Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of atorvastatin esters as prodrugs metabolically activated by human carboxylesterases

Kenta Mizoi, Masato Takahashi, Masami Haba, Masakiyo Hosokawa *

Faculty of Pharmacy, Chiba Institute of Science, 15-8, Shiomi-cho, Choshi, Chiba 288-0025, Japan

ARTICLE INFO

Article history:
Received 21 October 2015
Revised 28 November 2015
Accepted 19 December 2015
Available online xxxx

Keywords:
Carboxylesterase
CES1
Atorvastatin
Prodrug
Metabolism

ABSTRACT

We synthesized 11 kinds of prodrug with an esterified carboxylic acid moiety of atorvastatin in moderate to high yields. We discovered that they underwent metabolic activation specifically by the human carboxylesterase 1 (CES1) isozyme. The results suggested that these ester compounds of atorvastatin have the potential to act as prodrugs in vivo.

Carboxylesterases (CESs) are members of an α,β-hydrolase-fold family and play important roles in biotransformation of ester- or amide-type prodrugs. CESs can be classified into five major groups denominated CES1–CES5, and the majority of CESs belong to the CES1 or CES2 group in various mammals.[1] Drug-metabolizing enzymes that are present predominantly in the liver are involved in the biotransformation of both endogenous and exogenous compounds to polar products to facilitate their elimination. These reactions are categorized into phase I and phase II reactions. CESs are categorized as phase I drug-metabolizing enzymes that can hydrolyze a variety of ester-containing drugs and prodrugs, such as antitumor drugs (CPT-11 and Capecitabin),[2–6] angiotensin-converting enzyme inhibitors (temocapril, cilazapril, quinapril, and imidapril),[7–10] and narcotics (cocaine, heroin, and meperidine).[11–13]

Atorvastatin (**1**) has activity for a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor (statin), and it is therefore used as a therapeutic agent for dyslipidemia.[14] The total synthesis of atorvastatin, the most frequently used statin in a clinical setting, has been reported and has been attracting attention.[15–18] Statins have a 3,5-dihydroxycarboxylic acid moiety in their active center, but that moiety causes a decrease in lipophilicity and prevents the absorption from the small intestine, and it is thus one factor decreasing bioavailability (BA). Atorvastatin has the lowest BA among statins.[19] If its BA is increased by improvement of absorbency, it should be possible to reduce its dose.

Therefore, we focused on atorvastatin and the syntheses of prodrugs that have the potential for improved BA. Here, we report on two points. First, various compounds were synthesized from esterification of the carboxylic acid moiety of atorvastatin. Second, these compounds were metabolized to atorvastatin by CESs.

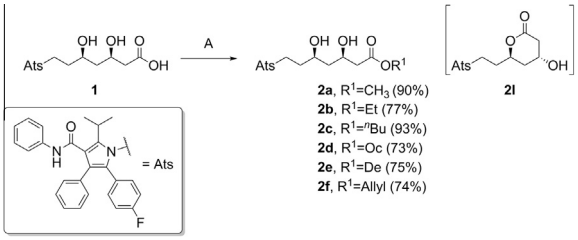
Primarily, we report on esterification of the carboxylic acid moiety of atorvastatin. We synthesized compounds (**2a**–**f**) using the Fischer esterification reaction.[20,21] As shown in Scheme 1, only straight-chain aliphatic primary alcohols were introduced into atorvastatin. When other kinds of alcohol were used, lactone (**2l**) was obtained as the major product because intramolecular condensation reaction with the 5-position of the hydroxyl group preferentially occurred. Therefore, we synthesized acetal-protected atorvastatin (**4**) from **2a** in two steps in order to suppress the generation of **2l**. In addition, a variety of alcohols and **4** were condensed by using EDC as a condensing agent.[20,22] Subsequently, the compounds (**2g**–**k**) were obtained by deprotecting the acetal group. The results are presented in Scheme 2. From the above results, 11 kinds of ester compounds (**2a**–**k**) were collected.

