Biochemical Pharmacology, Vol. 31, No. 22, pp. 3591–3596, 1982.
Printed in Great Britain.

0006-2952/82/223591-06 $03.00/0
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NON-STEROIDAL ANTI-INFLAMMATORIES—XII*

MODE OF ACTION OF ANTI-INFLAMMATORY METHANE SULFONANILIDES

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(*Received* 25 *February* 1982; *accepted* 4 *May* 1982)

Abstract—The influence of anti-inflammatory methane sulfonanilides on the arachidonic acid metabolism in ram seminal vesicle homogenate depends on the pK_a of the individual substance: more acidic compounds suppress, less acidic compounds stimulate PG synthesis and enhance the PGH_2/PGG_2 ratio. As oxygen radicals are liberated during the conversion of PGG_2 to PGH_2 it is suggested that the less acidic methane sulfonanilides are scavengers of the (pro-inflammatory) oxygen radicals.

Since the investigations of Vane *et al.* [2] it has become apparent that the anti-inflammatory and analgesic activity of the commonly used non-steroidal anti-inflammatory drugs (NSAID), e.g. indometacin (compound **1** in Table 1) is related to their ability to inhibit the biosynthesis of prostaglandins (PGs) from arachidonic acid (AA). NSAID are potent inhibitors of the enzyme cyclooxygenase which converts AA to the endoperoxide PGG_2 (Fig. 1).

However, the systemic reduction of PG levels is thought to be responsible not only for the therapeutic efficacy of NSAID, but also for the well-known side effects, especially their gastrointestinal intolerance [3]. It has also been shown that PGs, especially of the E-type, are involved in the regulation of various immunological reactions by activating T suppressor lymphocytes [4]. As rheumatoid arthritis and related diseases are associated with an overstimulation of several immunological reactions, which are at least in part responsible for disease-associated chronic destructive processes, it might be that PG synthesis inhibiting compounds even enhance these processes.

The current therapy with the only symptomatically acting PG synthesis inhibiting NSAID is not very satisfactory. To overcome these limitations, anti-inflammatory compounds displaying other modes of action have been intensively investigated within the last few years.

An interesting approach in this direction was the characterization of MK 447 (compound **9** in Table 1) by Kuehl and coworkers [5, 6]. This oxygen radical scavenging compound has been shown to be an anti-inflammatory, non-ulcerogenic compound in laboratory animals.

Theoretically, oxygen scavengers can influence AA metabolism at two distinct sites (Fig. 1). (1) Inhibition of oxygen incorporation into the endoperoxide PGG_2 (site A in Fig. 1) causing inhibition of PG synthesis [7]. (2) Enhancement of the conversion of PGG_2 to PGH_2 by oxygen radical trapping (site B in Fig. 1) causing a stimulated PG synthesis which has been demonstrated *in vitro* for MK 447 [5, 6]. As oxygen radicals are extremely cytotoxic and exhibit pro-inflammatory properties, the anti-inflammatory activity of compounds like MK 447 can be explained by their radical scavenging properties. This mechanism might therefore open the possibility to develop anti-inflammatory compounds which are non-ulcerogenic and do not suppress the synthesis of immunoregulatory PGs. According to the literature [8] the anti-inflammatory activity of some methane sulfonanilides (e.g. nimesulide, R 805 [9, 10], compound **4** in Table 1) might be related to oxygen radical scavenging properties. In the course of testing new *N*-methansulfonyl-indanylamines [11–13] we have found highly active anti-inflammatory compounds, some of them without acute ulcerogenic properties in animals. In this communication we are dealing with an analysis of the influence of these compounds on the arachidonic acid metabolism.

MATERIALS AND METHODS

Materials

Nimesulide, R 805 (**4**), was kindly provided by Riker Labs., Minnesota, U.S.A. Compound **2** was synthesized as described in Ref. 14. Compounds **3** [15], **5**, **6**, [13], **7** and **8** [11] stem from our own chemical labs.

Prostaglandin synthesis in ram seminal vesicle homogenate

Enzyme preparation. An acetone powder of ram seminal vesicles was prepared as described by Wallach and Daniels [16].

