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Communications to the Editor

Macrolides with Gastrointestinal Motor Stimulating Activity

Sir:

Erythromycin (EM), discovered in 1953,[1] was the first clinically useful macrolide antibiotic. It is still widely used today as an effective agent against infections with Gram-positive bacteria, Gram-negative cocci, and mycoplasmas. Recently, it has been found that EM produces increased activity of the gastrointestinal tract resembling the normal interdigestive motor activity of the stomach and upper intestine.[2,3] We report in this paper that the gastrointestinal motor stimulating (GMS) activity in vivo of some of the EM derivatives obtained in our laboratories is 60–2890 times stronger than that of erythromycin A (EM-A) while the antibacterial activities are lost.

Physiological interdigestive motor activity of the stomach and upper bowel is characterized by intermittant bursts of contractions in the stomach, which migrate caudad along the small bowel to the terminal ileum.[4] Normally, these coordinated bursts of activity occur at intervals of about 100 min and are associated with increases in the plasma concentration of the peptide hormone motilin.[5] EM precipitates this interdigestive motor activity in dogs at doses lower than those used to treat infections.

The GMS activity is specific for EM and closely related 14-membered macrolides as may be clear from the fact that other 16-membered macrolide and 12-membered macrolide antibiotics, having a similar antibacterial spectrum to EM, have no, or very weak, GMS activity.[6] We also have found that when erythromycin is given intravenously in far smaller than therapeutic doses, namely, 100 μg/kg per h for 20 min, it exactly mimics the effect of exogenous motilin in the dog.[3]

On the basis of these findings, we conducted chemical modification of erythromycin in order to obtain derivatives having stronger GMS activity and no antibacterial activity in order to pursue the possibility of developing derivatives devoid of antibiotic activity which might be useful in modulating gastrointestinal disorders.

The GMS activity in vivo was measured by means of chronically implanted force transducers on the serosa of the gastrointestinal tract positioned to record circular muscle contraction in the gastric body, the gastric antrum, and the small intestine in fasted conscious dogs. Changes in contractile activity were recorded as contractile waves on a polygraph through amplifiers.[2] Test materials were administered as an intravenous bolus injection 10–15 min after the termination of the interdigestive contractions in the stomach. Quantitative comparisons of GMS activity among the macrolide antibiotics were made by integrating their contractile waves in the gastric antrum for 10 min from the time of injection. Relative potency was estimated for 2 × 2 parallel line assay using five animals at each dose. EM served as the reference standard in each assay.

In order to confirm GMS activity of the derivatives, in vitro studies were carried out by using muscle strips (5 × 20 mm) of the rabbit duodenum. Muscle strips were mounted along their longitudinal axes in organ baths containing 20 mL of Tyrode's solution kept at 37 °C and bubbled continuously with 5% CO_2 and 95% O_2. Isotonic contractions of strips were recorded by means of isotonic transducers, which were preloaded with 1.0 g. A test material was first dissolved in ethanol (1 mg/0.05 mL), and the same amount of lactobionate was added. Then, the solution was diluted to the required concentration with normal saline.

Among the erythromycin A (EM-A) derivatives such as acyl and sulfonyl derivatives, obtained by first stage chemical modification, 8,9-anhydroerythromycin A 6,9-hemiketal (**1**),[7] obtained by mild acid treatment of EM-A, showed GMS activity (in vivo) 10 times as strong as that of EM-A[8] (Table I, Figure 1). The *N*-ethyl analogue of **1**, de-*N*-methyl-*N*-ethyl-8,9-anhydroerythromycin A 6,9-hemiketal (**2**),[9] obtained by a series of treatments of EM-A with I_2/NaOAc,[10] AcOH,[7] and EtI/diisopropylethylamine, showed GMS activity 18 times as strong as that of EM-A. The methyl quarternary ammonium derivative of **1**, 8,9-anhydroerythromycin A 6,9-hemiketal methyl iodide (**3**),[11] proved to be 20 times more potent than EM-A and was devoid of antimicrobial activity (Table I). Additional