As mentioned in the introduction, CESs have the ability to hydrolyze the ester structure. Thus, as the second point, it was examined whether atorvastatin esters (**2a**–**k**) and lactone (**2l**) are subjected to hydrolysis by CESs. In this study, it was investigated

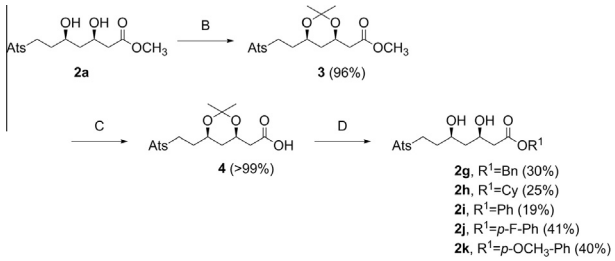
Abbreviations: DMP, 2,2-dimethoxypropane; TsOH, *p*-toluenesulfonic acid; THF, tetrahydrofuran; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide; DMAP, 4-dimethylaminopyridine.

* Corresponding author. Tel.: +81 479 30 4683; fax: +81 479 30 4683.
E-mail address: masakiyo@cis.ac.jp (M. Hosokawa).

http://dx.doi.org/10.1016/j.bmcl.2015.12.069
0960-894X/



Scheme 1. Esterification of atorvastatin. (a) Isolated yield. (b) Reagents and conditions: (A) R^1OH, H_2SO_4 (cat), rt.



Scheme 2. Synthesis of atorvastatin ester via an acetal—protected intermediate. (a) Isolated yield. (b) Reagents and conditions: (B) DMP, TsOH (cat), CH_2Cl_2, reflux. (C) 1 M NaOH aq, THF, rt. (D) (1) R^1OH, EDC, DMAP, CH_2Cl_2, rt. (2) 1 M HCl aq, MeOH, rt.

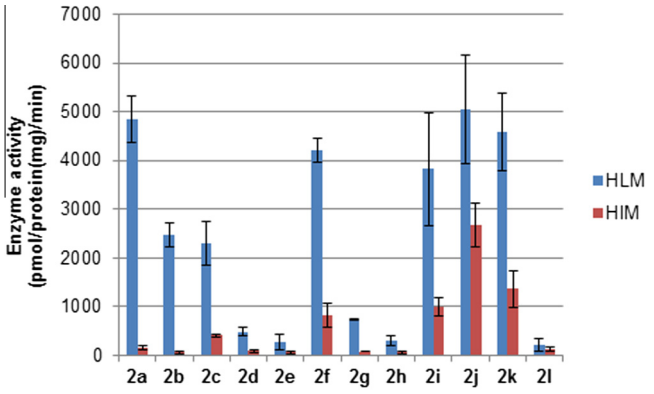


Figure 1. Enzyme activity assays of microsomes. Values are means ± S.D. (n = 3).

whether human liver microsomes (HLM) and/or human small intestine microsomes (HIM), tissues that express major CES isozymes, have hydrolytic activity. The results are summarized in Figure 1. The hydrolytic activity of primary linear aliphatic esters (**2a**–**e**) was reduced in accordance with a linear increase in HLM. It was presumed that this result was caused by steric repulsion of access to the active pocket or nucleophilic attack of water. Especially, despite a difference of one carbon atom, hydrolytic activity was significantly different compare with **2a** and **2b**. This result show that even the tiny difference of alcohol group in ester structure was recognized by CES1. This consideration is also supported by the fact that hydrolytic activity of **2g** was lower than that of **2f** for inversely correlated bulkiness despite the fact that there were nearly equal electronic properties of **2f** and **2g**. The hydrolytic activity of a secondary aliphatic ester (**2h**) was decreased in relation to bulkiness. Thus, we assume that these results, the biological hydrolysis reaction and the chemical hydrolysis reaction of esters, are correlated. We consider that high hydrolytic activity of the aromatic esters (**2i**–**k**) is natural because the phenoxide ion has higher ability than the alkoxide ion for elimination. Furthermore, these results are supported by the hydrolysis mechanism of CESs.[1] Hydrolysis reaction of CESs are involving the serine ester intermediate. This intermediate can be attacked by the phenoxide or alkoxide ion, instead of water, and then it proceed to the inverse reaction. Since phenoxide ion has a lower nucleophilic activity than alkoxide ion, the inverse reaction tends to not occur. Therefore, esters (**2i**–**k**) have a high hydrolytic activity despite having sterically–bulky aryl group in HLM and HIM. On the other hand, lactone (**2l**) was hardly hydrolyzed in either HLM or HIM.

