

Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. \_\_\_\_\_ S. \_\_\_\_\_ D. \_\_\_\_\_

Ref: Expediente No. 00.093.154

Solicitud de patente titulada: "COMBINACIÓN FARMACÉUTICA ÚTIL PARA EL TRATAMIENTO DE LA MOVILIDAD GASTROINTESTINAL ALTERADA, SENSIBILIDAD DE SECRECIÓN, O DESÓRDENES ABDOMINALES".

Solicitante: NOVARTIS AG.

**RESPUESTA AL OFICIO No. 9400, NOTIFICADO POR FIJACIÓN EN LISTA No. 293,**  
**DE FECHA 24 DE AGOSTO DE 2006,**  
**(SEGUNDO EXAMEN DE FONDO)**

CARLOS R. OLARTE, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal previsto en el artículo 45 de la Decisión 486, en los siguientes términos:

1. a- En su concepto técnico No. 1303 del 24 de agosto de 2006, el Examinador argumenta que el alcance de la reivindicación 3 no es claro, ya que ésta hace referencia a la aptitud de la combinación farmacológica reivindicada para participar en el tratamiento terapéutico de reflujo gastro-esofágico, materia que no sería patentable en virtud del artículo 20(d) de la Decisión 486, por corresponder a un método de tratamiento.

b- El Señor examinador también argumenta en contra del nivel inventivo, con base en las divulgaciones hechas por:

| No. | Título                                                         | Autor y publicación                                                                                                                   |
|-----|----------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| D1  | Effect of gastric acid suppressants on human gastric motility. | Parkman HP, Urbain JL, Knight LC, Brown KL, Trate DM, Miller MA, Maurer AH, Fisher RS.<br>Department of Medicine and Nuclear Medicine |

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|    |                                                                           |                                                                                                                                                                                                    |
|----|---------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    |                                                                           | Section, Temple University School of Medicine, Gut.<br>1998 Feb;42(2):243-50.                                                                                                                      |
| D2 | SDZ HTF 919 stimulates<br>canine colonic motility<br>and transit in vivo. | Nguyen A, Camilleri M, Kost LJ, Metzger A, Sarr MG,<br>Hanson RB, Fett SL, Zinsmeister AR.<br>Gastroenterology Research Unit, Mayo Clinic and<br>Mayo Foundation, Rochester, Minnesota 55905, USA. |

El Examinador establece que dado que D1 enseña el efecto sobre la motilidad gastrointestinal de las sustancias supresoras de la liberación de ácidos gástricos (ranitidina, famotidina y omeprazol), y que D2 muestra el efecto de tegaserod sobre la motilidad intestinal, los efectos benéficos de estos dos medicamentos son bien conocidos en el estado de la técnica.

Continúa el Examinador argumentando que las anterioridades D1 y D2 establecen la posibilidad de formular omeprazol y tegaserod de forma separada para el tratamiento de disfunciones en la movilidad gastrointestinal. Así las cosas, la acción de combinar tegaserod con omeprazol para tratar el síndrome de movilidad intestinal es una derivación evidente del estado de la técnica, pues la actividad alcanzada que los fármacos ejercen por separado no es inesperada. Si bien la descripción de la solicitud muestra ejemplos de la asociación farmacológica reivindicada, no se enseñan resultados sorprendentes o inesperados, como por ejemplo, un efecto acumulativo, sinérgico, de adición o dualismo que justifique técnicamente la invención reclamada. Se requerirían por ejemplo los resultados comparados de parámetros farmacocinéticos al administrar la combinación farmacológica de manera concomitante.

Adicionalmente, el Examinador anota que la forma de administración de los fármacos es imprecisa en la solicitud, pues no es claro si éstos se suministran, por ejemplo, de forma independiente, secuencial o concomitante.

2. a- Atendiendo la recomendación del Examinador y con el ánimo de brindar mayor claridad a la materia reivindicada, me permito presentar un **Capítulo Reivindicatorio Modificado**, que reemplaza en su totalidad al Capítulo Reivindicatorio radicado junto con el memorial del 07 de julio 2005. Para tal efecto, adjunto un recibo de pago que cubre la tasa de modificación correspondiente.

En el Capítulo Reivindicatorio Modificado adjunto se **ELIMINARON las reivindicaciones 3 y 4**. Así entonces, éste Capítulo Reivindicatorio se dirige a la protección específica de composiciones

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farmacéuticas que contienen tegaserod o una sal farmacéuticamente aceptable del mismo y omeprazol o una sal o enantiomero farmacéuticamente aceptable del mismo. Igualmente, la reivindicación 1 fue modificada de tal manera que ahora se especifica que la combinación reivindicada corresponde a una combinación farmacéutica en una única forma de dosificación, lo que implica que los fármacos se encuentran mezclados y su administración es posible solamente de manera simultánea.

Valga anotar que la materia que protege este Capítulo Reivindicatorio Modificado de ninguna manera constituye una ampliación a la divulgación original de la presente solicitud, por el contrario, lo ahora reivindicado corresponde a una franca limitación del alcance inicialmente pretendido.

b- De otra parte, respetuosamente difiero de la posición y los argumentos expuestos por el Examinador respecto del nivel inventivo de la presente invención.

En primer lugar, llamo la atención del Examinador sobre el contenido de las publicaciones D1 y D2. D1 se limita a determinar los efectos de ranitidina, famotidina y omeprazol en la motilidad y el vaciado gástrico, encontrando que la supresión de la secreción de ácidos gástricos, con dosis terapéuticamente efectivas de antagonistas de los receptores de histamina H<sub>2</sub>, está directamente asociada con un retardo del vaciado gástrico pero con un incremento en la motilidad antral.<sup>1</sup> A su vez, D2 demuestra que el agonista parcial no benzamídico de 5HT<sub>4</sub>, tegaserod, estimula el tránsito de residuos sólidos en el colon y también, aparentemente, estimula el vaciado gástrico, mostrando además que la aplicación de altas dosis no son más eficaces que las dosis bajas.

