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Synergistic and potentiating effects of ranitidine and two new anti-ulcer compounds from *Enantia chlorantha* and *Voacanga africana* in experimental animal models

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TN, an alkaloid from the fruit of *Voacanga africana* and a protoberberine-type alkaloid (7,8-dihydro-8-hydroxypalmatine) (**1**), obtained from the bark of *Enantia chlorantha* were tested for ulcer preventive and antisecretory activity in combination with ranitidine. When tested alone (50 and 100 mg/kg, *p.o.*), TN and **1** achieved their anti-ulcer actions through reduced gastric secretion and improved mucus production. 1 : 1 combinations of **1** and the antisecretory agents (25/25 and 50/50 mg/kg) resulted in significant reduction of ulceration under highly acidic conditions (50–70 mEq/l), suggesting potentiating effects. A combination of TN and ranitidine led to synergistic antisecretory effects.

1. Introduction

In a comprehensive review of the clinical pharmacology of the principal commercialized histamine$_2$-receptor antagonist drugs and their usefulness in the treatment of acid-peptic disorders, Feldman and Burton [1, 2] indicated that the four H_2 blockers (cimetidine, ranitidine, famotidine, nizatidine) are the standard therapy against which newer agents should be compared for efficacy and safety. The review cited cimetidine and nizatidine as the least and most potent, respectively. All the four drugs have the capacity to reduce peptic activity through lowered pepsin production and activity. The pharmacokinetic behaviour of these drugs leads to their presence in muscle, placenta, breast milk and cerebrospinal fluid, and in clinical use, age, hepatic and renal function are regarded as major risk factors for adverse drug reactions [1]. The most common adverse reactions include diarrhoea, headache, drowsiness, fatigue, muscular pain and constipation. Mental confusion, abnormalities in serum metabolite concentrations, impaired hepatic function, adverse hematological reactions and cardiac malfunction, are other less common adverse effects. H_2 blockers may also interact with other drugs by altering their clearance or absorption. On the other hand, some drugs can alter the disposition of H_2 blockers, for example, magnesium and aluminum hydroxide antacids which reduce their bioavailability, while other drugs can increase bioavailability by influencing absorption rates or hepatic metabolism [1]. Thus, an unexpectedly low response to a H_2 blocker when given concomitantly with another drug can be an indicator of a possible interaction. In two preceding studies [3, 4], we tested the anti-secretory activity of cimetidine and ranitidine at the dose of 50 and 100 mg/kg in rats in comparison with similar doses of two new anti-ulcer compounds, TN, and a protoberberine-type alkaloid (7,8-dihydro-8-hydroxypalmatine) (**1**), obtained from the fruit and bark of *Voakanga africana* and *Enantia chlorantha*. TN showed significant anti-secretory activity in pylorus ligated rats, as well as healing action on glacial acetic acid-induced chronic gastric ulcers at the dose of 100 mg/kg. It also showed significant prophylactic effects against gastro-duodenal lesions of cold restraint stress origin as well as against gastric mucosal lesions caused by irritants such as absolute ethanol, HCl/ethanol mixture, and indomethacin at the same dose [3]. On the other hand, **1** showed no anti-secretory activity. However, its possible anti-ulcer mode of action against irritant substances appeared to lie in its ability to strengthen gastric mucosal defenses through increased mucus production [4]. Since high dosage levels are usually responsible for most of the observed adverse drug effects, in the present study, we have investigated the gastric ulcer preventive and antisecretory activity of ranitidine when used in low doses in 1 : 1 combinations with TN and **1**. Results are interpreted as possible potentiating or synergistic effects between ranitidine and the two new anti-ulcer compounds.