Prostaglandin synthesis with cofactors. Enzyme powder (5 mg) was suspended in 0.5 ml buffer (62.5 μmoles/l. EDTA, 4 μmoles/l. glutathione, 1 μmole/l hydroquinone, pH adjusted to 8.0 with

* Part XI is in preparation [1].
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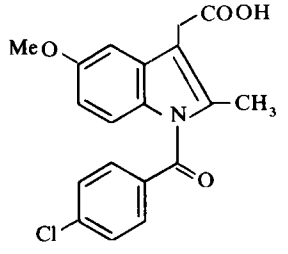
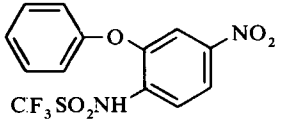
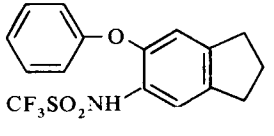
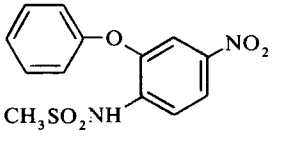
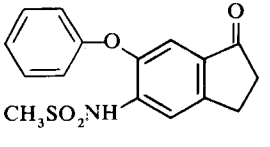
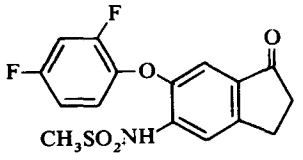
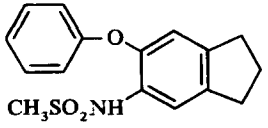
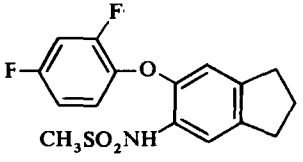
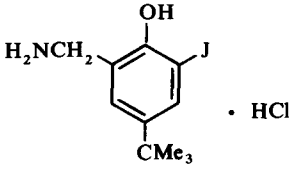
Table 1. Anti-inflammatory methane sulfonanilides (compounds **2–8**), pK_a values, anti-inflammatory activity *in vivo* and influence on the synthesis of prostaglandins *in vitro* in comparison to indometacin (**1**) and MK 447 (**9**)

Compound No.	Formula	pK_a	Adj.-Arthr. rat ED_{40} (mg/kg)	PG synthesis *in vitro* (% of control)						PGH_2/PGG_2 (control* = 1) without cofactors		
				with cofactors			without cofactors					
				10^{-5} M	10^{-4} M	10^{-3} M	4×10^{-5} M	2×10^{-4} M	10^{-3} M	4×10^{-5} M	2×10^{-4} M	10^{-3} M
1				39	19		14	10	10		+	
2		2.43	3–10	98	31	14	39	16	8		+	
3		4.65	10–30	99	88	30		ND		1.12	1.00	0.94
4		6.50	1–3	100	82	55	104	94	80	1.10	1.34	1.18
5		6.98	3–10	112	104	105	101	106	106	1.00	1.20	1.45

6		6.95	0.3–1	99	96	95	116	118	118	1.05	1.07	1.29
7		9.39	3–10	94	95	99	116	127	141	1.35	1.70	1.55
8		9.36	0.3–1	91	93	82	120	133	141	1.16	1.68	2.20
9				97	95	90	118	129	143	1.35	2.08	2.20

* Control: 20.0 ± 4.6% (N = 8) of arachidonic acid is converted into PGH_2 and PGG_2 whereby 60% was found to be PGH_2 and 40% PGG_2.
† Overall content of PGs too low for determination of PGH_2/PGG_2.

