Communications to the Editor

Selective Plasma Hydrolysis of Glucocorticoid γ-Lactones and Cyclic Carbonates by the Enzyme Paraoxonase: An Ideal Plasma Inactivation Mechanism

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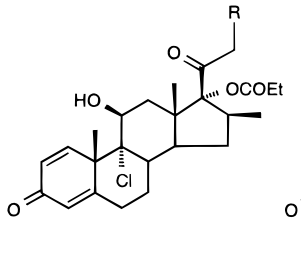
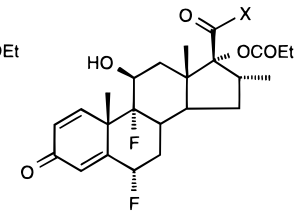
The terms "antedrug"[1] or "soft" drug[2] have been applied to compounds designed to exert their desired effect locally but which are inactivated in the circulation to reduce unwanted systemic effects. This inactivation may be achieved in the liver but the *ideal* antedrug would combine stability in the target tissue with very rapid inactivation in the blood.

The introduction in 1972 of the first inhaled corticosteroid beclomethasone dipropionate (BDP, **1a**) revolutionized asthma therapy.[3] Delivery of the compound topically to the lungs allowed the dose to be greatly reduced, thereby resulting in a dramatic reduction in the unwanted systemic effects associated with oral steroid treatment. Further reduction in systemic exposure has been achieved with the introduction of fluticasone propionate **2**[4] in which hepatic inactivation has been essentially optimized to give the inactive carboxylic



1a R = OCOEt
1b R = OH

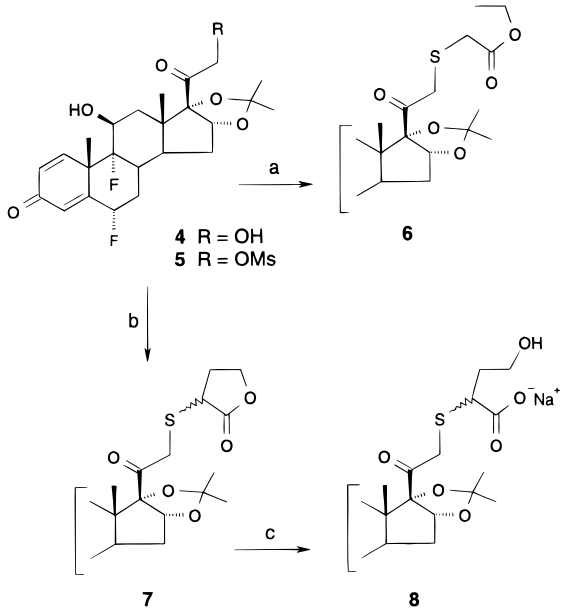


2 X = SCH_2F
3 X = OH

acid **3**. More recently, the search for even safer topical steroids has been directed toward *plasma labile* antedrugs with attention focused on carboxylic ester and thioester derivatives.[5] However, the ubiquitous tissue distribution of esterase activity makes it unlikely that such ester based antedrugs will deliver the ideal profile due to premature inactivation in the target tissue. In the case of inhaled steroids, this is most clearly illustrated by the diester prodrug BDP which is relatively stable in plasma but which is rapidly converted into the more active monopropionate BMP (**1b**) by esterases in the lung.[6]

In this communication we report preliminary results which indicate that incorporation of a γ-lactone or cyclic carbonate moiety onto the glucocorticoid nucleus provides compounds with the ideal antedrug profile as the result of a novel inactivation mechanism.

Scheme 1[a]



4 R = OH
5 R = OMs

6

7

8

[a] Reagents: (a) $HSCH_2CO_2Et$, iPr_2NEt, DMF, rt, 63%; (b) α-bromo-γ-butyrolactone, NaH, THF, 0 °C to rt, 51%; (c) NaOH, aq MeOH, rt, 88%.

While investigating 21-thio derivatives of fluocinolone acetonide **4** we discovered a remarkable difference in plasma lability between an acyclic ester and the corresponding γ-lactone. The ethyl ester **6**[7] and the analogous α-linked γ-lactone **7** were readily prepared by displacement of the 21-mesylate **5**[7] with the appropriate thiols (Scheme 1). Whereas the ester **6** showed some lability in human plasma at 37 °C ($t_{1/2}$ 24 min), the mixture of lactone diastereoisomers **7** was almost instantly hydrolyzed under the same conditions ($t_{1/2}$ < 1 min). The lactone mixture was essentially stable in a control incubation with Krebs buffer, indicating this facile plasma hydrolysis to be enzyme mediated. The metabolite formed in plasma was identified as the hydroxy-acid **8** by comparison with an authentic sample prepared by a simple base catalyzed hydrolysis of the lactone **7**.