(1) Flynn, E. H.; Sigal, M. V., Jr.; Wiley, P. E.; Gerzon, K. *J. Am. Chem. Soc.* **1954**, *76*, 3121–3131.
(2) Itoh, Z., Suzuki, T.; Nakaya, M.; Inoue, M.; Mitsuhashi, S. *Antimicrob. Agents Chemother.* **1984**, *26*, 863–869.
(3) Itoh, Z.; Nakaya, M.; Suzuki, T.; Arai, H.; Wakabayashi, K. *Am. J. Physiol.* **1984**, *247*, G688–694.
(4) Itoh, Z.; Takeuchi, S.; Aizawa, I.; Takayanagi, R. *Am. J. Dig. Dis.* **1978**, *23*, 229–238.
(5) Itoh, Z.; Honda, R.; Hiwatashi, K.; Takeuchi, S.; Aizawa, I.; Takayanagi, R.; Couch, E. F. *Scand. J. Gastroenterol., Suppl.* **1976**, *11*(39), 93–110.
(6) Ōmura, S.; Tsuzuki, K.; Sunazuka, T.; Toyoda, H.; Takahashi, I.; Itoh, Z. *J. Antibiot.* **1985**, *38*, 1631–1632.
(7) Stephens, V. C.; Conine, J. W. *Antibiot. Annu.* **1958–1959**, 346–353.
(8) Ōmura, S.; Tsuzuki, K.; Sunazuka, T.; Takahashi, I.; Itoh, Z. *Abstracts of the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Minneapolis, 1985; p 302.
(9) All new compounds being reported have been fully characterized by ^1H and ^{13}C NMR and high-resolution mass spectroscopy.
(10) Freiberg, L. A. *Japan Kokai Patent* 47-9129, 1972.
(11) Wiley, P. F.; Gerzon, K.; Flynn, E. H.; Sigal, M. V., Jr.; Weaver, O.; Quarck, U. C.; Chauvette, R. R.; Monahan, R. *J. Am. Chem. Soc.* **1957**, *79*, 6062–6070.

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Table I. Antimicrobial Activity (MIC) and Gastrointestinal Motor Stimulating (GMS) Activity in the Dog (in Vivo) of Erythromycin and Its Derivatives

compd[a]	antibacterial activity: MIC,[b] μg/mL					GMS activity[c]
	SA	BS	BC	EC	KP	
EM-A	0.2	0.1	0.1	12.5	6.25	1
1	50	25	25	>100	>100	10
2	>100	>100	>100	>100	>100	18
3	>100	>100	>100	>100	>100	21
4	>100	>100	>100	>100	>100	111
5	100	>100	>100	>100	>100	256
6	100	100	100	>100	>100	2890
7	>100	>100	>100	>100	>100	65
8	100	100	100	>100	>100	115
9	>100	>100	>100	>100	>100	202

[a] The structures of the various compounds are shown in Figure 1. [b] Minimum inhibitory concentration (MIC) was estimated by agar dilution method. SA: *Staphylococcus aureus* ATCC 6538P. BS: *Bacillus subtilis* ATCC 6633. BC: *Bacillus cereus* IFO 3001. EC: *Escherichia coli* NIHJ. KP: *Klebsiella pneumoniae* ATCC 10031. [c] GMS activity in vivo was estimated by 2 × 2 points parallel line assay. The activity of EM-A[2] was assumed to be 1.0.

