
INHIBITION OF PROSTAGLANDIN BIOSYNTHESIS BY A NEW ANTI-INFLAMMATORY DRUG, TA-60

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Effect of a new anti-inflammatory drug which has a little ulcer inducing property on gastrointestinal tract, TA-60 (2-[4-(3-methyl-2-butenyl) phenyl] propionic acid), on the prostaglandin (PG) metabolism was investigated. TA-60 inhibited the PGE₂ biosynthesis of the bovine seminal vesicle microsome dose-dependently. The inhibition constant (Kᵢ) of TA-60 was approximately 8 μM. The potency of TA-60 was approximately the same and two times that of ibuprofen (IP) and phenylbutazone (PBZ), respectively. TA-60 did not show the time dependent inhibition of the PGE₂ biosynthesis unlike to indomethacin (IM). The decrease in the PGE₂ contents in the stomach of the rats by TA-60 reached a plateau and the content was not decreased to less than a certain level. The PGE₂ content of the intestine was not changed by TA-60. TA-60 did not inhibit the activity of the PG degrading enzyme, 15-hydroxy PG dehydrogenase (15-OH-PG-DH) of the gastric mucosa like the other non-steroidal anti-inflammatory drugs (NSAIDs): IP, PBZ and IM. These results suggest that the slight ulcerating effect of TA-60 on the gastrointestinal tract might be resulted from the small decreasing effect of TA-60 on the gastrointestinal level of PGE₂.

Keywords — TA-60; non-steroidal anti-inflammatory drug (NSAID); prostaglandin E₂; gastrointestinal tract

INTRODUCTION

The inhibition of prostaglandin (PG) biosynthesis has been well established as the mechanism of actions for most non-steroidal anti-inflammatory drugs (NSAIDs). These NSAIDs also cause injury to the gastrointestinal tract as a side effect. It has been reported that the incidence of this side effect of ibuprofen (IP) is less than most other NSAIDs. Recently, we synthesized a new NSAID, TA-60. The ulcerating effect of TA-60 is significantly less than that of IP, while the potencies of the anti-inflammatory and analgesic activities are the same or a little weak compared with IP.

Furthermore, TA-60 did not induce the lesion which is caused by the necrotizing agents to a more intensive degree or caused healing of the lesion whereas the other NSAIDs made the lesion more intensive. TA-60 is a propionic acid derivative containing an isoprene unit in its molecule. It has been reported that the isoprene unit(s) containing compounds have an anti-ulcerating effect. On the other hand, it is generally believed that the PG deficiency in the gastric tract induce the ulceration. In the present study, we investigated the effects of TA-60 on the metabolism of PG comparing with those of the other NSAIDs.

MATERIALS AND METHODS

Enzyme of PG Biosynthesis — Microsomal fraction of bovine seminal vesicles (PG synthetase) was obtained from the Miles laboratories.

Enzyme Preparation of 15-Hydroxy PG Dehydrogenase (15-OH-PG-DH) from Rat Gastric Mucosa — The enzyme preparation was obtained from rat gastric mucosa as previously described. Rat was killed by decapitation and the stomach was removed. After the stomach was washed with 0.9% NaCl, the fundal mucosa was
scraped from the underlying musclar layer. The
mucosa was homogenized with 4 vol of an ice-
cold 150 mM potassium phosphate buffer (pH
7.4, containing 2 mM ethylenediaminetetra acetic
acid (EDTA), 2 mM mercaptoethanol, 300 μM
nicotinamide adenine dinucleotide (NAD) and
200 mg/l bovine serum albumin)) by mechan-
ical homogenizer (Ultra-Turrax® (Janke &
Kunkel) ) for 10 s. Following centrifugation at
100000 × g for 60 min, the resultant superna-
tant was used as the enzyme source of the gastric
mucosa.