2. Investigations and results

2.1. Effect of the drugs on gastric lesion formation

Tables 1, 2 and 3 show the macromorphological results obtained after submitting the experimental animals to pyloric ligature after they had earlier received the test compounds alone or in combinations. The accumulated gastric juice (77 mEq/l) caused extensive ulceration of the stomach mucosa in the control animals. TN and **1** both dose-dependently inhibited ulcer formation when given alone at doses of 50–100 mg/kg. TN was the more potent inhibitor (100% inhibition) than **1** (50% inhibition) at the highest dose. 1 : 1 combinations of the two compounds (25/25 and 50/50 mg/kg) also led to a dose-dependent inhibition of ulceration but the inhibition obtained at the 50/50 combination (78%) was lower than that obtained with 100 mg/kg of TN alone (Table 1). A combination of **1** and ranitidine also reduced ulcer formation dose-dependently, with lesion indices reducing from 3.74 ± 0.48 in the controls to 1.55 ± 0.40 and 1.10 ± 0.40 for the 25/25 and 50/50 mg/kg doses (Table 2). Ranitidine alone (100 mg/kg) totally inhibited ulcer formation. When TN was combined with ranitidine (25/25 mg/kg), the inhibition achieved (70.6%) was greater than that obtained either with 50 mg/kg of TN alone (41.7%) or of ranitidine alone (58.0%) (Table 3).

2.2. Effect of the drugs on gastric acid secretion and ulcer formation

Tables 3, 4 and 5 show the biochemical results obtained after pylorus ligation.

When given alone at the dose of 50 mg/kg, TN reduced gastric acidity to 47 mEq/l but **1**, at the same dose, did not affect gastric acid levels (83 mEq/l) compared with the

Table 1: Effects of combinations of TN and 1 on gastric lesion formation in rats

Treatment	Dose (mg/kg)	N	Lesion index (mean ± SEM)	Inhibition (%)	Ulcerated Surface (%)
Control	–	6	3.74 ± 0.48	–	0.36
TN	50	6	2.81 ± 0.68*	41.7	1.25
TN	100	6	0.00 ± 0.00*	100	0.00
1	50	6	3.01 ± 0.69	19.5	2.60
1	100	6	1.87 ± 0.54*	50.0	0.61
TN/**1**	25/25	6	1.98 ± 0.52	47.1	1.36
TN/**1**	50/50	6	0.84 ± 0.36*	77.8	0.36

* Statistically significant relative to control ($P < 0.01$); N, number of rats

Table 2: Effects of combinations of 1 and ranitidine on gastric lesion formation in rats

Treatment	Dose (mg/kg)	N	Lesion index (mean ± SEM)	Inhibition (%)	Ulcerated surface (%)
Control	–	6	3.74 ± 0.48	–	0.36
1	50	6	3.01 ± 0.69	19.5	2.60
1	100	6	1.87 ± 0.54*	50.0	0.61
1/Ranitidine	25/25	6	1.55 ± 0.40*	58.6	0.59
1/Ranitidine	50/50	6	1.10 ± 0.40*	70.6	0.18
Ranitidine	100	6	0.00 + 0.00*	100	0.00

* Statistically significant relative to control ($P < 0.01$); N, number of rats

controls. However, a 1 : 1 combination of **1** and TN (50/50 mg/kg) reduced gastric acidity to 50 mEq/l compared with 77 mEq/l for the controls (Table 4). A similar reduction in gastric acidity was achieved (52 mEq/l) when the lower dose (25/25 mg/kg) of **1** and ranitidine was tested (Table 5). TN alone 100 mg/kg) significantly reduced acid production to 25 mEq/l with total inhibition of ulceration. However, although a 50/50 combination with ranitidine

Table 3: Effects of combinations of TN and ranitidine on gastric lesion formation in pylorus ligated rats

Treatment	Dose (mg/kg)	N	Lesion index (mean ± SEM)	Inhibition (%)	Ulcerated surface (%)
Control	–	6	3.74 ± 0.48	–	0.36
TN	50	6	2.81 ± 0.68*	41.7	1.25
TN	100	6	0.00 ± 0.00*	100	0.00
TN/Ranitidine	25/25	6	1.10 ± 0.44	70.6	0.50
TN/Ranitidine	50/50	6	0.35 ± 0.05*	90.64	0.12
Ranitidine	50	6	1.57 ± 0.32*	58.00	0.72

*Statistically significant relative to control ($P < 0.01$); N, number of rats

Table 4: Effects of combinations of TN and PAL on gastric acidity and mucus secretion in pylorus ligated rats