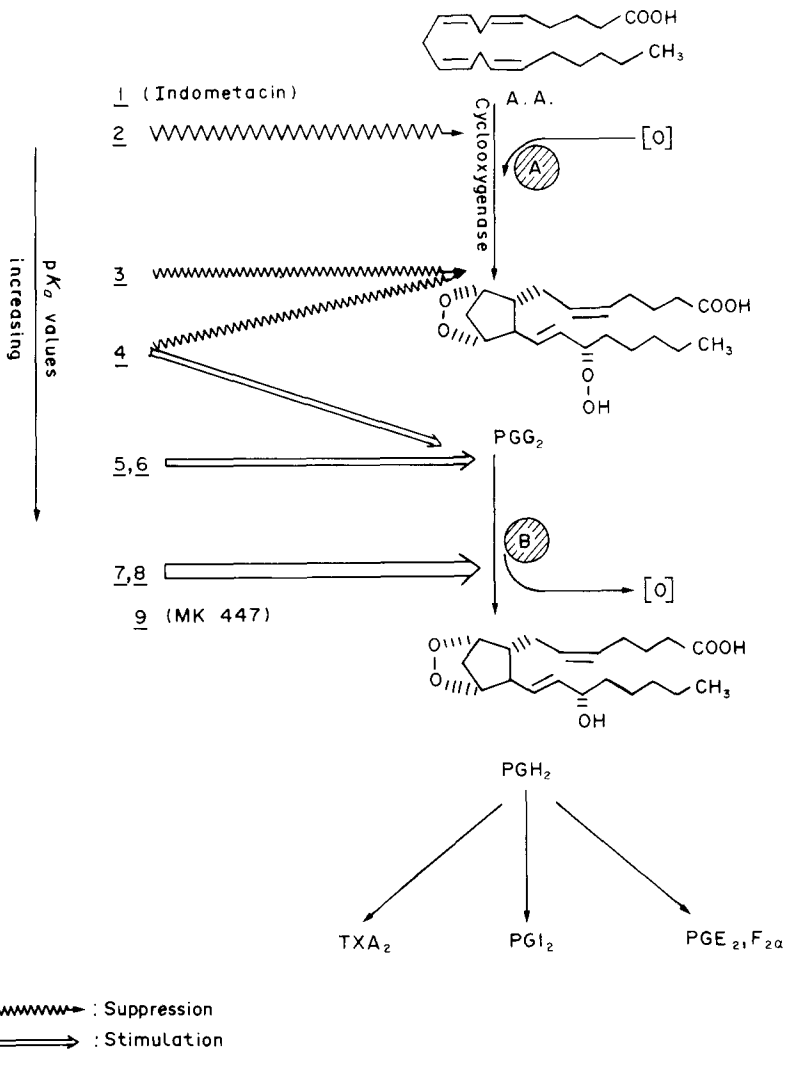


Fig. 1. Anti-inflammatory methane sulfonanilides (compounds **2**–**8**) (Table 1), hypothetical sites of their interference with the arachidonic acid cascade in comparison with indometacin (**1**) and MK 447 (**9**).

NaOH). The enzyme was preincubated with NSAID (10^{-3}–10^{-5} mole/l.) for 15 min at 37°. After addition of 10 µl of substrate (3.2 µmoles arachidonic acid containing 3 mCi [^{14}C]arachidonic acid) the reaction was continued for 20 min at 37° and then stopped with 1 ml of 1 N citric acid. Prostaglandins were extracted two times with 3 ml of ethyl ether, concentrated in a stream of nitrogen and applied to TLC plates. The chromatograms were developed with a solvent system consisting of ethyl ether:petrol ether:methanol:acetic acid (50:40:5:5, v/v). After monitoring the radioactivity with a TLC scanner, the peaks for arachidonic acid and prostaglandins (in total) were scraped off and measured in a Tricarb® scintillation counter (Fa. Packard).

Prostaglandin synthesis without cofactors. Enzyme powder (5 mg) was suspended in 0.5 ml of 0.1 moles/l. Tris-HCl buffer, pH 8.0, in the presence of 0.16 µmole of [^{14}C]arachidonic acid. After incubation with NSAID (10^{-3}–4×10^{-5} moles/l.) for 5 min at ambient temp the reaction was stopped with 50 µl 2 N citric acid. Prostaglandins were extracted three times with 2 ml of ethyl ether, concentrated in a stream of nitrogen and applied to TLC plates. The chromatograms were developed with a solvent system consisting of ethyl ether:petrol ether:acetic acid (85:15:0.1, v/v), the zones of radioactivity were monitored with a TLC scanner, scraped off and measured in a Tricarb® scintillation counter (Fa. Packard).

Determination of pK_a values according to Albert and Serjeant [17]

Spectrophotometric determination (compounds **2**, **4**, **5**, **6**, and **7**). A vol. of 0.1 ml of a stock solution in methanol of the compound under investigation were added with stirring to 20.0 ml buffer solution (ready-made buffer solution of various pH values, Riedel de Haen).

The u.v. spectra (240–500 nm) were measured following mixing (1 cm cuvettes, reference solution: same composition without the corresponding compound). The final concns were between 2×10^{-5} and 8×10^{-5} moles/l. Isobestic points were observed for

all compounds except compound **7**. The extinction at a certain wavelength was plotted against the pH values. The pK_a value was obtained from the point of inversion of the extrapolated curve which resembles a titration curve in shape.

(b) *Solubility method (compounds* **3**, **7** and **8**). In the case of compounds **3** and **8** in the unionized form, the solubility was too small for direct spectrophotometric measurements. Therefore the pH dependency of the solubility was used to calculate the pK_a value. The reliability of this method was demonstrated with compound **7**, where a good agreement of the pK_a values of both methods was obtained. The solubility of the compounds was measured in buffer solutions ($c < 5 \times 10^{-3}$ moles/l., ionic strength adjusted to $I = 0.1$ with NaCl).