Así las cosas, es evidente que aunque D1 y D2 se refieren al mismo campo técnico que trata la presente solicitud, estos documentos no anticipan de ninguna manera la materia aquí reivindicada, que corresponde específicamente a combinaciones de tegaserod y omeprazol. A pesar de que D1 y D2 hacen referencia de manera independiente al tegaserod y al omeprazol para el tratamiento de desórdenes gastrointestinales, en particular para estimular la motilidad gástrica, no hay sugerencia alguna en dichas referencias que conduzcan inequívocamente a una persona medianamente versada en la materia a combinar estos dos fármacos, tal como se encuentra en la materia del Capítulo Reivindicatorio, con el fin de buscar un incremento significativo de la movilidad gastrointestinal.

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<sup>1</sup> La cavidad antral es la parte terminal del estómago, que se cierra parcialmente durante la digestión mediante esfínteres que se encuentran en la pared estomacal.

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Igualmente, no hay indicación en el estado de la técnica representado por D1 y D2 que enseñe que la combinación reivindicada de tegaserod y omeprazol tendría un efecto mejorado en el tratamiento de desórdenes gastrointestinales, respecto de la utilización de dichos fármacos individualmente. En primer lugar, estos agentes actúan mediante diferentes mecanismos de acción, interviniendo sobre receptores diferentes, y el arte previo no sugiere que éstos puedan tener un efecto aditivo al administrarlos en combinación.

Para ilustrar al Examinador acerca de los efectos mejorados e inesperado que se derivan de la combinación que reivindica la presente solicitud, me permito anexar el borrador del artículo "PROTECTIVE EFFECT OF TEGASEROD AGAINST INDOMETHACIN INDUCED INJURY AND GASTRIC MUSCLE DISFUNCTION", que se encuentra pendiente por ser publicado y que se refiere al estudio del efecto de tegaserod y la combinación de tegaserod y omeprazol contra las lesiones en las mucosas inducidas por indometacina y la disfunción del músculo gástrico. Los datos mostrados por este estudio demuestran el efecto sinérgico que se produce por la administración de tegaserod y omeprazol en modelos animales con lesión gástrica.

El artículo adjunto proporciona evidencia suficiente del efecto sinérgico y los resultados benéficos sobre ulceraciones. Es claro que el tegaserod o el omeprazol individualmente producen una reducción de las úlceras, sin embargo, se observó sorpresivamente que aún es posible encontrar lesiones en ratas tratadas únicamente con omeprazol o tegaserod, mientras que en animales tratados con omeprazol en combinación con tegaserod, la actividad de la mieloperoxidasa (MPO)<sup>2</sup> se inhibe a niveles que resultan similares a la actividad natural determinada para los animales control (a los que no se les provocaron lesiones con indometacina). El artículo concluye claramente que el tegaserod puede ofrecer un efecto protector aditivo al ofrecido por el omeprazol individualmente, cuando ambos fármacos se administran en combinación, proporcionando una protección óptima a la mucosa gástrica.

en qué folio lo conduce?

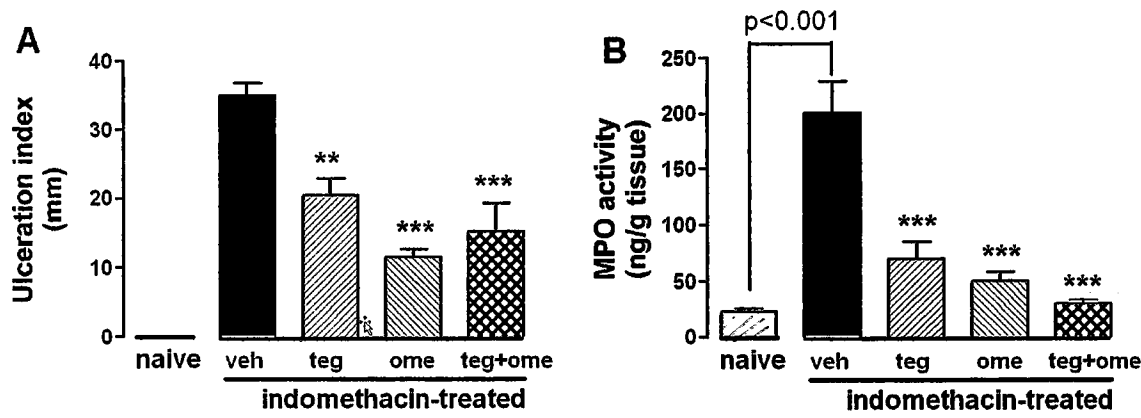
En primer lugar, los estudios demuestran que el tratamiento de ratas con tegaserod (teg), omeprazol (ome) o una combinación de ambos fármacos (teg+ome), reduce significativamente la formación de lesiones gástricas, en comparación con ratas tratadas únicamente con el vehículo (veh). Sin embargo, aunque hay una reducción importante del índice de ulceración, las lesiones hemorrágicas aun están presentes en animales que reciben solamente omeprazol o tegaserod; en contraste, la combinación de tegaserod con omeprazol inhibieron la actividad de la MPO de una

inhibió

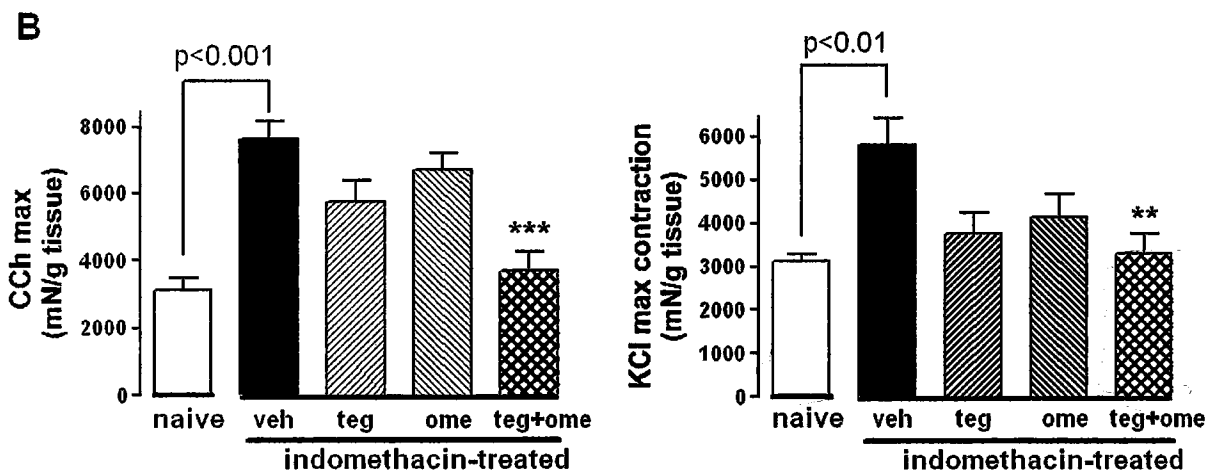
<sup>2</sup> La MPO es un marcador de la infiltración de neutrófilos e indica inflamación y daño gástrico.

