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Effects of cupric chloride and tamrabhasma, a traditional Indian preparation of copper, on eicosanoid production by human gastric and colonic mucosa

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Abstract—Tamrabhasma is a traditional copper oxide-containing Indian preparation which has anti-ulcer activity. We have studied the effect of tamrabhasma and $CuCl_2 \cdot 2H_2O$ on prostaglandin formation by human gastric and colonic mucosa and submucosa, as prostaglandins have mucosal protective activity, and their release may contribute to the anti-ulcer effect. With the gastric mucosa, tamrabhasma 10 $\mu g\ mL^{-1}$, but not 0·1 or 1 $\mu g\ mL^{-1}$, increased prostaglandin E (PGE) concentration by 38% ($P < 0.05$), with little or no effect on 6-keto-$PGF_{1\alpha}$, thromboxane B_2 or the leukotriene (LT) LTC_4/LTD_4. $CuCl_2 \cdot 2H_2O$ (10 $\mu g\ mL^{-1}$) increased 6-keto-$PGF_{1\alpha}$ by 46% ($P < 0.05$), but 0·1, 1, 50 and 250 $\mu g\ mL^{-1}$ did not significantly affect any of the prostanoids, and only the highest concentration reduced the amount of LTC_4/LTD_4. In the colon mucosa, tamrabhasma (0·1–10 $\mu g\ mL^{-1}$) or $CuCl_2 \cdot 2H_2O$ (10–50 $\mu g\ mL^{-1}$) increased all the prostanoids and this effect was greater than in the gastric mucosa but there was no significant change in LTC_4/LTD_4. $CuCl_2 \cdot 2H_2O$ showed a bell-shaped concentration-effect curve, with no significant effect at lower and higher amounts. Indomethacin (0·1–10 $\mu g\ mL^{-1}$) caused a concentration-dependent reduction in the prostanoid amounts. The effect of tamrabhasma was probably not only due to the presence of Cu^{2+}, as tamrabhasma was more effective than $CuCl_2 \cdot 2H_2O$ alone; in addition the solubility of CuO is very low. Increased prostanoid levels might explain, at least partly, the gastric mucosal protection by tamrabhasma. The results in the colon, however, raise the possibility that tamrabhasma should be examined for the treatment of inflammatory bowel disease.

The medicinal use of metals in India was cited as long ago as the Vedic and Samhite periods (4500–2500 BC and 2500–500 BC, respectively). Copper, in the form of the complex mixture tamrabhasma has been used for treating various ailments including 'amlapitta', a clinical entity resembling peptic ulceration (Misra & Vaisya 1969; Sanyal et al 1982). The complex Ayurvedic processes for manufacturing tamrabhasma are said to reduce toxicity and enhance the therapeutic quality of the copper compound (Dixit 1980). Tamrabhasma contains 44–66% CuO, Fe_2O_3 (up to 6%), sulphur (up to 2·75%), and unidentified material (Raghunathan 1976).

The anti-ulcer effect of tamrabhasma has been demonstrated, using various models of experimental gastric and duodenal ulceration in rats and guinea-pigs (Sanyal et al 1982; Pandey et al 1983; Das et al 1983). Tamrabhasma decreased acid and pepsin secretion in rats, increased mucin, and promoted defensive mucosal resistance factors for up to 5 days after stopping the treatment (Pandey et al 1983).

In rats, the effective anti-ulcer dose of tamrabhasma is 1 mg kg^{-1} (Sanyal et al 1982); no toxicity was found with acute (1 g kg^{-1}) or subacute (100 mg kg^{-1} for 7 days) oral administration. Tamrabhasma can be bought without prescription in India, and since no serious toxicity has been reported it appears that excessive copper absorption does not occur from the preparation.

Tamrabhasma from three different sources showed only slight quantitative differences in anti-ulcerogenicity, and all of them were better than either the pure copper compound or a mixture of the known ingredients (Pandey et al 1983; Mahendri & Rao 1981).

Eicosanoids are thought to play roles in gastrointestinal inflammation and ulceration, and prostaglandin E (PGE) compounds can protect the gastrointestinal mucosa from damage and can exert both pro- and anti-inflammatory actions (Scratcherd 1987; Zurier 1988). Decrease of prostaglandin breakdown has been suggested as a mechanism by which sulphasalazine exerts its therapeutic effect in ulcerative colitis (Hoult & Moore 1978). $CuCl_2$ (0·1 mM) inhibited the conversion of arachidonic acid to PGE_2, but stimulated the formation of $PGF_{2\alpha}$ by homogenates of sheep seminal vesicular tissue (Maddox 1973). A similar result was obtained with higher concentrations of $CuCl_2$ in a microsomal preparation of bovine seminal vesicles (Boyle et al 1976). The present study compares the effect of tamrabhasma and Cu^{2+} on the accumulation of eicosanoids in the fluid incubating pieces of human gastric and colonic mucosa/submucosa. A brief account of the gastric work has been presented by Tavares et al (1990).