Assay of PG Biosynthesis — PG synthesis was
carried out in 0.5 ml of a reaction mixture consist-
ing of 1-14C-arachidonic acid (S.A.: 52.9
mCi/mmol), enzyme (100 μg protein), hematin
(1 μM), reduced glutathion (1 mM), tryptophan
(1 mM) and pH 7.4 potassium phosphate buffer
(50 mM) as previously described.7) The reaction
medium incubated in the presence or absence of
the inhibitors at 30 °C. Ethanol was used to dis-
solve the arachidonic acid and the inhibitors. No
ethanol effect on the PG synthesis was observed.
Unless otherwise noted, 0.38 μM 14C-arachidonic
acid was used. The inhibition of the PG bio-
synthesis by the NSAIDs was investigated with
two conditions: treatments of no preincubation
and preincubation of the enzyme with the
NSAIDs for 10 min. The reaction was stopped by
an addition of 2 ml of n-hexane-ethyl acetate
(2:1, v/v) according to the method of Yanagi and
Komatsu.8) The remaining arachidonic acid was
extracted from the reaction medium by mixing
with the n-hexane-ethyl acetate and centrifuga-
tion. After the centrifugation at 10000 × g for 10
min, the aqueous phase was frozen at –20 °C and
the organic solvent phase was discarded. The
extraction was repeated three times. The resultant
aqueous phase was mixed with 1.0 ml ethanol and
centrifuged at 10000 × g for 10 min to precipitate
the 14C-arachidonic acid which was bound to the
protein non-specifically. Since PGE2 selectively
remained in the aqueous phase, the PG bio-
synthesis activity was determined by measuring
the radioactivity in the aqueous phase. Under
these conditions, the PGE2 was the main product
which occupied more than 93% of the all pro-
ducts. By this selective extraction, more than 90% of
the PGE2 was able to be recovered from the
reaction medium. A bovine seminal vesicle
microsomal fraction (2.8 mg/ml) was solubilized
with 1% Nonidet P-40 (Nakarai) and it was left
to stand for 20 min at 0 °C. The solubilized frac-
tion was obtained by centrifugation at 100000 ×
g for 30 min and used for the enzyme prepara-

Assay of 15-OH-PG-DH Activity — The
15-OH-PG-DH activity was determined as pre-
viously reported.6,9) As a substrate, PGE1 was
incubated with the enzyme preparations. The
reaction mixture contained, in a total volume of 2
ml, the same buffer used for the homogenization
of the tissues. Fifty μM of PGE1 and 300 μM of
NAD were used as the substrate unless otherwise
mentioned. The reaction was initiated by an addi-
tion of PGE1. Incubation was performed at 37 °C
and terminated by an addition of 0.5 ml of 2 N
NaOH. The maximum absorbance at 500 nm was
measured about 2 min later using a spectro-
photometer (Shimazu UV 240). The product, 15-
keto-PGE1, was quantitated from the extinction
coefficient (30300 M⁻¹/cm) of chromophore
generated in the base.

Determination of the PGE2 Contents of the
Stomach and Intestine of Rats — A certain hours
after an administration of TA-60 and IP, the
stomach and intestine of the rats were quickly
removed and immersed in an ice-cold solution
of 4 ml of ethanol containing 20 μM indomethacin
(1M). After being left to stand for a few minutes,
the tissues were homogenized and filtrated. The
filtrate was evaporated with a Savant Concentra-
tor under reduced pressure. The residues were
dissolved in 0.5 ml of water and the PGs were
extracted from the water to 2 ml of ethyl acetate
by mixing them with 60 μl of 1 N formic acid.
Prior to the PGs extraction from the tissue
homogenate, 9000 dpm of 3H-PGE2 (S.A.: 165
Ci/mmol) was added to the homogenate to deter-
mine the recovery. The ethyl acetate containing
the PGs extracted from the tissue was evaporated
with a Savant Concentrator under reduced
pressure. The resultant residues were dissolved in 100 µl of methanol-ethyl acetate (1:1, v/v) and half of this solution was spotted on a thin layer chromatographic (TLC) plate of silicagel (Kieselgel 60 (Merk, Darmstadt)). The TLC plate was developed with an organic phase of ethyl acetate-iso-octane-acetic acid-H₂O (11:5:2:10, v/v) to separate PGE₂ from the other PGs and other substances. The authentic PGE₂ on the plate was identified by spraying 10% phosphomolybdic acid in ethanol and heating. The portion of the TLC plate having the same Rf value as the authentic PGE₂ in the sample line was scraped off and PGE₂ was extracted with ethyl acetate from the silicagel. The ethyl acetate was evaporated with a Savant Concentrator under reduced pressure. The residues were dissolved in 1 ml of the Tris-HCl buffer containing 1 mg of gelatin for the radioimmunoassay of PGE₂.

_Determination of PGE₂_ — PGE₂ was determined using a radioimmunoassay kit (Clinical Assays). The recovery of added ³H-PGE₂ from the homogenates of the stomach and intestine of the rats were 10—40% and 30—50%, respectively. Protein concentration was determined by the method of Lowry et al.¹⁰ using bovine serum albumin as the standard.

