C.; Adams, K. *Arch. Pathol. Lab. Med.* **1985**, *109*, 735.

4. Edwards, K. J.; Jenkins, T. C.; Neidle, S. *Biochemistry* **1992**, *31*, 7104.

5. Jenkins, T. C.; Lane, A. N.; Neidle, S.; Brown, D. G. *Eur. J. Biochem.* **1993**, *213*,1175.

6. Lane A. N.; Jenkins, T. C.; Brown, T.; Neidle, S. *Biochemistry* **1991**, *30*,1372.

7. Bailly C.; Donkor, I. O.; Gentle, D.; Thornalley, M.; Waring, M. J. *Molecular Pharmacol.* **1994**, *41*, 313.

8. Donkor, I. O.; Tidwell, R. R.; Jones, S. K. *J. Med. Chem.* **1994**, *37*, 4554.

9. Tidwell, R. R.; Jones, S. K.; Geratz, J. D.; Ohemeng, K. A.; Cory, M.; Hall, J. E. *J. Med. Chem.* **1990**, *33*, 1252.

10. Queener, S. F.; Dean, R. A.; Bartlett, M. S.; Milhous, W. K.; Berman, J. D.; Ellis, W. Y.; Smith, J. W. *J. Infect. Dis.* **1992**,*165*, 764.

11. Berger, B. J.; Naiman, N. A.; Hall, J. E.; Peggins, J.; Brewer, T. G.; Tidwell, R. R. *Antimicrob. Agents Chemother.* **1992**, *36*, 1825.

12. Yeh, T.-K.; Dalton, J. T.; Au, J. L.-S. *J. Chromatogr. Biomed. Appl.* **1993**, *622*, 255.

(*Received in USA* 10 *June* 1996; *accepted* 22 *July* 1996)