The extreme plasma lability of the lactone diastereoisomers **7** compared with the corresponding ester **6**

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Table 1. Stability of Compounds **1a**, **6**, and **7** When Incubated with Human Plasma and Human Lung S9 at 37 °C[a]

compound	human plasma $t_{1/2}$ (min)	human lung S9 $t_{1/2}$ (min)
6	24	6
1a (BDP)	>120	10
7 isomer A	<1	>480
isomer B	<1	>480

[a] Full assay details given in Supporting Information.

suggested that the lactone hydrolysis is mediated by a different enzyme. This hypothesis was further supported by the remarkable observation that the lactone isomers **7** were **stable** on incubation with a human lung S9 preparation whereas the esters **6** and **1a** (BDP) were rapidly hydrolyzed under the same conditions (Table 1). Thus, despite their extreme lability in plasma the lactone isomers **7** appear stable to the esterase activity in the human lung.

Importantly, hydrolysis of the lactone moiety was shown to result in a marked inactivation. Thus, the separate diastereoisomers of the lactone **7** and of the hydroxy-acid **8** were obtained by preparative HPLC and their affinities for the human glucocorticoid (GC) receptor were compared (Table 2). The lactone diastereoisomers both show high affinity for the GC receptor whereas the corresponding hydroxy-acids show much lower affinity. The lactone isomers **7** therefore display the ideal lung selective antedrug profile of rapid inactivation in plasma combined with stability in the target tissue.

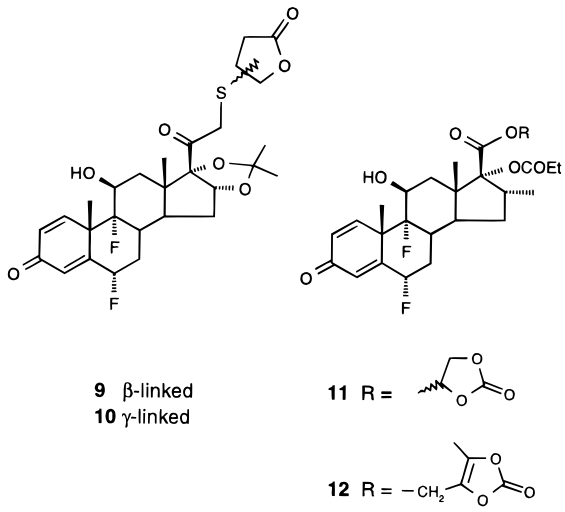
Table 2. Human Glucocorticoid Receptor Affinities of Compounds **7**, **8**, and FP **2**[a]

compound	IC_{50} (nM)
7 isomer A	3.4 ± 0.2
isomer B	6.6 ± 2.0
8 isomer A	194 ± 24
isomer B	827 ± 41
2 (FP)	0.5 ± 0.2

[a] Determined in competition studies with ^{3}H dexamethasone (n = 2, ±SEM). Full assay details in Supporting Information.

Preliminary investigation of the scope of this discovery has revealed similar plasma lability with compounds linked through the β- and γ-positions of the lactone moiety (e.g. **9**, $t_{1/2}$ ca. 2 min; **10**, $t_{1/2}$ ca. 10 min). Furthermore, these properties are not confined to lactones. Thus, both saturated (e.g. **11**) and unsaturated (e.g. **12**) cyclic carbonates have been shown to be rapidly hydrolyzed ($t_{1/2}$ ca. 2 min) in human plasma. In these cases, hydrolysis is accompanied by loss of the carbonate moiety to regenerate the inactive carboxylic acid **3**.[4]