_Drugs Used_ — IP (Hokuko), IM (Sumitomo) and phenylbutazone (PBZ) (Sigma) were used. TA-60 (dl-TA-60), d- and l-TA-60 were prepared at our laboratory.

RESULTS

_Inhibition of PGE₂ Biosynthesis by TA-60_ — TA-60 inhibited the PGE₂ biosynthesis of the

FIG. 1. Inhibition of Cyclooxygenase Activity by TA-60
Specific activity of control value was 90.5 ± 5.2 pmol/mg prot/min
Reaction was carried out for 2 min at 30 °C.

FIG. 2. Stereospecificity of TA-60 on Inhibition of Cyclooxygenase Activity
Reaction was carried out for 2 min at 30°C.

FIG. 3. Double-Reciprocal Plots of Inhibition of Cyclooxygenase Activity by TA-60 and IP
Reaction was carried out for 2 min at 30°C.
Concentrations from 22 to 400 µM of ¹⁴C-arachidonic acid were used. Each point is the mean value of the duplicate determinations.
bovine seminal vesicle microsomes in a dose-dependent manner (Fig. 1). The IC₅₀ of TA-60 was 9 μM. The other NSAIDs, IP and PBZ also similarly inhibited the PGE₂ synthesis (Fig. 1). As

**FIG. 4.** Protective Effect of IP on Inhibition of Cyclooxygenase Activity by TA-60
Enzyme was incubated in the absence and presence of 8 μM IP for 2 min at 30°C.

**FIG. 5.** Protective Effect of IM on Inhibition of Cyclooxygenase Activity by TA-60
Enzyme was incubated in the absence and presence of 3 μM IM for 2 min at 30°C.
previously reported, the inhibitory potency of IM was increased by preincubation or prolongation of the incubation time,\textsuperscript{7,8,11} while the potency of TA-60, IP and PBZ were not altered by these treatments. The IC\textsubscript{50} of IP and PBZ were 8 and 57 \( \mu M \), respectively. The IC\textsubscript{50} of IM was decreased from 6.4 to 0.1 \( \mu M \) by the preincubation. It is well known that most NSAIDs do not have any effect on hydroxylase but cyclooxygenase,\textsuperscript{12} and the Hill coefficient of the inhibition curves of the NSAIDs were less than 1.0 as was previously reported.\textsuperscript{7} These results indicate

**FIG. 6. Dixon Plots Analysis of Effect of IM on Inhibition of Cyclooxygenase Activity by TA-60**

Enzyme was incubated with \( ^{14} C \)-arachidonic acid of 0.38 and 3.4 \( \mu M \) in the presence and absence of 3 \( \mu M \) IM for 2 min at 30\(^\circ\)C.

**FIG. 7. Inhibition of Cyclooxygenase Activity by TA-60 in Solubilized Enzyme**

Specific activity of control was \( 124.0 \pm 3.6 \) pmol/mg prot./min.

**FIG. 8. Double-Reciprocal Plots of Inhibition of Cyclooxygenase Activity by TA-60 in Solubilized Enzyme**

Concentrations from 17 to 200 \( \mu M \) of \( ^{14} C \)-arachidonic acid were used. Each point is the mean value of the duplicate determinations.

**FIG. 9. Effect of TA-60 on 15-OH-PG-DH Activity of Rat Gastric Mucosa**

Specific activity of control was 254 pmol/mg prot./min. Reaction was carried out for 30 min at 37\(^\circ\)C.
that the inhibition of PGE$_2$ biosynthesis by the NSAIDs represents the inhibition of one main reaction. Therefore, in this study we used the cyclooxygenase activity to represent the activity of the enzyme of the PGE$_2$ biosynthesis. 

**Stereospecificity of TA-60 on the Inhibition of PG Biosynthesis**

TA-60 has an asymmetric carbon in its molecule. The inhibitory effect of $d$- and $l$-TA-60 on the PG biosynthesis was investigated. $d$-TA-60 inhibited cyclooxygenase activity more significantly than $l$-TA-60 (Fig. 2). The IC$_{50}$ of $d$- and $l$-TA-60 were 8 and 260 $\mu$M, respectively. These results suggest that the inhibitory effect of $dl$-TA-60 was represented only by the effect of $d$-TA-60.