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manera significativa hasta alcanzar niveles que son comparables a los naturales en animales no tratados (naive), esto es, a los que no se les provocaron úlceras con indometacina.



De la misma forma, la siguiente gráfica enseña que las contracciones musculares inducidas por exposición a KCl son mayores en animales tratados solamente con tegaserod (teg) u omeprazol (ome); los niveles de contracciones alcanzados en animales tratados con la combinación tegaserod-omeprazol (teg-ome) fueron similares a los observados en ratas que no fueron expuestas a indometacina para producir lesiones gástricas. En conclusión, solamente con la combinación tegaserod-omeprazol se alcanza una disminución estadísticamente significativa de las contracciones inducidas por KCl. Es importante resaltar que este ensayo demuestra la conveniencia de la administración de la combinación reivindicada, que proporciona un efecto sinérgico en la disminución de la contractibilidad, ya que la contractibilidad anormal del músculo liso en el estómago puede producir disfunción de la musculatura gástrica, resultando en motilidad anormal que contribuye al desarrollo de lesiones de la mucosa gástrica cuando hay exposición a agentes ulcerosos. Resultados similares se observaron cuando los tejidos se expusieron a carbacol (CCh), que es un agonista de los receptores muscarínicos.



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Los anteriores ejemplos de los efectos farmacológicos en la protección de lesiones en la mucosa gástrica, son el resultado de la combinación inventiva de tegaserod y omeprazol, cuya asociación en ningún momento fue siquiera sugerida por el arte previo y cuya actividad sinérgica no hubiera podido ser derivada de ninguna manera de las propiedades individuales de estos fármacos individualmente, ni anticipada a partir de las enseñanzas del estado de la técnica citado por el Examinador, pues una persona medianamente versada en la materia no hubiera podido predecir que dicha combinación sería altamente efectiva en la reducción de las ulceraciones e inflamación causadas por indometacina, atenuando la hipercontractibilidad inducida del músculo liso. Por el contrario, el tratamiento individual con omeprazol o tegaserod es efectivo en la corrección de algunas pero no de todas las anormalidades inducidas en un nivel significativo, como lo resumo en los resultados de la siguiente tabla:

| Anormalidades inducidas por exposición a indometacina: | Prevención por tratamiento con |           |           |                       |
|--------------------------------------------------------|--------------------------------|-----------|-----------|-----------------------|
|                                                        | Vehículo                       | Tegaserod | Omeprazol | Omeprazol + tegaserod |
| Ulceración gástrica                                    | -                              | +         | +         | +                     |
| Inflamación (MPO)                                      | -                              | +         | +         | +                     |
| Hipercontractibilidad por KCl                          | -                              | +         | -         | +                     |
| Hipercontractibilidad por carbacol                     | -                              | +         | -         | +                     |
| Hipercontractibilidad por EFS                          | -                              | ⊕ ?       | +         | +                     |

3. En conclusión, el nivel inventivo de la presente invención no se ve afectado por las anterioridades citadas por el Señor Examinador. Como ya se dijo anteriormente, la materia comprendida en D1 y D2 no anticipa o siquiera sugiere el objeto de la presente solicitud, dado que en ningún momento se refiere a la posibilidad de combinar tegaserod con omeprazol y aún menos a las ventajas y resultados inesperados de tipo sinérgico obtenidos con esta invención.

Es importante recordar que para juzgar si una invención se deriva evidentemente del estado de la técnica, el Examinador tiene la carga de probar que la invención carece de nivel inventivo, y no puede solo limitarse a establecer las diferencias o similitudes entre la solicitud y dicho estado de la técnica.<sup>3</sup> En el presente caso, no basta con citar la simple existencia de fármacos y sus propiedades individuales, debe denotarse una sugerencia clara que indique a una persona medianamente versada en la materia una expectativa de éxito fundada que conlleve

<sup>3</sup> MANUAL PARA EL EXAMEN DE SOLICITUDES DE PATENTES DE INVENCION. Numeral 10.1.

inevitablemente a las combinaciones que reivindica la presente invención y los resultados obtenidos.

En el análisis del nivel inventivo de una solicitud de patente de invención no es aceptable hacer referencia a elementos aislados del estado de la técnica, como si de armar una colcha de retazos se tratara. Si ese fuese el criterio de nivel inventivo nada sería patentable ya que, como lo ha reconocido el Tribunal Andino de Justicia, nada se inventa de la nada.<sup>4</sup> Por el contrario, para que pueda desvirtuarse la altura inventiva de un invento (carga procesal que recae sobre ese Despacho), el Examinador debe poder citar anterioridades que específicamente motiven a una persona versada en el arte a combinar los elementos del estado de la técnica para llegar a la solución inventiva reclamada. No es suficiente alegar que la persona versada podría llegar, puesto que, como se ha dicho, este criterio desdibujaría por completo el sistema de patentes, elevando el requisito de nivel inventivo a tal punto que nada sería patentable.