Treatment	Dose	N	Volume of gastric juice (mean ± SEM)	Gastric acidity (mEq/l) (mean ± SEM)	Mucus production (mg) (mean ± SEM)
Control	–	6	3.38 + 0.83	77.20 ± 5.80	96.51 ± 24.51
TN	50	6	3.17 ± 0.25	47.45 ± 6.72*	96.37 ± 12.25
TN	100	6	3.00 ± 0.31	24.91 ± 4.83*	100.23 ± 14.83
1	50	6	5.50 ± 0.43*	83.60 ± 13.20*	98.12 ± 26.50
1	100	6	6.50 ± 0.43*	82.50 ± 11.28*	129.55 ± 10.41
TN/**1**	25/25	6	4.86 ± 0.73	70.77 ± 5.80	96.51 ± 24.51
TN/**1**	50/50	6	5.34 ± 0.63*	49.70 ± 6.38*	95.56 ± 12.49

* Statistically significant relative to control ($P < 0.01$); N, number of rats

Table 5: Effects of combinations of PAL and ranitidine on gastric acidity and mucus secretion in pylorus ligated rats

Treatment	Dose	N	Volume of gastric juice (ml) (mean ± SEM)	Gastric acidity (mEq/l) (mean ± SEM)	Mucus production (mg) (mean ± SEM)
Control	–	6	3.38 + 0.83	77.20 ± 5.80	96.51 ± 24.51
1	50	6	5.50 ± 0.43*	83.60 ± 13.20*	98.12 ± 26.50
1	100	6	6.50 ± 0.43*	82.50 ± 11.28*	129.55 ± 10.41*
1/Ranitidine	25/25	6	3.50 ± 0.34*	52.50 ± 0.07	95.85 ± 11.30
1/Ranitidine	50/50	6	5.80 ± 0.91*	51.25 ± 3.67*	99.40 ± 15.42
Ranitidine	100	6	3.36 ± 0.25*	9.58 ± 0.05*	105.46 ± 12.26

* Statistically significant relative to control ($P < 0.01$); N, number of rats

Table 6: Effects of combinations of TN and ranitidine on gastric acidity and mucus secretion

Treatment	Dose	N	Volume of gastric juice (ml) (mean ± SEM)	Gastric acidity (mEq/l) (mean ± SEM)	Mucus production (mg) (mean ± SEM)
Control	–	6	3.38 + 0.83	77.20 ± 5.80	96.51 ± 24.51
TN	50	6	3.17 ± 0.25	47.45 ± 6.72*	96.37 ± 12.25
TN	100	6	3.00 ± 0.31	24.91 ± 4.83*	100.23 ± 14.83
TN/Ranitidine	25/25	6	4.48 ± 0.44*	52.10 ± 0.09	89.40 ± 8.49*
TN/Ranitidine	50/50	6	4.36 ± 0.64*	41.20 ± 5.80*	96.60 ± 15.20
Ranitidine	50	6	3.45 ± 0.35	44.46 ± 3.45*	91.26 ± 8.27

* Statistically significant relative to control ($P < 0.01$); N, number of rats

reduced acid levels only to 41 mEq/l, the level of inhibition of ulcer formation was still significantly high (90%) (Table 6). Mucus secretion was significantly high for all the preparations used although **1** (100 mg/kg) gave the highest values.

3. Discussion

1 : 1 combination (25/25 mg/kg) of **1** and the potent well-known anti-secretory agent, ranitidine, resulted in significant reduction of ulceration (59% I). The results show that **1**, when given alone, does not show anti-secretory effects. However, addition of **1** in small amounts to compounds with potential anti-secretory activity leads to significant reduction of gastric ulceration at gastric acid levels (50–70 mEq/l) that have previously been shown [5, 6] to result in severe gastric ulceration. Compound **1** therefore appears to have a potentiating mucosal protective effect when used in combination with anti-secretory preparations. Since pepsin and HCl are important for the formation of pylorus ligated ulcers, the effect of **1** when used alone in increasing doses could arguably be achieved either through increased mucus secretion that re-enforces gastric mucous defenses, or through the reduction of the proteolytic activity of the pepsin in the gastric juice. However, the potentiating effect of **1** does not appear to be linked to mucus production alone since combinations of **1** and the anti-secretory agents did not significantly increase mucus production compared with the controls. Additional cytoprotective mechanisms involving the physico-chemical re-enforcement of the gastric mucous layer or direct protective effects similar to endogenous prostaglandins may be

involved. Akhtar and Ahmad [7] found a similar activity for the methanolic extract of *Trianthema pentandra* which did not show any decrease in aspirin-induced acid secretion or pepsin content or an increase in mucin but showed a highly significant decrease in the ulcer index.