**The Active Site of TA-60 on the Enzyme**

The TA-60 inhibited cyclooxygenase activity competitively for the substrate arachidonic acid (Fig. 3). IP also inhibited the enzyme activity competitively for arachidonic acid (Fig. 3). $K_i$ values of TA-60 and IP were 14.0 and 9.6 $\mu$M, respectively. The inhibition curve of cyclooxygenase by TA-60 was significantly shifted to the right in the presence of 4 $\mu$M IP (Fig. 4). A similar result was obtained in the presence of IM, but this effect was smaller than that of IP (Fig. 5). Dixon plots analysis clearly showed that the $K_i$ value of TA-60 was not changed by the presence of IM (Fig. 6). $K_i$ values of TA-60 were 5.0 and 5.2 $\mu$M in the concentrations of 0.38 and 3.4 $\mu$M arachidonic acid, respectively. These results indicate that active site of TA-60 on the enzyme is the

![Graph showing the effect of TA-60 on PG Contents of Stomach and Intestine of Rats (Time Dependency)](image)

**FIG. 10. Effect of TA-60 and IP on PG Contents of Stomach of Rats (Time Dependency)**

TA-60 and IP were administered per os. Each value is the mean $\pm$ S.E.M. obtained from 5 separate experiments.

- $a$) $p < 0.05$ compared with non-drug administered control, $b$) not significant compared with non-drug administered control.

$\square$ : TA-60, $\square$ : IP.

![Graph showing the effect of TA-60 on PG Contents of Stomach and Intestine of Rats (Dose Dependency)](image)

**FIG. 11. Effect of TA-60 on PG Contents of Stomach and Intestine of Rats (Dose Dependency)**

TA-60 and IP were administered per os 4h before. Each value is the mean $\pm$ S.E.M. obtained from 5 separate experiments.

Stomach $\square$ : TA-60, $\square$ : IP. Intestine, $\square$ : TA-60, $\square$ : IP.
same as that of IP and IM, namely the catalytic site of the enzyme in which arachidonic acid is converted to PG endoperoxides.

**Inhibition of PGE\(_2\) Biosynthesis by TA-60 in the Solubilized Enzyme**

By solubilization of the enzyme, the inhibitory effect of the NSAIDs were significantly increased as previously reported.\(^7\) The potency of TA-60 became 35 times greater than that of the non-solubilized enzyme (Figs. 7 and 8). \(K_P\) values of TA-60 and IP were 0.43 and 0.35 \(\mu\)M, respectively. However, the \(K_m\) value of the arachidonic acid was increased by the solubilization from 23 to 54 \(\mu\)M. These results indicate that the binding sites of arachidonic acid and the NSAIDs were not completely the same even the NSAIDs competitively inhibited the enzyme activity for the arachidonic acid.

**Effect of TA-60 on 15-OH-PG-DH Activity of Rat Gastric Mucosa**

All the NSAIDs including IP, TA-60, IM and PBZ did not inhibit the 15-OH-PG-DH activity (Fig. 9). At a high concentration of \(10^{-3}\) M, however, PBZ and IM inhibit the enzyme activity by 50 and 65% of the control.

**Effect of TA-60 on the Contents of PGE\(_2\) of the Stomach and Intestine of Rats**

TA-60 and IP decreased the PGE\(_2\) contents of the stomach of rats significantly. Two hours after the administration of the drugs, the maximum effects of the drugs in decreasing PGE\(_2\) contents of the stomach were observed (Fig. 10). At this time, the drugs did not show the dose-dependent effect in PGs contents. These results suggest that the level of PGE\(_2\) in stomach at 2 h after administration of TA-60 and IP were the lowest level induced by each drug. At low dose of TA-60 (40 mg/kg), the recovery of PGE\(_2\) contents from low level was faster than that of IP (Fig. 10). Similar results were obtained from the other experiments. IP decreased the PGE\(_2\) contents dose-dependently, however TA-60 did not decrease the contents less than a certain level (Fig. 11). In regard to the decreasing effects of PGE\(_2\) contents, the potency of TA-60 was significantly weaker than that of IP. In the intestine of rats, TA-60 did not decrease the PGE\(_2\) contents but IP decreased the contents significantly (Fig. 11). These results suggest that TA-60 have small decreasing effect of PGE\(_2\) in gastrointestinal tract compared with IP.