In contrast, the hydrolytic activity of these esters (**2a**, **2c**) toward HIM was increased in relation to chain length. We have already reported that human CES1A was highly expressed in the liver and that human CES2A was highly expressed in the small intestine.[1] The substrate specificities of CES1 and CES2 are significantly different. CES1 isozymes mainly hydrolyze a substrate with a small alcohol group and large acyl group, whereas CES2 isozymes hydrolyze a substrate with a small acyl group and large alcohol group.[1,23] Our results for linear aliphatic esters are in agreement with the theory of substrate specificity of CESs. From the above results, since these ester compounds (**2a**–**k**) have hydrolytic activity in HLM, they may undergo metabolic activation in the CES1 isozyme.

Furthermore, the results presented in Table 1 show that atorvastatin ethylester (**2b**) has the highest hydrolyzing activity ratio of HLM/HIM. Hence, this compound has the best utility as a prodrug among these esters (**2a**–**l**) because this prodrug is required to be metabolized in the human liver after it has been absorbed in the human small intestine without being metabolized. We obtained new findings regarding the structure activity relationship for CESs, due to differences in the size and electron density of the ester moiety for atorvastatin.

However, there was no evidence that the ester compounds were activated by CESs because these metabolic reactions were performed in microsomes that contained various enzymes. Finally, we compared the hydrolyzing activities of several CESs including human CES1b, human CES1c, human CES2, and human arylacetamide deacetylase (AADAC). Esters **2a**, **2f**, and **2j**, which showed the highest activity among the aliphatic esters (**2a**–**e**, **2h**, **2l**), the allylic or benzylic esters (**2f**, **2g**), and the aromatic esters (**2i**–**k**), respectively, were used as a substrate. As shown in Figure 2, the metabolic activity of these esters (**2a**, **2f**, **2j**) was specific to CES1. This result corresponds to previously reported observations.[1,23] In addition, it has been reported that AADAC is expressed in the human liver and gastrointestinal tissues. The characteristic of AADAC is that it recognizes the substrates including an *N*-arylamide and/or ester with a small acyl group and large alcohol group.[23,24] Accordingly, it was appropriate result that these esters (**2a**, **2f**, **2j**) having a small alcohol group had low hydrolytic activity in the presence of AADAC. From these results, it is suggested that the hydrolysis experiment of esters (**2a**, **2f**, **2j**) in HLM were selectively hydrolyzed by CES1.

In conclusion, CESs have an important role in the metabolic activation of prodrugs. There is the possibility that the ester com-

Table 1
Hydrolyzing activity ratio of HLM/HIM[a]

Compound	R^1=	Ratio (HLM/HIM)	Compound	R^1=	Ratio (HLM/HIM)
2a	–CH_3	30.5	**2g**	–Bn	9.1
2b	–Et	40.1	**2h**	–Cy	4.9
2c	–^{n}Bu	5.7	**2i**	–Ph	3.8
2d	–Oc	5.3	**2j**	–*p*-F-Ph	1.9
2e	–De	4.4	**2k**	–*p*-OCH_3-Ph	3.4
2f	–Allyl	5.1	**2l**	Lactone	1.7

[a] The data were calculated from Figure 1.

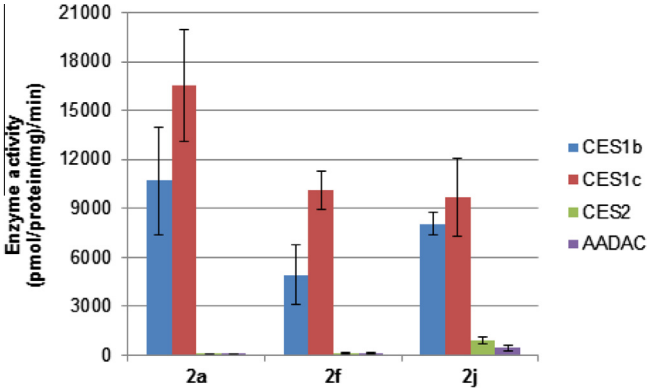


Figure 2. Enzyme activity assays of CESs. Values are means ± S.D. (n = 3).

pound of atorvastatin can be used as a prodrug. This possibility is supported by results showing that these compounds were specifically metabolized by CES1, which is mainly expressed in the human liver. Thus, atorvastatin esters can behave as prodrugs that have been hydrolyzed not by the human small intestine but by the human liver. The results of further studies should contribute to the design of ester prodrugs in various substrates that are highly metabolized in the human liver.