In peptic ulcer therapy, the modern standard drug combinations include antacids, antisecretory agents as well as antibiotics for *H. pylori* eradication. Constipation constitutes a frequent side effect brought about by antacids and anti-secretory drugs such as ranitidine, cimetidine and famotidine. The role of antacids in peptic ulcer therapy is to provide the necessary acid neutralizing capacity against abnormally high gastric secretions and thus prevent the necrotizing effect of the stomach acid secretions. The cationic antacids (magnesium and aluminum hydroxides), unlike the anionic ones (calcium and sodium carbonates), in addition to the irreversible acid neutralization effect, can also undergo hydrolysis. The result is the formation of variable proportions of chlorides, hydroxychlorides and hydroxide complexes, which provide the buffering power in the gastrodoudenal environment [8]. However, these cationic antacids can reduce the bioavailability of ranitidine, cimetidine and famotidine by 30 to 40% [2]. Due to their effects on gastric pH, the antacids also modify proteolytic activity since pepsine becomes inactive at pH 4–6 [8]. The high gastric acidities that accompany the anti-ulcer effects observed when **1** is combined with the anti-secretory agents therefore imply that the combinations may not interfere with protein digestion in the stomach, a process that requires an optimum pH range of 1.6 to 3.2. The combination of a product like **1**, which has stomach mucosal strengthening potency, with antisecretory agents like TN and ranitidine may thus comprise alternative therapeutic combinations where the products maintain stomach acid concentrations at levels physiologically adequate for peptic digestion, but at the same time prevent the high acid levels from causing mucosal ulceration. The healing effects of TN and **1** on chronic acetic acid induced ulcers have been shown [3, 4].

The results also showed significant decreases in ulcer indices from 3.74 in the controls to 1.1 and 0.35 when increasing doses (25/25 and 50/50 mg/kg) of ranitidine and TN were given to the test animals. This was associated with a dose-dependent reduction in gastric acidity. Ranitidine is a known potent anti-secretory drug with histamine H_2 blocking activity. The potential anti-secretory actions of TN have recently been demonstrated using a modified augmented histamine test, with the results suggesting that similar histamine H_2 receptor blocking actions may be involved [3]. The dose-dependent reduction in gastric acidity therefore suggests synergistic anti-secretory effects of the two compounds. The dose-dependent increase in % I (70–90%) which accompanied the declining gastric acid levels (52–41 mEq/l) also suggest possible synergistic gastric defense actions of TN and ranitidine.

The results of this study confirm the anti-secretory actions of TN and ranitidine, as well as the gastric cytoprotective actions of **1**. The results also demonstrate the possibility of using the two new anti-ulcer compounds and ranitidine in combination at doses lower than the effective therapeutic level of each compound when used alone. In particular, combinations between **1** and any of the anti-secretory products may be useful in reducing digestive side effects related to physiologically low acid secretion. Further work is envisaged in order to evaluate the toxicity profiles of the two new anti-ulcer compounds.

4. Experimental

4.1. Materials

Ranitidine (Glaxo Wellcome) and Betadine (Asta Medica, Labo. Darge, Cedex, France), were obtained from a local pharmacy. Dimethyl sulphoxide and Tween-20 were obtained from Sigma Chemical Co.

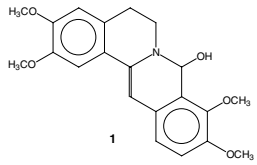
4.2. Animals

Male Wistar rats (160–200 g) raised in the animal house of the Faculty of Medicine and Biomedical Sciences, University of Yaounde I, were used. They were fed a standard laboratory diet (S.P.C. Ltd, Bafoussam, Cameroon) and given fresh water *ad libitum*. Before the experiments, they were starved for 48 h in wire mesh bottom cages to prevent coprophagy but allowed free access to water.