El hecho de implementar herramientas conocidas en el estado de la técnica no desvirtúa automáticamente el nivel inventivo de una invención. En efecto, dado que es necesario valerse de las herramientas existentes en la técnica para así poder seguir avanzando en los desarrollos tecnológicos, el Tribunal de Justicia Andino, con relación a nivel inventivo en el área de los inventos químicos y biotecnológicos, ha manifestado que frecuentemente **“aplicando procedimientos conocidos pueden obtenerse resultados inesperados para una persona normalmente versada en la materia”**, pero que **“por ello, el juicio del nivel inventivo no puede ser elaborado con criterios generales, sino que dependerá de las especiales circunstancias de cada caso”**.<sup>5</sup>

En conclusión, es claro que las anterioridades citadas por el Señor Examinador no brindan enseñanzas o sugerencias claras que forzosamente condujeran a una persona medianamente versada en la materia al desarrollo de las combinaciones que reivindica la presente solicitud. Por lo tanto, el nivel inventivo de la presente invención no se ve afectado por el arte previo citado.

4. Por todo lo anterior, considero que las objeciones presentadas por el Examinador han sido resueltas y por consiguiente solicito respetuosamente que se proceda con la concesión del privilegio de patente, siendo que como se demostró anteriormente, la presente solicitud cumple a cabalidad con los requisitos impuestos por la Decisión 486, particularmente en cuanto a nivel

<sup>4</sup> Proceso 21-IP-2000, página 8, línea 8.

<sup>5</sup> Proceso 105-IP-2000, página 7, líneas 45 a 50.

inventivo se refiere. Adicionalmente, si el Examinador considera que surgen nuevas objeciones o que algunas continúan pendientes, le ruego emita un tercer examen con el fin de permitir al solicitante proporcionar mayores aclaraciones.

5. Para terminar, si bien es cierto que las concesiones de patentes en oficinas de otros países no son vinculantes en el sentido que obliguen a la Superintendencia a tomar una decisión en el mismo sentido, sí considero que dichas concesiones sugieren el cumplimiento de los requisitos de patentabilidad de la materia reivindicada, ya que los criterios de evaluación y estudio aplicados para determinar la novedad y nivel inventivo resultan equivalentes a los que la Superintendencia debe emplear. Tan cierto es lo anterior, que el Manual Andino de Patentes, al comentar el Artículo 46 de la Decisión 486, establece que una Oficina nacional competente podrá reconocer los resultados de exámenes de novedad o de patentabilidad efectuados respecto a la solicitud extranjera equivalente, como suficientes para acreditar el cumplimiento de las condiciones de patentabilidad.<sup>6</sup> En el mismo sentido, es importante anotar que el mismo Tribunal Andino de Justicia ha citado a la jurisprudencia de las Cámaras de Apelación de Oficina Europea de Patentes como antecedentes aplicables a la Decisión 486.<sup>7</sup>

Así las cosas, deseo poner bajo su conocimiento el hecho de que las siguientes patentes relacionadas con la presente solicitud fueron otorgadas en diversas legislaciones:

| País               | Número Patente |
|--------------------|----------------|
| Perú               | 4147           |
| New Zealand        | 531489         |
| European Procedure | 1286668        |

<sup>6</sup> Manual Andino de Patentes. Sección 7.3.

<sup>7</sup> Ver por ejemplo:

- Proceso 193-IP-2005, página 12, donde se cita que “el Tribunal a manera de orientación ha tomado como referencia criterios de las Cámaras de Recursos de la Oficina Europea de Patentes (OEP)” para el análisis de la falta de novedad.
- Proceso No. 216-IP-2003, página 11, donde se menciona a manera de referencia la interpretación y el alcance que dan las directivas de la Oficina Europea de Patentes al término terapia.
- Proceso No. 118-IP-2005, página 15, donde el Tribunal cita a manera de referencia que “a partir de la “decisión adoptada en el caso T-506/95, las Cámaras han establecido que el estado de la técnica más próximo es aquel que...”.
- Proceso 69-IP-2005, página 12, donde el Tribunal explica que “para analizar la existencia de nivel inventivo, las Cámaras de Recursos han adoptado el método llamado de “acercamiento problema-solución” (problem and solution approach)”.
- Proceso No. 101-IP-2005, página 9, donde el Tribunal cita “a título de referencia, el criterio de las Cámaras de Recursos, según el cual los manuales u obras básicas deben formar parte de los conocimientos generales del experto medio”.

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## Abstract

Treatment with non-steroidal anti-inflammatory drugs (NSAIDs) is associated with gastric mucosal injury and often requires concurrent gastroprotective therapy. Proton pump inhibitors (PPI) suppressing gastric acid secretion are used to avoid the complications. However, gastric muscle hypercontractility also contributes to the NSAIDs-induced injury and drugs which promote normal gastric motility may have a beneficial effect. We investigated whether tegaserod, a partial 5-HT<sub>4</sub> receptor agonist and inhibitor of 5-HT<sub>1B</sub> and 5-HT<sub>2B</sub> receptors, applied alone or in combination with a PPI, has a protective effect against mucosal injury and gastric muscle dysfunction induced by indomethacin. Rats were treated with tegaserod (1 mg/kg), omeprazole (20 mg/kg), a combination of both drugs or the vehicle one day prior to the experiment and 30-min prior to intragastric infusion of indomethacin (50 mg/kg). Animals were euthanized 6 h post-indomethacin and gastric ulceration and inflammation were evaluated. Circular muscle strips from the gastric antrum of indomethacin-treated rats receiving vehicle showed increased contractile responses to KCl, carbachol, 5-HT and electrical field stimulation (EFS, 1-15 Hz). Pretreatment with tegaserod and omeprazole applied individually or in combination, reduced gastric ulceration and mucosal inflammation. Indomethacin-induced hypercontractility was unaffected by omeprazole, while it was significantly attenuated by tegaserod in the presence or absence of omeprazole. The protective effect of tegaserod appears to result mainly from improvement of gastric muscle contractility via stimulation of 5-HT<sub>4</sub> receptors and/or potential antagonism of 5-HT<sub>1B</sub> and 5-HT<sub>2B</sub> receptors. Thus tegaserod may offer an additive protective effect to that of omeprazole when both drugs are administered in combination.