The unique properties of these compounds prompted us to identify the enzyme responsible for this facile plasma hydrolysis. Human plasma was sequentially chromatographed on cibachrome blue, monoQ, hexyl-agarose, hydroxyl-apatite, and Superdex S-200 and then passed over wheatgerm and lentil lectin columns. The purified protein was separated by native gel electrophoresis followed by SDS-polyacrylamide gel electrophoresis and then digested with trypsin. Sequence analysis using nano-spray mass spectroscopy identified the protein as the known enzyme human serum paraoxonase (EC 3.1.8.1).[8] Further evidence that paraoxonase was the enzyme involved was obtained when the lactone



isomers **7** were shown to be essentially stable on incubation (37 °C, 1 h) with chicken plasma which is known to contain only very low levels of paraoxonase.[8]

Paraoxonase is so-called because of its ability to hydrolyze organophosphates including paraoxon but it also displays arylesterase activity. Importantly the enzyme is known to display genetic polymorphism which affects its hydrolytic activity but in a substrate dependent manner.[8] Thus, whereas paraoxon is hydrolyzed less rapidly by Pon A (192-Gln) than by Pon B (192-Arg), these two common polymorphs hydrolyze phenyl acetate at a similar rate. Reassuringly, no marked inter-individual variation was observed when the lactone **7** (isomer A) was incubated with plasma from 52 healthy individuals. Confirmation of the involvement of paraoxonase and further reassurance of the generality of this inactivation mechanism was obtained when the lactone isomers **7** and the cyclic carbonates **11** and **12** were shown to be rapidly hydrolyzed (**7** $t_{1/2}$ < 1 min; **11** and **12** $t_{1/2}$ ca. 5 min) by recombinant polymorphs Pon A and Pon B.[9]

Paraoxonase is of considerable current interest through its apparent involvement in the prevention of atheroma formation, although its precise role has still to be established.[10] Lactone hydrolysis by paraoxonase has not previously been reported. However, lactonase activity (EC 3.1.1.25) in human plasma was reported in 1966 by Fishbein[11] who partially purified the enzyme responsible for the plasma hydrolysis of simple aliphatic γ-lactones. Like paraoxonase, this enzyme was reported to be a calcium dependent hydrolase, distinct from esterase, and studies with the rat enzyme indicated this activity to be confined to plasma and liver. Paraoxonase has also been shown to be absent from many tissues,[12] and it seems likely, therefore, that these calcium dependent hydrolases are one and the same enzyme. Interestingly, a very recent publication from Kobayashi et al.[13] has highlighted significant sequence homology (ca. 25%) between paraoxonase and a fungal lactonase from *Fusarium oxysporum* AKU 3702.

In conclusion, a novel antedrug mechanism has been identified in which glucocorticoid γ-lactones and cyclic carbonates are extremely rapidly inactivated by the plasma enzyme paraoxonase. The absence of this enzyme activity from the lung and other tissues could open the way to even safer steroid treatment of asthma and

other local inflammatory diseases. Furthermore it is likely that this mechanism will be applicable to other drug series.[14]

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Supporting Information Available: Experimental details and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Lee, H. J.; You, Z.; Ko, D.-H.; McLean, H. M. Recent Advances in Prodrugs and Antedrugs. *Curr. Opin. Drug Discovery Dev.* **1998**, *1*, 235−244.

(2) Bodor, N.; Buchwald, P. Drug Targeting via Retrometabolic Approaches. *Pharmacol. Ther.* **1997**, *76*, 1−27.

(3) Barnes, P. J.; Pedersen, S.; Busse, W. W. Efficacy and Safety of Inhaled Corticosteroids. *Am. J. Respir. Crit. Care Med.* **1998**, *157*, S1−S53.

(4) Hogger, P.; Rohdewald, P.; McConnell, W.; Howarth, P.; Meibohm, B.; Mollmann, H.; Wagner, M.; Hochhaus, G.; Mollmann, A.; Derendorf, H.; Neil Johnson, F.; Barnes, N. C.; Hughes, J.; Williams, J.; Pearson, M. G. Fluticasone Propionate in Asthma. *Rev. Contemp. Pharmacother.* **1998**, *9*, 501−599.