Materials and methods

Materials. Tamrabhasma powder (Dabur Private Ltd) was obtained from a batch (S6–150, MED 10/1985) known to have anti-ulcer activity. An aqueous suspension was homogenized (1 mg mL^{-1}, Silverson homogenizer) diluted in water to give stock suspensions of 1, 10 and 100 $\mu g\ mL^{-1}$, and stored at −20°C. For incubation, these suspensions were diluted 10-fold in phosphate-buffered saline pH 7·4 (PBS), to give final concentrations of 0·1, 1 and 10 $\mu g\ mL^{-1}$. Aqueous $CuCl_2 \cdot 2H_2O$ (Sigma) was diluted in water to give stock concentrations of 1, 10, 100, 500 and 2500 $\mu g\ mL^{-1}$, and diluted 10-fold with PBS on the day of incubation to give final concentrations of 0·1, 1, 10, 50 and 250 $\mu g\ mL^{-1}$. All amounts of $CuCl_2$ refer to the hydrated salt. Tamrabhasma has an overall solubility in water of approximately 1%, whereas 12 ng mL^{-1} CuO is a saturated solution at 50°C (Hayward et al 1967), and $CuCl_2$ is very soluble. We do not know about other compounds or complexes that might be present in tamrabhasma and may add to the amount of released copper.

Human gastric and colonic mucosa/submucosa. Tissues were taken at least 5 cm from any macroscopically detected lesions in surgical specimens removed from patients with benign or malignant disease. As far as we know, the patients had not consumed, within the previous 4–6 days, any drug known to affect eicosanoid synthesis. The mucosa/submucosa was carefully cut away from the underlying muscle while the tissue was bathed in PBS. Carefully weighed pieces of mucosa (3–5 mm^2, 100 ± 5 mg) were pre-incubated (1 mL PBS, 0°C, 30 min) in the absence or presence of drugs (indomethacin or tamrabhasma, 0·1, 1 and 10 $\mu g\ mL^{-1}$, or $CuCl_2$ 0·1, 1, 10, 50 and 250 $\mu g\ mL^{-1}$). This pre-incubation fluid was discarded and replaced with fresh PBS, with or without drug as appropriate. After further incubation at 37°C for 30 min, the fluid which contained the released eicosanoids was removed and stored at −20°C for up to 2 weeks before the assay. The effects of tamrabhasma and $CuCl_2$ on eicosanoid production were studied separately using different specimens.

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Radioimmunoassay. The measurements were carried out in duplicate using suitable dilutions of specific antisera and tritiated standards (Jaffe & Behrman 1974). Assay sensitivities were about 20 pg for prostanoids and 50 pg for the leukotrienes LTC_4/LTD_4. Intra- and inter-assay coefficients of variation were 5–9% and 10–11% depending on the eicosanoid measured.

Tritiated prostanoids were purchased from Amersham Radiochemical Centre (Amersham, UK). The PGE antiserum was from ICN (High Wycombe, UK); this antiserum does not distinguish between PGE_1 and PGE_2. TXB_2 and 6-keto-$PGF_{1\alpha}$ antisera were from Wellcome (Beckenham, UK). Cross-reactions were as previously reported (Tavares et al 1987; Goel et al 1989).

Tritiated LTC_4 was purchased from Dupont (UK) Ltd (Stevenage). The percent cross-reactions of the antiserum, provided by Dr J. Zakrzewski, Department of Thoracic Medicine, King's College School of Medicine and Dentistry, were: LTC_4 100; LTD_4 29·4; LTE_4 0·7; LTB_4 0·05; PGE < 0·05; arachidonic acid < 0·05.

Results are shown as means ± s.e. and were analysed statistically by Student's *t*-test for paired data (2-tailed).

Results and discussion

Gastric mucosa; the effect of tamrabhasma. The amounts of prostanoids accumulating in the fluid incubating gastric mucosal pieces, measured by radioimmunoassay (ng (g wet wt tissue)$^{-1}$/30 min) were: PGE 139·4 ± 13·6; TXB_2 68·9 ± 13·4; 6-keto-$PGF_{1\alpha}$ 64·5 ± 14·0 (n = 7). Tamrabhasma 10 μg mL^{-1}, increased the amount of PGE by 38% ($P < 0.05$), but had no significant effect on the other prostanoids measured (TXB_2 and 6-keto-$PGF_{1\alpha}$). Lower concentrations of tamrabhasma, 0·1 and 1·0 μg mL^{-1} had little or no effect on any of the eicosanoids studied (Table 1). Indomethacin, 0·1–10 μg mL^{-1}, caused a concentration-related inhibition of prostanoid formation (PGE by 40–79%, $P < 0.02$ to $P < 0.01$; TXB_2 39–81%, $P < 0.1$ to $P < 0.05$; 6-keto-$PGF_{1\alpha}$ 38–83%, $P < 0.01$; Table 1).