## Introduction

NSAIDs are commonly used to treat pain and inflammation despite a recognized risk of developing major upper gastrointestinal complications. Dyspepsia occurs in up to 60% and ulcers are found by endoscopy in 15-30% of patients regularly using NSAIDs (Laine, 2004). A history of ulcer disease, high doses or use of multiple NSAIDs and advanced age are considered risk factors for the development of NSAID-related gastric injury (Peng and Duggan, 2005). To avoid such complications, patients with one or more risk factors usually receive concurrent gastroprotective therapy. Several options for pharmacological protection of the upper gastrointestinal tract against NSAID injury have been used with various rates of success. Clinical studies showed that histamine-2 ( $H_2$ ) receptor antagonists promote the healing of gastric and duodenal ulcers after discontinuation of the NSAID. However, ulcer prevention requires high doses of  $H_2$  antagonist and there is a relatively poor protective effect against NSAID-induced gastric ulcers (Lazzaroni and Bianchi Porro, 1999; Agrawal, 1995). Mucosal prostaglandin replacement by treatment with misoprostol offers an effective method to prevent gastric and duodenal ulcers associated with long-term NSAIDS therapy (Raskin et al., 1995). However, misoprostol treatment can itself produce adverse symptoms, has a risk of inducing abortion if taken early during pregnancy, has no efficacy for treatment of established mucosal lesions and shows poor protection from or reduction in severity of NSAID-associated dyspeptic symptoms (La Corte et al., 1996).

To date, the maximal protective effects are found clinically with proton pump-inhibitors (PPI) when prescribed as co-therapy with the NSAID. Omeprazole (20 mg/day) has been

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shown to prevent recurrence of ulcer bleeding in high-risk patients (Hawkey et al., 1998; Chan et al., 2005). At present concomitant gastroprotective therapy with omeprazole or other PPIs is a common strategy used to reduce the risk of developing adverse upper gastrointestinal symptoms and ulceration or bleeding in patients using NSAIDs. The mechanism responsible for the protective effect of PPIs is the effective suppression of gastric acid release, which leads to a less injurious environment at the gastroduodenal mucosal surface. However, a breakdown in the mucosal barrier against gastric acid is not the only mechanism contributing to mucosal injury during NSAIDs administration.

Abnormal smooth muscle contractility induced by inhibition of prostaglandin synthesis can produce dysfunction of gastric muscle and the resulting abnormal motility has been shown to contribute to the development of gastric mucosal injury (Takeuchi et al., 2004). Moreover, omeprazole itself has been shown to cause a delay in gastric emptying in healthy subjects (Rasmussen et al., 1991; Rasmussen et al., 1999; Benini et al., 1996; Parkman et al., 1998; Tougas et al., 2005). Thus, the multiplicity of mechanisms involved in NSAIDs-induced gastric injury raises an important question of whether treatment with a prokinetic such as tegaserod may itself have a protective effect. In addition, when administered with a PPI, the promotility drug could have an additive effect providing optimal mucosal protection.

The overall goal of our study was to investigate whether tegaserod, which is a potent partial agonist of peripheral 5-HT<sub>4</sub> receptors (Camilleri, 2001) and antagonist of 5-HT<sub>1B</sub> and 5-HT<sub>2B</sub> receptors (Beattie et al., 2004, Weber et al., 2006) would have a protective effect against NSAID-induced gastric mucosal injury and muscle dysfunction when

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equilibration. Optimal tension was adjusted by loading the preparation and examining the contractile response to 80 mM KCl. The experimental protocols were designed to characterize indomethacin-induced changes in smooth muscle contractility and the ability of tegaserod, omeprazole and the combination to prevent smooth muscle dysfunction. Experiments were performed to investigate: contractile responses induced by receptor-independent KCl depolarization; concentration-response curves to carbachol or 5-HT due to activation of smooth muscle muscarinic receptors or 5-HT receptors; contractions induced by electrical field stimulation (EFS) of enteric nerves regulating gastric contractility. A pair of platinum electrodes positioned parallel to the muscle strip and a Grass S88 stimulator (Grass Institute Division, W. Warwick, RI) were used to deliver 10-s trains of rectangular pulses with 0.5 ms pulse-duration applied at increasing frequencies of 1, 5, 10 and 15 Hz. Tetrodotoxin (1  $\mu$ M) was added to the bathing solution to verify that the responses to EFS are neurally mediated. At the end of the experiment the muscle strips were blotted dry and weighed.

## 2.7. Solutions and drugs

The Krebs buffer was of the following composition (mM): NaCl 120, KCl 6, MgCl<sub>2</sub> 1.2, NaH<sub>2</sub>PO<sub>4</sub> 1.2, CaCl<sub>2</sub> 2.5, NaHCO<sub>3</sub> 14.4 and glucose 11.5 (pH 7.3-7.4 when aerated with 95%O<sub>2</sub> and 5% CO<sub>2</sub>). Tegaserod maleate was supplied by Novartis Pharmaceuticals (East Hanover, NJ). Omeprazole, indomethacin, carbamylcholine chloride, atropine sulfate, 5-hydroxytryptamine creatinine sulfate and tetrodotoxin were obtained from Sigma (St. Louis, MO) and were dissolved in distilled water. All drugs were added to the baths in volumes less than 1% of the total bath volume.

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receiving only omeprazole or only tegaserod treatment. In contrast, pretreatment with the combination of omeprazole and tegaserod inhibited MPO activity to levels that were not significantly different from those observed in naïve animals. No significant differences in the level of MPO activity were found between the groups pretreated with omeprazole or tegaserod administered alone or in combination.

### **3.2. Protective effects against indomethacin-induced gastric muscle dysfunction**

**3.2.1. Hypercontractility in response to KCl.** The spontaneous contractile activity of the antral smooth muscle was characterized by low basal tone and phasic contractions developing at a frequency of 4-6 per minute. Exposure to high KCl induced a tonic contraction in all circular muscle strips isolated from the gastric antrum. However, the maximal amplitude of KCl-induced contractions was significantly higher ( $p < 0.01$ ) in the strips isolated from indomethacin-treated rats undergoing pretreatment with the vehicle compared to naïve rats. Tegaserod and omeprazole administered individually showed a tendency to prevent the development of hypercontractile responses to KCl, but the suppressive effect of neither drug reached statistical significance when compared to the vehicle. In summary, only when tegaserod and omeprazole were administered in combination was the amplitude of KCl-induced contractions significantly lower compared to the responses observed in vehicle-treated animals (Figure 2).