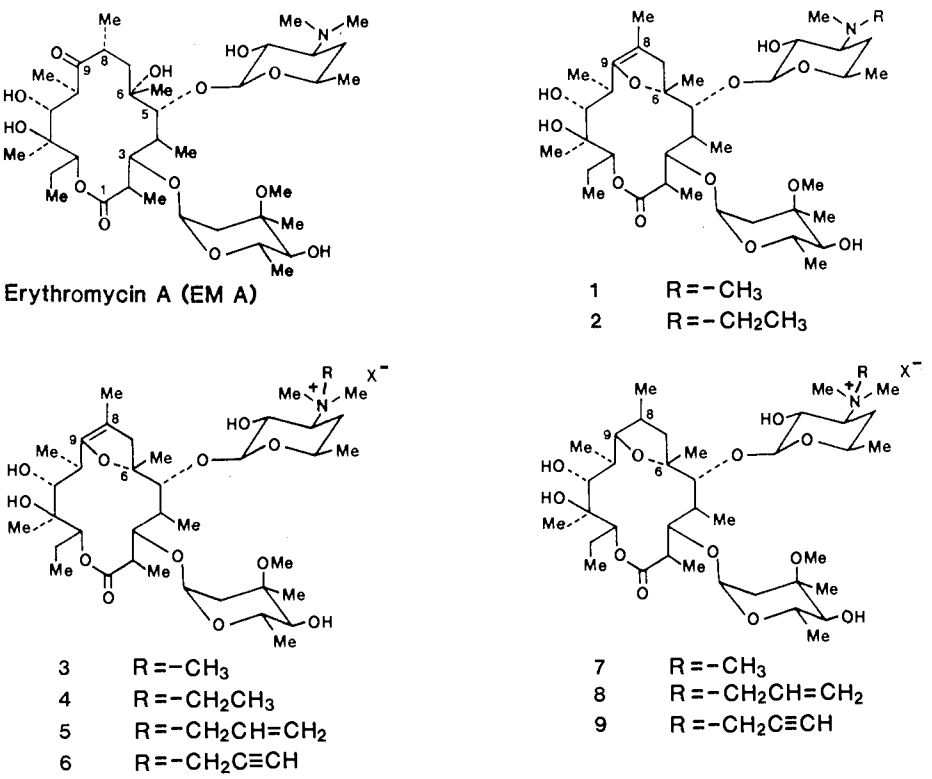

Figure 1. Structures of EM derivatives.

quarternary amines containing groups other than methyl were prepared. Three derivatives, 8,9-anhydroerythromycin A 6,9-hemiketal ethyl iodide (**4**),[9] 8,9-anhydroerythromycin A 6,9-hemiketal allyl bromide (**5**),[9] and 8,9-anhydroerythromycin A 6,9-hemiketal propargyl bromide (**6**),[9] were obtained by the treatment of EM-201 with the corresponding alkyl halides. Finally, stable dihydro derivatives, 9,9-dihydroerythromycin A 6,9-epoxide methyl iodide (**7**),[9] 9,9-dihydroerythromycin A 6,9-epoxide allyl bromide (**8**),[9] and 9,9-dihydroerythromycin A 6,9-epoxide propargyl bromide (**9**),[9] corresponding to **3**, **5**, and **6**, respectively, were obtained by the N-alkylation of 9,9-dihydroerythromycin A 6,9-epoxide.[12] The GMS activity of **6**, the most potent derivative, was found to be 2890 times as potent as that of EM-A. Most of the derivatives listed in Table I were remarkably active and were free of antibacterial activity.

When **6**, the most potent derivative, was given as an intravenous bolus dose of 0.1 μg/kg to conscious dogs, a series of strong contractions was induced in the gastric body and antrum and the contractions migrated along the small intestine in a caudad direction as shown in Figure 2. Although the GMS activity was different quantitatively

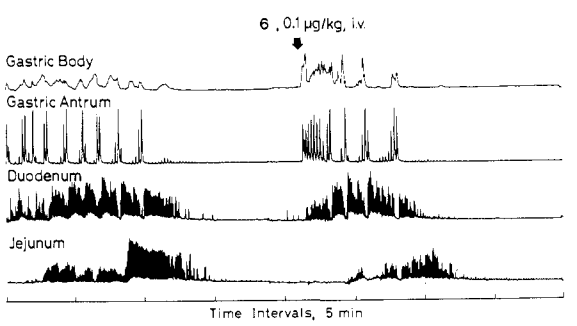

Figure 2. Effect of an intravenous bolus injection of **6** (0.1 μg/kg) at 10 min after the termination of the natural interdigestive contractions in a conscious dog. Compound **6** induced a series of strong contractions in the stomach, which migrated in a caudal direction along the small intestine. This contractile pattern is quite similar to the natural interdigestive contractions, which are seen on the left-hand side of this figure.

(12) Kurath, P.; Egan, R. S.; Jones, P. H. *Japan Kokai Patent* 47-1588, 1972.