Acknowledgment

We thank the Human and Animal Bridging (HAB) Research Organization (Japan) for providing human livers.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.12.069.

References and notes

1. Hosokawa, M. *Molecules* **2008**, *13*, 412.
2. Sanghani, S. P.; Quinney, S. K.; Fredenburg, T. B.; Sun, Z.; Davis, W. I.; Murry, D. J.; Cummings, O. W.; Seitz, D. E.; Bosron, W. F. *Clin. Cancer Res.* **2003**, *9*, 4983.
3. Humerickhouse, R.; Lohrbach, K.; Li, L.; Bosron, W. F.; Dolan, M. E. *Cancer Res.* **2000**, *60*, 1189.
4. Kojima, A.; Hackett, N. R.; Ohwada, A.; Crystal, R. G. *J. Clin. Invest.* **1998**, *101*, 1789.
5. Satoh, T.; Hosokawa, M.; Atsumi, R.; Suzuki, W.; Hakusui, H.; Nagai, E. *Biol. Pharm. Bull.* **1994**, *17*, 662.
6. Tabata, T.; Katoh, M.; Tokudome, S.; Nakajima, M.; Yokoi, T. *Drug Metab. Dispos.* **2004**, *32*, 1103.
7. Geshi, E.; Kimura, T.; Yoshimura, M.; Suzuki, H.; Koba, S.; Sakai, T.; Saito, T.; Koga, A.; Muramatsu, M.; Katagiri, T. *Hypertens. Res.* **2005**, *28*, 719.
8. Furihata, T.; Hosokawa, M.; Koyano, N.; Nakamura, T.; Satoh, T.; Chiba, K. *Drug Metab. Dispos.* **2004**, *32*, 1170.
9. Mori, M.; Hosokawa, M.; Ogasawara, Y.; Tsukada, E.; Chiba, K. *FEBS Lett.* **1999**, *458*, 17.
10. Takai, S.; Matsuda, A.; Usami, Y.; Adachi, T.; Sugiyama, T.; Katagiri, Y.; Tatematsu, M.; Hirano, K. *Biol. Pharm. Bull.* **1997**, *20*, 869.
11. Brzezinski, M. R.; Spink, B. J.; Dean, R. A.; Berkman, C. E.; Cashman, J. R.; Bosron, W. F. *Drug Metab. Dispos.* **1997**, *25*, 1089.
12. Kamendulis, L. M.; Brzezinski, M. R.; Pindel, E. V.; Bosron, W. F.; Dean, R. A. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 713.
13. Zhang, J.; Burnell, J. C.; Dumaual, N.; Bosron, W. F. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 314.
14. Endo, A. *J. Lipid Res.* **1992**, *33*, 1569.
15. Chen, X.; Xiong, F.; Chen, W.; He, Q.; Chen, F. *J. Org. Chem.* **2014**, *79*, 2723.
16. Kawato, Y.; Chaudhary, S.; Kumagai, N.; Shibasaki, M. *Chem. Eur. J.* **2013**, *19*, 3802.
17. Andrushko, N.; Andrushko, V.; Tararov, V.; Korostylev, A.; König, G.; Börner, A. *Chirality* **2010**, *22*, 534.
18. George, S.; Sudalai, A. *Tetrahedron Lett.* **2007**, *48*, 8544.
19. Christians, U.; Jacobsen, W.; Floren, L. C. *Pharmacol. Ther.* **1998**, *80*, 1.
20. Smith, M. B.; March, J. *March's Advanced Organic Chemistry*, 6th ed.; Wiley: New York, 2007; pp 1414–1419.
21. McMurry, J. *Organic Chemistry*, 6th ed.; Thomson: Belmont, CA, 2004; pp 781–782.
22. Sheehan, J. C.; Cruickshank, P. A.; Boshart, G. L. *J. Org. Chem.* **1961**, *26*, 2525.
23. Fukami, T.; Yokoi, T. *Drug Metab. Pharmacokinet.* **2012**, *27*, 466.
24. Fukami, T.; Kariya, M.; Kurokawa, T.; Iida, A.; Nakajima, M. *Eur. J. Pharm. Sci.* **2015**, *78*, 47.