**3.2.2. Hypercontractility in response to carbachol.** Circular muscle strips isolated from the gastric antrum of both indomethacin-treated and naïve rats responded to increasing concentrations of carbachol (1 nM-10 mM) added to the bathing solution with concentration-

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dependent contractions. Comparison of the carbachol concentration-response curves showed a significant increase in the amplitude of the contractile responses in muscle strips from indomethacin-treated rats which received vehicle compared to untreated naïve rats (Figure 3 A). The increased reactivity of antral muscle appeared without a significant shift in sensitivity to the muscarinic receptor agonist and similar  $EC_{50}$  values were observed in naïve and indomethacin-treated rats (naïve rats:  $\log EC_{50} = 6.95$ , 95% CL -7.38 to -6.51, Hill slope 1.07 vs. vehicle pretreated rats receiving indomethacin:  $\log EC_{50} = 7.32$ , 95% CL -7.62 to -7.01, Hill slope 1.11). The effects of tegaserod or omeprazole administered individually were not significantly different from the effect observed with vehicle treatment alone. In contrast, pretreatment of the rats with the combination of tegaserod plus omeprazole resulted in a significant attenuation of maximal carbachol-induced muscle contraction compared to the effect of the vehicle (Figure 3 B).

**Hypercontractile responses to 5-HT.** Antral smooth muscle strips from naïve or indomethacin-treated rats responded to addition of 5-HT to the bathing solution with concentration-dependent contractions. The amplitude of contractile responses in muscle strips from naïve rats was significantly ( $p < 0.05$ ) lower compared to the responses in strips from indomethacin-treated rats receiving vehicle. However, there was no significant shift in the concentration response curve to 5-HT ( $\log EC_{50} = 6.299$ , 95% CL -7.097 to -5.776, Hill slope 0.48 in naïve rats vs.  $\log EC_{50} = 6.839$ , 95% CL -8.028 to -5.805, Hill slope 0.65) (Figure 4 A). Pretreatment of the rats with tegaserod alone or a combination of tegaserod and omeprazole caused a significant reduction in the amplitude of the maximal response to 5-HT compared to the effect of the vehicle (Figure 4B). In

comparison, the contractile response in strips from omeprazole-only pretreated rats did not differ significantly from the response in preparations from vehicle-treated animals.

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#### **3.2.4. Hypercontractile responses to electrical field stimulation (EFS)**

EFS applied at frequencies of 1-15 Hz induced frequency-dependent responses characterized by an initial inhibition of spontaneous activity or a low amplitude relaxation followed by a pronounced contraction. Both the inhibitory and the excitatory responses were abolished by addition of tetrodotoxin (1  $\mu$ M) to the bathing solution, indicating that the EFS-induced responses are neurogenic. In muscle strips isolated from naïve rats EFS-induced contractile responses increased in proportion to the frequency of stimulation. A comparison between the amplitude of EFS-induced contractions in muscle strips from naïve and indomethacin-treated animals demonstrated a significant increase in the indomethacin-treated rats dosed with vehicle (Figure 5). Moreover, these preparations responded with high-amplitude contractions in response to low frequency stimulation and showed no linear increase of the contractile responses with an increase in EFS frequency (Table 1, slope not significantly different from zero). The effects of tegaserod and omeprazole on indomethacin-induced hypercontractility in responses to EFS were evaluated comparing the results obtained by linear regression of frequency-response data (Figure 6). Pretreatment of the rats with tegaserod or the combination of tegaserod and omeprazole prevented the increase of contractile responses to EFS and preserved the linear frequency-response relationship (Table 1). Omeprazole applied individually preserved the linear frequency-response relationship, but was not able to prevent the increase in amplitude of the contractile responses (Figure 6).

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A summary of the protective effects of tegaserod, omeprazole or their combination against the development of indomethacin-induced abnormalities in gastric mucosa and muscle function are presented in Table 2. Gastric damage induced by acute intragastric administration of indomethacin in the rat was characterized by mucosal ulceration and inflammation in the glandular stomach and hypercontractile muscle responses to high KCl, carbachol, 5-HT and EFS. As seen in the Table 2, the combined treatment with tegaserod and omeprazole was most effective in reducing indomethacin-induced ulceration and mucosal inflammation and attenuating the induced smooth muscle hypercontractility. Treatment with either omeprazole or tegaserod alone was effective in correcting some but not all of the induced abnormalities. The most complete protection against mucosal injury and abnormal muscle contraction was afforded by the tegaserod plus omeprazole combination treatment.

## Discussion

The results of this study demonstrate that tegaserod has a protective effect against gastric mucosal injury induced by indomethacin in a rodent model. This protective effect was manifest by both a decrease in indomethacin-induced antral mucosal ulceration and suppression of abnormally increased gastric antral muscle contractions. Furthermore, when tegaserod was administered with omeprazole, an effective PPI which suppresses gastric acid secretion, the combination therapy demonstrated better protection against indomethacin-induced gastric mucosal damage and smooth muscle dysfunction than the protection observed with omeprazole alone. The main pathophysiological event considered important for development of NSAID-induced gastric mucosal injury is a

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deficiency of endogenous prostaglandins in the stomach wall caused by blockade of cyclooxygenase activity by the NSAID. This effect in turn leads to a breakdown in various mucosal factors which normally provide an effective barrier against back-diffusion of luminal HCl causing tissue damage, blood vessel injury, tissue ischemia, ulceration and bleeding. Additional factors related to the reduction of tissue prostaglandins are neutrophil activation and an increase in gastric motility (Takeuchi et al., 1986; Takeuchi et al., 1997 a,b; Suzuki et al., 2000). Mucosal protection by omeprazole treatment is thought to result from the PPI-induced reduction of gastric acid secretion, which in turn results in less luminal hydrogen ions being available for back-diffusion and tissue damage. However, the protective effect is not complete, as clinical studies document that ulceration and bleeding, while greatly reduced, can occur despite concomitant PPI use. Thus, application of a more potent protective mechanism or addition of a second protective factor which causes mucosal protection by a different mechanism could be helpful in providing more complete protection against mucosal injury, especially in high risk patients.