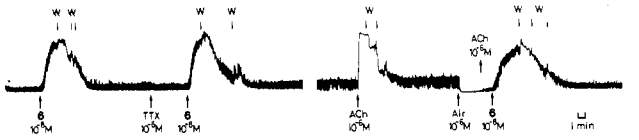

Figure 3. Comparison of contractile response of the isolated rabbit duodenum in a longitudinal axis to **6** and acetylcholine (ACh) in vitro. The contractile response induced by **6** (10^{-8} M) was phasic with a gradual tonal increase while that induced by ACh (10^{-6} M) was a rapid tonic contraction. The effect of **6** on smooth muscle contraction was not inhibited by the pretreatment of the muscle preparation with tetrodotoxin (TTX, 10^{-6} M) or atropine (Atr, 10^{-6} M). The minimum effective concentration of **6** measured in this system was found to be 10^{-9} M. W with arrows indicates repeated washing of preparation.

among the derivatives as shown in Table I, the qualitative characteristics of the contractile patterns induced by these derivatives were quite similar to each other; namely, all these derivatives induced a series of contractions in the gastrointestinal tract which were quite similar to the natural interdigestive contractions. The in vitro study, moreover, indicated that **6** caused contractions of the rabbit duodenum in a concentration of 10^{-9} M (the minimum effective concentration).[13] The contractile pattern induced by **6**, as shown in Figure 3, was quite different from that caused by acetylcholine, and the contractions produced by this compound were not blocked by pretreatment with tetrodotoxin (10^{-6} M) and atropine (10^{-6} M). The EM derivatives illustrated here may be useful to modulate the contractile activity in the gastrointestinal tract. Such agents may alone be useful tools to study the physiology and controlling mechanism of gastrointestinal motility.

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Registry No. 1, 33396-29-1; 2, 110205-60-2; 3 (X = I^-), 110205-61-3; 4 (X = I^-), 110205-62-4; 5 (X = Br^-), 110205-63-5; 6 (X = Br^-), 110205-64-6; 7 (X = I^-), 110205-65-7; 8 (X = Br^-), 110205-66-8; 9 (X = Br^-), 110205-67-9; EM-A, 114-07-8; 8,9-dihydroerythromycin A 6,9-epoxide, 42853-24-7.

Satoshi Ōmura,* Kazuo Tsuzuki, Toshiaki Sunazuka Shogo Marui, Hajime Toyoda

The Kitasato Institute and School of Pharmaceutical Sciences Kitasato University Minato-ku, Tokyo 108, Japan

Nobuhiro Inatomi, Zen Itoh

College of Medical Technology of Gunma University Maebashi 371, Japan

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Articles

Antimalarial Activity of 2-(Substituted amino)-4,6-bis(trichloromethyl)-1,3,5-triazines and *N*-(Chlorophenyl)-*N'*-[4-(substituted amino)-6-(trichloromethyl)-1,3,5-triazin-2-yl]guanidines[1,2]

Leslie M. Werbel,* Edward F. Elslager, Carolyn Hess, and Marland P. Hutt

Department of Chemistry, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105.
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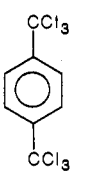
A series of 2-[[(dialkylamino)alkyl]amino]-4,6-bis(trichloromethyl)-1,3,5-triazines (III) and *N*-(4-chlorophenyl)-*N'*-[4-[[(dialkylamino)alkyl]amino]-6-(trichloromethyl)-1,3,5-triazin-2-yl]guanidines (IV) were prepared from 2,4,6-tris(trichloromethyl)-1,3,5-triazine and 2-chloro-4,6-bis(trichloromethyl)-1,3,5-triazine. Compounds of type III showed modest antimalarial activity while XIa with the camoquin side chain was more potent. Analogues of type IV broadly exhibited modest antimalarial activity.

The continuing problem of drug resistance in the successful treatment of malaria mandates further exploratory studies for novel structural classes that exhibit even moderate antimalarial activity.

The importance of the trichloromethyl group has been implicated in several instances in conferring antimalarial activity on a molecular species. Thus both aromatic and heterocyclic structures (I, II) have been shown to possess strong suppressive activity against the malaria parasite.[3-5]



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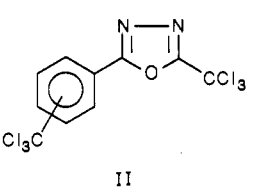

II

In the course of these investigations patents[6,7] came to our attention indicating that certain trichloromethyl-

(1) This is paper 64 of a series on antimalarial drugs. For paper 63, see: Werbel, L. M.; Degnan, M. J. *J. Med. Chem.* **1987**, *30*, 2151.

(2) This investigation was supported in part by U.S. Army Medical Research and Development Command Contract DA-49-193-MD-2754. This is Contribution No. 1815 to the U.S. Army Drug Development Program.

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(4) Elslager, E. F.; Hutt, M. P.; Werbel, L. M. *J. Med. Chem.* **1970**, *13*, 542.

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