RESULTS

The influence of various methane sulfonanilides on PG synthesis in ram seminal vesicle homogenates is shown in Table 1.

The PG synthesis has been tested in this system either in the absence or in the presence of cofactors (glutathione, hydroquinone). The major PGs synthesized are PGE_2 and $PGF_{2\alpha}$. In the presence of cofactors, AA is converted very rapidly and almost completely (>80%) to PGs. A stimulating effect on PG synthesis or an influence on the PG synthesis intermediates, the endoperoxides PGG_2 and PGH_2, is therefore much better demonstrated in a system with a much slower rate of AA metabolism (system without cofactors).

Indometacin (**1**) displays strong inhibitory activity in both systems, which is characteristic for classical cyclooxygenase inhibitors. On the other hand, the oxygen radical scavenger MK 447 (**9**) clearly stimulates the PG synthesis in the system without cofactors.

Various *in vivo* anti-inflammatory active methane sulfonanilides have been tested in both systems. In this connection we paid attention to the acidity of these compounds (Table 1).

As expected, compounds **2** and **3** bearing a CF_3SO_2NH group are much more acidic than the corresponding compounds **4** and **7** with CH_3SO_2NH moieties. The order of pK_a values within these classes of compounds is evidently caused by the order of electron attracting potency of the substituents in *p*-position to the RSO_2NH group falling from NO_2 over CO to CH_2 (**2**→**3** and **4**→**5**, **6**→**7**, **8**, respectively).

Interestingly, the influence on PG synthesis by the methane sulfonanilides has been shown to be closely correlated to the acidity of these compounds: only the more acidic methane sulfonanilides display PG synthesis-inhibiting activity; the potency decreases from compound **2** over **3** to **4** (Table 1) with rising pK_a values. Compounds with a pK_a around 7 (**5** and **6**) did not influence the PG synthesis even in concns as high as 10^{-3} moles/l. In contrast, methane sulfonanilides with $pK_a > 7$ (compounds **7** and **8**) enhanced the conversion of AA to PGs, as shown in the system without cofactors, very similar to the stimulating effect of the oxygen radical scavenger MK 447 (compound **9** in Table 1).

Looking for the PG synthesis intermediates PGG_2 and PGH_2 in the system without cofactors the following correlations have been found: according to the literature [5] and also shown in Table 1, MK 447 strongly enhances the formation of PGH_2 at the expense of PGG_2, i.e. PGH_2/PGG_2 increases. Within the series of methane sulfonanilides the enhancement of PGH_2/PGG_2 correlates very well with increasing pK_a and the stimulating effect on PG synthesis: whereas compound **3** ($pK_a = 4.65$) did not change the PGH_2/PGG_2 ratio,* R 805 (compound **4**, $pK_a = 6.5$) and compounds **5** and **6** ($pK_a = 7.0$) caused a slight enhancement. Finally, compounds **7** and **8** with pK_a values of 9.4 reached a potency in enhancing the PGH_2/PGG ratio which is comparable to MK 447.

CONCLUSION

The described methane sulfonanilides represent a class of anti-inflammatory compounds which influence at least in part the PG synthesis by other mechanisms than classical NSAID. The site of interference with the AA cascade is obviously determined by the acidity of the individual compound: the more acidic methane sulfonanilides (compounds **2** and **3**) inhibit the transformation of AA to PGG_2 (site A in Fig. 1), whereas compounds with $pK_a > 7$ (**7** and **8**) enhance the transformation of PGG_2 to PGH_2 (site B in Fig. 1). Compounds with pK_a 6.5–7 (**4**–**6**) hold an intermediate position.

It can be suggested that the weakly acidic methane sulfonanilides ($pK_a \geq 7$) accelerate the transformation of PGG_2 to PGH_2 by scavenging oxygen radicals liberated during this process.

O-Radicals are not only liberated during AA metabolism which is stimulated during inflammatory processes, they are also created during various defence mechanisms by several cell types, especially by neutrophiles. As oxygen radicals are extremely cytotoxic, oxygen scavenging might prevent tissue destruction which is associated with acute and chronic inflammatory processes.

Therefore, compounds like the weakly acidic methane sulfonanilides could provide a better gastrointestinal tolerance and perhaps a better therapeutical profile than the PG synthesis inhibiting classical NSAID.

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* Because of the almost complete inhibition of PG synthesis with compound **2** and indometacin (**1**) in the concns tested, the PGH_2/PGG_2 ratios could not be accurately estimated. However, it is known for indometacin and other cyclooxygenase inhibitors that they do not change the PGH_2/PGG_2 ratio.

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