**DISCUSSION**

TA-60 inhibited the PGE\(_2\) biosynthesis via inhibition of the cyclooxygenase activity. The potency of the TA-60 was a little weaker than that of IP. The active site of TA-60 on the enzyme was the same as the other NSAIDs and it also was the site in which the arachidonic acid was bound. However, the site of the arachidonic acid was not completely the same as that of the NSAIDs, since the solubilization of the enzyme did not influence the affinities of the NSAIDs and arachidonic acid in the same way. TA-60 completely inhibited the cyclooxygenase activity at 1 mM similar to the other NSAIDs. On the other hand, TA-60 did not decrease the PGE\(_2\) contents of the gastrointestinal tract below a certain level differently from IP. As described previously,\(^3\) TA-60 has no severe side effect in the gastrointestinal tract compared with IP known as a useful drug having a lesser side effect than the other NSAIDs.

As the mechanism of the action of most NSAIDs, the inhibition of the PG biosynthesis has been well established.\(^1\) It is also generally believed that both actions of anti-inflammation and ulceration on gastrointestinal tract are due to the inhibition of the PG biosynthesis in each organ.\(^2,5,13\) Recently, some drugs were synthesized which have a sufficient anti-inflammatory action but do not have such severe gastrointestinal side effects compared with the well-known NSAIDs.\(^8,14\) The mechanisms of these drugs have been investigated but it has not been clarified as yet.\(^8,14\) Bhattacharjee and Eakins\(^5\) indicated that the inhibitory potency of IM varied greatly when tested against enzyme preparations from different tissues. From these results, Flower and Vane\(^1\) suggested that the existence of the multiple form of the PG biosynthesis system within the organism and that each has its own
drug specificity. Yanagi\textsuperscript{14a)} reported that the inhibitory potencies of the NSAIDs differed in the enzyme sources of the PG synthetase from the bovine seminal vesicle and the intestine of the rats. However, the relative potencies of the NSAIDs were the same in both enzyme sources. Rainsford \textit{et al.}\textsuperscript{10} suggested that the inhibition of the PG biosynthesis and gastric mucosal damage induced by the NSAIDs depend on the drugs and species of animals used. Another suggested possibility for the differences in severity of the gastrointestinal complications due to the NSAIDs is the irreversibility and time-dependency of the inhibition of the PG biosynthesis by the IM-type drugs.\textsuperscript{8}) However, gastrointestinal injury is the most common side effect of the NSAIDs even when they do not show irreversible and time-dependent inhibition of the PG biosynthesis.\textsuperscript{20} Therefore, these types of action regarding the NSAIDs are not considered to be an important factor concerning the side effect of NSAIDs. It is well known that PGs prevent ulceration and accelerate ulcer healing.\textsuperscript{17} Anti-ulcer drugs, SU-88 and carbenoxolone, inhibit the activity of PG metabolizing enzyme 15-OH-PG-DH.\textsuperscript{4}) To clarify the involvement of the inhibition of 15-OH-PG-DH activity in the slight ulcerating effect of TA-60, the effect of TA-60 on the activity of the enzyme was investigated. TA-60, however, did not inhibit the enzyme activity. These results indicate that the inhibition of PG degradation also is not involved in the little side effect of the NSAIDs including TA-60. As another possibility of the different ulcerating effects of TA-60 and IP, the difference of the amount of these drugs which distribute to the gastrointestinal tract can be considered. However, the amount of TA-60 that distribute to the gastrointestinal tract is much greater than that of IP (in preparation). These results suggest that the difference of the amount of the drugs in stomach and intestine also is not involved in the slight ulcerating effect of TA-60.

TA-60 has an isoprene unit in its molecular structure. Some compound containing isoprene unit(s) in their molecular structure (gefarnate, sofalcon and geranylgeranylaceetone) have an anti-ulcer effect.\textsuperscript{4}) TA-60 does not have such an anti-ulcer effect but does not cause any severity to the lesion induced by necrotizing agents in contrast to the actions of the other NSAIDs which cause more severity to the lesion.\textsuperscript{20} So, these effects might be partially involved in the TA-60 action. However, there is no clear explanation for the slight side effect to gastrointestinal tract and the small decreasing effect on the PG contents of the stomach and intestine. Anyway, TA-60 did not so significantly decrease the PG contents of the stomach and intestine compared with IP. Therefore, unless the well-established hypothesis in which the PG deficiency results in ulceration is not true, the slight effect of TA-60 on the PG contents in the gastrointestinal tract might be related to the slight ulcerating effect of TA-60 on the gastrointestinal tract.

**REFERENCES**


TA-60 and Prostaglandin