LTC_4/LTD_4 accumulation in control gastric mucosal incubates was 3·7 ± 1·0 ng (g wet tissue)$^{-1}$/30 min. The 13–37% lower mean amounts of LTC_4/LTD_4 with tamrabhasma 0·1–10 μg mL^{-1} were not statistically significant ($P < 0.5$ to $P < 0.2$, Table 1).

Gastric mucosa; effect of $CuCl_2$. In a separate study with $CuCl_2$ the amounts of prostanoids accumulating in the control incubates of human gastric mucosa were (ng (g wet tissue)$^{-1}$/30 min): PGE 97·7 ± 13·4; TXB_2 78·3 ± 8·6; 6-keto-$PGF_{1\alpha}$ 53·4 ± 6·7) (n = 5). $CuCl_2$ 10 μg mL^{-1} increased the amount of 6-keto-$PGF_{1\alpha}$ by 46% ($P < 0.05$), and there was a similar trend with PGE and TXB_2 (mean amounts respectively 28 and 33% higher; $P < 0.1$ and $P < 0.2$; Table 2).

LTC_4/LTD_4 accumulation in control gastric mucosal incubates was 3·9 ± 0·7 ng (g wet tissue)$^{-1}$/30 min. With $CuCl_2$ 0·1, 1 or 10 μg mL^{-1} there was little or no change, but 250 μg mL^{-1} caused a decrease of 39% ($P < 0.05$; Table 2).

Colon mucosa; effect of tamrabhasma. The amounts of prostanoids accumulating in incubates of colonic mucosal pieces (ng (g wet tissue)$^{-1}$/30 min) were: PGE 33·9 ± 7·2; TXB_2 25·8 ± 4·2; 6-keto-$PGF_{1\alpha}$ 54·4 ± 11·8 (n = 6). Tamrabhasma 0·1–10 μg mL^{-1} increased the amount of PGE (34–80%, $P < 0.05$) and 6-keto-$PGF_{1\alpha}$ (19–46%, $P < 0.05$ to $P < 0.02$), but only 10 μg mL^{-1} tamrabhasma significantly increased TXB_2 accumulation (Table 3). The effect of 0·1 μg mL^{-1} tamrabhasma on colonic PGE and 6-keto-$PGF_{1\alpha}$ was greater than with gastric tissue (both $P < 0.05$).

Indomethacin 10 μg mL^{-1} inhibited colonic prostanoid accumulation (PGE 77% inhibition, $P < 0.02$; TXB_2 92%, $P < 0.05$, and 6-keto-PGF_{α} 87%, $P < 0.02$; Table 3). Similar trends occurred with indomethacin 1 and 0·1 μg mL^{-1}.

LTC_4/LTD_4 accumulation in control incubates of colonic

Table 1. Effects of different concentrations of tamrabhasma and indomethacin on the accumulation of endogenous eicosanoids in incubates of human gastric mucosal pieces.

Drugs (μg mL^{-1})	PGE	TXB_2	6-Keto-$PGF_{1\alpha}$	LTC_4/LTD_4
Tamrabhasma				
0·1	99·1 ± 9·1	81·9 ± 15·2	83·5 ± 17·1	86·8 ± 13·1
1·0	103·1 ± 11·3	89·2 ± 10·6	94·6 ± 10·3	95·6 ± 12·0
10·0	138·2 ± 9·7[a]	101·2 ± 8·7	110·3 ± 7·9	63·1 ± 11·1
Indomethacin				
0·1	60·1 ± 11·2[b]	61·6 ± 10·3	62·1 ± 7·8[c]	—
1·0	31·5 ± 8·0[c]	21·3 ± 4·1[a]	30·3 ± 5·9[c]	—
10·0	21·3 ± 8·7[c]	19·2 ± 5·7[a]	17·0 ± 3·8[c]	—

The values are expressed as percent PBS control, means ± s.e., n = 7, and analysed by Student's *t*-test for paired data. The highest concentration of tamrabhasma, 10 μg mL^{-1}, increased the amount of PGE, whereas indomethacin caused concentration-dependent decreases in all the prostanoids. *P* values: [a] < 0·05, [b] < 0·02, [c] < 0·01 compared with the PBS control. Amounts of eicosanoids (ng (g wet tissue)$^{-1}$/30 min) in the PBS controls were: PGE 139·4 ± 13·6; TXB_2 68·9 ± 13·4; 6-keto-$PGF_{1\alpha}$ 64·5 ± 14·0; LTC_4/LTD_4 3·7 ± 1·0.