The results of this study demonstrate that tegaserod exerts a protective effect against indomethacin-induced gastric mucosal injury as well as enhances the protective effect of omeprazole when both drugs are administered together. The mechanism(s) responsible for the effect of tegaserod is unclear but it is most likely that it acts through an effect on gastric muscle function. In our experiments, indomethacin induced mucosal ulceration and a significant increase in neutrophil infiltration (measured as an increase in MPO activity) in the antrum. Others have also noted (Morini et al., 1995) that NSAID-induced

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ulceration is confined to the glandular stomach, namely the gastric corpus, antrum and pyloric region, and that no visible lesions or significant increase in inflammatory cell infiltrates are present in the fundus. As a functional correlate of the mucosal lesions we observed an increased contractility in muscle strips from the gastric antrum. The antral muscle showed hypercontractility in response to high  $K^+$  depolarization, carbachol, 5-HT or EFS of enteric nerves. Earlier studies implied that increased contractility of the antral muscle contributes significantly to development of NSAIDs-induced gastric mucosal damage, moreover, antral hypermotility itself was sufficient to induce ulcerative damage to the gastric mucosa (Okada et al., 1989; Mersereau et al., 1988; Takeuchi et al., 1986).

In view of such findings, one might expect that prokinetic drugs would enhance the mucosal ulcerogenic response to NSAIDs. In fact, Takeuchi et al. (1997,a) demonstrated that metoclopramide, which exerts a gastroprokinetic effect by blocking inhibitory dopamine  $D_2$  receptors, and ondansetron which is a 5-HT<sub>3</sub> receptor antagonist, both provoke hemorrhagic mucosal lesions in the stomach of rats pretreated with a low dose of indomethacin which had no ulcerogenic effect. In the same model, co-treatment with cisapride, a gastokinetic which has both 5-HT<sub>3</sub> antagonist and 5-HT<sub>4</sub> agonist activity and whose mechanism of action involves activation of 5-HT<sub>4</sub> receptors (Katayama et al., 1995; Yamano et al. 1997), resulted in no mucosal ulceration. In our experiments, tegaserod that activates 5-HT<sub>4</sub> receptors but has no 5-HT<sub>3</sub> antagonist properties showed significant protective effect against mucosal injury in indomethacin-treated rats. Thus, it is logical to assume that the activation of 5-HT<sub>4</sub> receptors, shown to normalize antral muscle contractility and enhance gastric emptying, plays a role in the protective effect.

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In support of such mechanism of action is the fact that tegaserod has been shown to enhance gastric emptying (Degen et al., 2001; Degen et al., 2005). Moreover, the protective effect of tegaserod enhanced the protective effect of omeprazole, which itself is known to decrease the rate of gastric emptying (Rasmussen et al., 1991; Rasmussen et al., 1999; Parkman et al., 1998). Recently, it has been shown by results of both animal and human studies that tegaserod can reverse an omeprazole-induced delay in gastric emptying (Cowan et al., 2005; Tougas et al., 2005).

Although an accelerated emptying of indomethacin from the gastric lumen could contribute to the protective effect of tegaserod, it is unlikely that this is the only mechanism of tegaserod-induced mucosal protection, since mucosal injury and increased gastric antral contraction can occur when NSAIDs are administered peripherally via subcutaneous injection (Suzuki et al., 2000). In addition, indomethacin is rapidly absorbed and high plasma levels are quickly reached after a single oral dose (Ogiso et al., 1983). Thus, future experiments, which use a peripheral route for NSAID administration thereby avoiding direct instillation into the stomach, will be required to prove whether or not faster gastric evacuation of orally administered NSAID is responsible for at least part of the protective effect of tegaserod.

In the present study, tegaserod treatment prevented the development of antral muscle hypercontractility to nerve stimulation and normalized the responses to muscarinic or 5-HT receptors in indomethacin-treated rats, thus eliminating the pathogenic influence of the indomethacin induced gastric antral hypercontractility. Although the mechanism by

which this occurs is unclear, we suspect that the effect of tegaserod on smooth muscle contractility is the major factor responsible for the mucosal protection. Stimulation of 5-HT<sub>4</sub> receptors by tegaserod has been shown to inhibit contractility in human colonic circular muscle (Tam et al., 1994). An effect involving inhibition by tegaserod of 5-HT<sub>2B</sub> receptors is also possible, since a recent *in vitro* study in the rat clearly demonstrated that tegaserod antagonizes contractions of muscle strips from the gastric fundus which were induced by a selective 5-HT<sub>2B</sub> receptor agonist (McCullough et al., 2006). However, to date there is insufficient information documenting 5-HT<sub>2B</sub> receptor activity in gastric muscle in humans. A different potential protective mechanism may involve the effect of tegaserod on the function of sensory neurons in the mucosa and its action as an antagonist at 5-HT<sub>1B</sub> receptors regulating mucosal blood flow. Mucosal blood flow is an important factor maintaining integrity of the gastric mucosal barrier to acid back diffusion and indomethacin treatment has been shown to have an adverse effect manifested by a reduction in gastric mucosal blood volume (Yokoyama et al., 1994). Sensory neurons in the mucosa play a key role in the protective hyperemic response of submucosal arterioles to luminal irritants which is disturbed under prostaglandin-deficient conditions (Wallace and Granger, 1996). The protective effect of tegaserod against NSAID-induced gastric damage could thus involve its interaction with visceral afferent nerves, including enteric afferents, which regulate mucosal microcirculation. Tegaserod is known to interact with 5-HT<sub>4</sub> receptors on intrinsic primary afferent neurons (Camilleri, 2001) and more recently has been shown to regulate visceral sensitivity most probably affecting peripheral afferent pathways (Greenwood-Van Meerveld et al., 2006). A more speculative effect of tegaserod which could potentially increase mucosal blood flow is its

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5-HT<sub>1B</sub> receptor antagonist activity. In isolated human and non-human primate mesenteric arteries, vasoconstriction can be induced by exposure to sumatriptan, a known agonist of 5-HT<sub>1B</sub> receptor activity. Tegaserod, which has affinity for the 5-HT<sub>1B</sub> receptor demonstrates antagonist activity at this receptor by preventing vasoconstriction induced by sumatriptan (Weber et al., 2006).