(5) (a) Kwon, T.; Heiman, A. S.; Oriaku, E. T.; Yoon, K.; Lee, H. J. New Steroidal Antiinflammatory Antedrugs: Steroidal [16α,17α-*d*]-3′-Carbethoxyisoxazolines. *J. Med. Chem.* **1995**, *38*, 1048−1051 and references therein. (b) Druzgala, P.; Hochhaus, G.; Bodor, N. Soft Drugs-10. Blanching Activity and Receptor Binding Affinity of a New Type of Glucocorticoid: Loteprednol Etabonate. *J. Steroid Biochem. Mol. Biol.* **1991**, *38*, 149−154 and references therein. (c) Ueno, H.; Maruyama, A.; Miyake, M.; Nakao, E.; Nakao, K.; Umezu, K.; Nitta, I. Synthesis and Evaluation of Antiinflammatory Activities of a Series of Corticosteroid 17α-Esters Containing a Functional Group. *J. Med. Chem.* **1991**, *34*, 2468−2473. (d) Chanoine, F.; Grenot, C.; Heidmann, P.; Junien, J.-L. Pharmacokinetics of Butixocort 21-Propionate, Budesonide and Beclomethasone Dipropionate in the Rat after Intratracheal, Intravenous and Oral Treatments. *Drug Metab. Dispos.* **1991**, *19*, 546−553. (e) Moodley, I.; Grouhel, A.; Lelievre, V.; Junien, J.-L. Antiinflammatory Properties of Tixocortol 17-Butyrate, 21-Propionate (JO 1222), a Novel, Locally Acting Corticosteroid. *J. Lipid Mediators* **1991**, *3*, 51−70. (f) Milioni, C.; Jung, L.; Koch, B. Synthesis of New Local Antiinflammatory Thiosteroids Based on Antedrug Concept. *Eur. J. Med. Chem.* **1991**, *26*, 947−951.

(6) Wurthwein, G.; Rohdewald, P. Activation of Beclomethasone Dipropionate by Hydrolysis to Beclomethasone-17-Monopropionate. *Biopharm. Drug Dispos.* **1990**, *11*, 381−394.

(7) Mitsukuchi, M.; Ikemoto, T.; Taguchi, M.; Higuchi, S.; Abe, S.; Yasui, H.; Hatayama, K.; Sota, K. Studies on Topical Antiinflammatory Agents II. Synthesis and Vasoconstrictive Activity of 21-Substituted Corticosteroids with Sulphur-Containing Moieties. *Chem. Pharm. Bull.* **1989**, *37*, 1795−1801.

(8) Mackness, B.; Durrington, P. N.; Mackness, M. I. Human Serum Paraoxonase. *Gen. Pharmacol.* **1998**, *31*, 329−336.

(9) Sorenson, R. C.; Primo-Parmo, S. L.; Kuo, C.-L.; Adkin, S.; Lockridge, O.; La Du, B. N. Reconsideration of the Catalytic Centre and Mechanism of Mammalian Paraoxonase/Arylesterase. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7187−7191.

(10) Shih, D. M.; Gu, L.; Xia, Y.-R.; Naveb, M.; Li, W.-F.; Hama, S.; Castellani, L. W.; Furlong, C. E.; Costa, L. G.; Fogelman, A. M.; Lusis, A. J. Mice Lacking Serum Paraoxonase are Susceptible to Organophosphate Toxicity and Atherosclerosis. *Nature* **1998**, *394*, 284−287.

(11) Fishbein, W. N.; Bessman, S. P. Purification and Properties of an Enzyme in Human Blood and Rat Liver Microsomes Catalysing the Formation and Hydrolysis of γ-Lactones. *J. Biol. Chem.* **1966**, *241*, 4835−4841.

(12) Kelso, G. J.; Stuart, W. D.; Richter, R. J.; Furlong, C. E.; Jordon-Starck, T. C.; Harmony, J. A. K. Apolipoprotein J is Associated with Paraoxonase in Human Plasma. *Biochemistry* **1994**, *33*, 832−839.

(13) Kobayashi, M.; Shinohara, M.; Sakoh, C.; Kataoka, M.; Shimizu, S. Lactone-ring-cleaving Enzyme: Genetic Analyis, Novel RNA Editing and Evolutionary Implications. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12787−12792.

(14) During the preparation of this manuscript, paraoxonase was identified as the enzyme responsible for the metabolic activation of an unsaturated cyclic carbonate prodrug of the quinolone antibiotic Prulifloxacin. Tougou, K.; Nakamura, A.; Watanabe, S.; Okuyama, Y.; Morino, A. Paraoxonase has a Major Role in the Hydrolysis of Prulifloxacin (NM441), A Prodrug of a New Antibacterial Agent. *Drug Metab. Dispos.* **1998**, *26*, 355−359.

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