4.3. Preparation of the anti-ulcer compounds

The mature fruits of *V. africana* were harvested in Yaounde in August/September 1998 and sun dried. A voucher specimen No. HNC/1949 (P. Nana: Collector), has been deposited at the National Herbarium, Yaounde. A 1 : 1 mixture of methylene chloride and methanol (5 l) was used to extract 5 kg of dried ground powder. The resulting solid (300 g) was fractionated by bioassay-guided procedure to obtain 5 g of a pure active component (TN). The Meyer test gave a positive reaction indicating that TN is an alkaloid but the $FeCl_3$ test (characteristic of phenolic or enolic hydroxy groups) gave a negative reaction. Preliminary chemical and physical data suggest that TN may correspond to tabersonine hydrochloride (m.p. 196 °C. $[K]_D$ −307, elemental analysis: $C_{20}H_{26}O_2N_2 \cdot HCl$). The detailed structural elucidation of the compound will be published later. 50 mg of TN were dissolved in 3 to 5 drops of DMSO and the solution made up to 100 ml using a 0.25% solution of Tween®-20. A 1% solution of DMSO in 0.25% Tween®-20 was prepared and used as the vehicle control.

E. chlorantha was collected in May 1997 by B. Sonke at Edéa (Littoral Province, Cameroon) and a voucher specimen No. 25918/SRFCAM was deposited in the National Herbarium, Yaounde. A detailed description of the extraction and isolation, as well as the characterisation of **1** has been given by Wafo et al. [9]. Briefly, 200 g of the crude material, resulting from the evaporation of the methanol extract of *E. chlorantha* bark, was treated with 10% aqueous HCl (2 l) to yield 30 g of yellow solid precipitate. The filtrate was extracted with methylene chloride and subsequent lyophilisation gave a fluffy solid (80 g), which was chromatographed on silica gel (600 g), using hexane/methylene chloride mixtures of increasing polarity and collecting 200 ml fractions. Identical fractions by TLC obtained upon elution with hexane/$CHCl_3$ (7 : 3) were pooled and the solvent removed. The semi solid thereby obtained was further purified on a smaller silica gel column using the same eluting system as above to give a solid (2 g) that was recrystallised from hot methanol to yield yellow crystals of **1** (1.2 g): m.p. 188–190 °C. $[K]_D$ −35.0 (c 0.08 $CHCl_3$). IR ν max (KBr) cm^{-1}: 3421, 1606, 1511, 1490, 1343, 1190, 1036, 1001, 975, 830, 807. EIMS m/z: 352 $[M\text{-}17]^+$. 750 mg of **1** were dissolved in a few drops of DMSO and the solution made up to 50 ml using Tween-20 (0.25%). The yellow solution obtained (15 mg/ml) was given to the rats by oral route.



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4.4. Pylorus ligated gastric secretion and ulceration

A literature method by [10] was used. Following the 48 h fast, test compounds and vehicle were administered orally to the test and control rats, respectively. The stomachs of the rats were opened 1 h later. The pylorus of each rat was tied under light ether anaesthesia and the abdominal incisions were closed. The rats were killed 6 h later and the gastric juice produced by each was collected, centrifuged, and the volume measured. Ulcers formed in the glandular portion of the stomachs 6 h after pyloric ligation were scored using a modification of literature methods [11, 12]: 0, no ulcer; 1, vessel dilatation and pointed ulcers; 2.5, small ulcers < 4mm long; 5, large ulcers > 5 mm long. Ulcer index for each animal was calculated as the mean ulcer score and %-inhibition and%-ulcerated surface were estimated as before [9].

4.5. Measurement of acid content of gastric juice

Samples of gastric contents (1 ml) were analyzed for hydrogen ion concentration by pH-metric titration with 0.1 N NaOH solution using a digital pH meter. The acid content was expressed as mEq/l.

4.6. Measurement of mucus production

The gastric mucosa of each rat was gently scraped using a glass slide and the mucus obtained was weighed using a precision electronic balance. The same experimenter performed this operation each time.

4.7. Statistical analysis

Values in tables are given as arithmetic means ± standard error of the mean (S.E.M.) The significance of differences between means was calculated using the student's *t*-test.

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