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In summary, our results demonstrate that tegaserod has a protective effect against gastric mucosal damage and smooth muscle dysfunction induced by intragastric administration of indomethacin in the rat. The protective effect of tegaserod most likely results from a complex action modulating smooth muscle contractility and enhancing factors which contribute to gastric mucosal defense against intraluminal acid. Data from the literature suggests that the pharmacological mechanism(s) responsible for these effects in humans are derived mainly from tegaserod's activation of 5HT<sub>4</sub> receptors, but there is intriguing evidence, at least in the rat, that tegaserod may also exert a protective effect through an antagonism at 5-HT<sub>1B</sub> (Weber et al., 2006) and 5-HT<sub>2B</sub> receptors (Beattie et al., 2004; McCullough et al., 2006). Moreover, when tegaserod was administered as a combination with the PPI omeprazole, the combined mucosal protective effect against indomethacin-induced injury was greater than that observed with either drug alone and was accompanied by preserved normal antral muscle contractility. This novel finding suggests that in addition to its prokinetic action tegaserod may regulate serotonergic mechanisms providing protection against the harmful effect of NSAIDs. The positive results in rats support that further investigation is warranted to establish whether the observed

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☐ 1: Am J Health Syst Pharm. 1996 Nov 15;53(22 Suppl 3):S5-12.

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**Influence of pathophysiology, severity, and cost on the medical management of gastroesophageal reflux disease.****Reynolds JC.**

Division of Gastroenterology and Hepatology, Allegheny University of Health Sciences, Philadelphia, PA 19102, USA.

This article summarizes the pathophysiology of gastroesophageal reflux disease (GERD) and the wide spectrum in disease and symptom severity as they influence the selection of cost-effective treatment strategies. The vast majority of patients with GERD have mild symptoms, no gross endoscopic evidence of esophagitis, and little risk of developing complications. More than 85% of patients with GERD symptoms have uncomplicated disease. Diffuse ulcerations or complications (grade III or IV esophagitis) occur in only 3.5% of patients < 65 years of age. However, some patients with GERD can develop severe complications, including esophageal obstruction, significant blood loss, and, in rare circumstances, perforation. Furthermore, adenocarcinoma of the esophagus, which is increasing in incidence faster than any other cancer, is caused by GERD. Although severe ulcerations are uncommon in young patients, they occur in 20-30% of patients over age 65. Patients with ulcerative esophagitis are not only more prone to develop complications, they are also more resistant to treatment. Cost-effective medical management of GERD must take into account the wide spectrum of symptom and disease severity. Therapy consists of both nonpharmacologic treatment and the appropriate use of medications from several classes of drugs, either alone or in combination. Traditionally, prokinetic agents or histamine receptor antagonists have been used as primary therapy; proton-pump inhibitors are reserved for more resistant cases. The rationale for this and for alternative approaches is discussed.

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### [Gastroesophageal reflux]

[Article in German]

**Schwizer W, Fried M.**

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Universitätsklinik Zurich.

Gastroesophageal reflux disease (GERD) is a common, typically chronic recurring disorder. The majority of patients with heartburn and regurgitation have intermittent symptoms for which they do not consult their physicians. The main long-term risk of esophagitis is adenocarcinoma arising from Barrett's metaplasia. There are two principle therapeutic strategies in the treatment of GERD. The use of prokinetic drugs aims at treating the primary motility disorder leading to reflux, whereas acid-suppressive therapy targets at the reduction of gastric acid production to prevent symptoms and complication of GERD. The cornerstone in the treatment of GERD are proton pump inhibitors (PPI). Patients with mild symptoms and rarely relapsing disease may be best treated intermittently. Longterm maintenance acid-suppressive therapy with PPIs is necessary in GERD patients with immediate and severe relapse. At present, eradication of *Helicobacter pylori* in GERD patients is not recommended. Antireflux surgery is an effective treatment to control gastroesophageal reflux. However, surgery is associated with a low, but still substantial morbidity, a low mortality of up to 0.5% and is only indicated in patients with pharmacological refractory reflux disease.

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### A prokinetic approach to treatment of gastroesophageal reflux disease.

**Achem SR, Robinson M.**

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Currently available medications for gastroesophageal reflux disease (GERD) vary in mechanisms of action from neutralization or suppression of gastric acid to improving underlying upper gastrointestinal dysmotility. This article reviews the clinical efficacy of pharmacological agents used to treat GERD and provides a rationale for considering a primary prokinetic approach to antireflux treatment which will be applicable to many patients. Treatment trials in acute GERD have demonstrated unexpectedly prolonged maintenance of high esophageal pH with tablet and liquid antacid formulations. However, there are no well-designed placebo-controlled trials of antacids for esophageal mucosal healing. H2 receptor antagonists (H2RAs) at conventional doses relieve reflux symptoms in at least 50% of reported series of GERD patients, and they can also provide endoscopic healing in 27-45% of the cases. Therapy with more potent acid-suppressive agents such as proton pump inhibitors (PPIs) may lead to improved symptomatic relief and to superior healing compared with H2RA therapy, especially in those patients with more advanced erosive esophagitis. Promotility agents, particularly cisapride, offer symptom relief and healing rates which are quite similar to standard H2RA treatment. GERD tends to be a chronic and relapsing condition. Cisapride has been shown to be quite effective in maintaining remission in GERD patients, including endoscopic remission in the lesser degrees of esophagitis. This may be accomplished with relatively low and cost-effective dosing in many individuals. For the small proportion of patients who manifest severe grades of esophagitis, PPI therapy is associated with lower relapse rates than either H2RA or prokinetic treatment. Overall, a strong case can be made for the empirical selection of promotility therapy for the large numbers of GERD patients who do not have documented severe erosive disease.

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