Table 2. Effects of different concentrations of $CuCl_2 \cdot 2H_2O$ on the accumulation of endogenous eicosanoids in incubates of human gastric mucosal pieces.

$CuCl_2 \cdot 2H_2O$ (μg mL^{-1})	PGE	TXB_2	6-Keto-$PGF_{1\alpha}$	LTC_4/LTD_4
0·1	90·4 ± 6·3	97·3 ± 4·8	101·9 ± 19·3	96·7 ± 7·4
1·0	94·5 ± 6·7	109·0 ± 10·1	116·1 ± 17·8	107·1 ± 13·1
10·0	128·3 ± 14·7	133·2 ± 20·1	146·1 ± 14·1[a]	97·3 ± 9·1
50·0	103·2 ± 17·6	101·2 ± 12·3	139·8 ± 23·6	75·1 ± 15·2
250·0	114·1 ± 23·9	111·3 ± 21·5	99·3 ± 31·3	60·9 ± 10·1[a]

Values are expressed as percent PBS control, means ± s.e. (n = 5), and analysed by Student's *t*-test for paired data. The 10 μg mL^{-1} concentration increased the amount of 6-keto-$PGF_{1\alpha}$, and there was a trend for increases of the other prostanoids, whereas 250 μg mL^{-1} decreased the amount of LTC_4/LTD_4. *P* value: [a] < 0·05 compared with the PBS control. Amounts of eicosanoids (ng (g wet tissue)$^{-1}$/30 min) in the PBS controls were: PGE 97·7 ± 13·4; TXB_2 78·3 ± 8·6; 6-keto-$PGF_{1\alpha}$ 53·4 ± 6·7; LTC_4/LTD_4 3·9 ± 0·7.

Table 3. Effects of tamrabhasma and indomethacin on the accumulation of prostanoids in incubates of pieces of human colonic mucosa/submucosa.

Drugs (μg mL^{-1})	PGE	TXB_2	6-Keto-$PGF_{1\alpha}$	LTC_4/LTD_4
Tamrabhasma				
0·1	133·8 ± 9·9[a]	86·5 ± 7·0	126·2 ± 6·1[b]	86·8 ± 18·8
1·0	138·4 ± 15·9[a]	112·6 ± 15·3	118·6 ± 7·8	88·7 ± 32·2
10·0	179·5 ± 35·2[a]	130·0 ± 6·9[c]	145·5 ± 10·2[a]	69·4 ± 17·3
Indomethacin				
0·1	72·8 ± 18·9	62·3 ± 13·6	70·4 ± 11·7	—
1·0	42·0 ± 13·4[a]	20·6 ± 9·1	34·1 ± 10·5[a]	—
10·0	22·7 ± 9·0[b]	8·2 ± 2·8[a]	12·8 ± 3·8[c]	—

Results are expressed as percent control (means ± s.e., n = 6) and analysed by Student's *t*-test for paired data. *P* values: [a] < 0·05, [b] < 0·02, [c] < 0·01 compared with PBS control. Amounts of eicosanoids (ng (g wet tissue)$^{-1}$/30 min) in the PBS controls were: PGE 33·9 ± 7·2 TXB_2 25·8 ± 4·2; 6-keto-$PGF_{1\alpha}$ 54·4 ± 11·8; LTC_4/LTD_4 1·70 ± 0·51.

Table 4. Effects of $CuCl_2 \cdot 2H_2O$ on the accumulation of endogenous eicosanoids in incubates of human colonic mucosal pieces.

$CuCl_2 \cdot 2H_2O$ ($\mu g\ mL^{-1}$)	PGE	TXB_2	6-keto-$PGF_{1\alpha}$	LTC_4/LTD_4
0·1	84·4±7·6	87·1±9·7	77·2±17·6	101·2±21·2
1·0	94·6±11·3	96·5±7·7	114·5±14·6	131·5±33·5
10·0	127·3±15·7[a]	125·9±12·5	163·5±12·8[a]	61·6±20·7
50·0	115·2±13·3	130·9±36·1	168·4±40·8	52·7±18·1
250·0	73·2±14·0	72·5±12·6	112·0±25·2	55·5±16·5

$CuCl_2 \cdot 2H_2O$ 10 $\mu g\ mL^{-1}$ increased the amounts of PGE and 6-keto-$PGF_{1\alpha}$ in incubates of human colonic mucosal pieces. Results are expressed as percent control (means ± s.e., n = 6) and analysed by Student's *t*-test for paired data. *P* value: [a] < 0.05 compared with respective PBS controls. Amounts of eicosanoids (ng (g wet tissue)$^{-1}$/30 min) in the PBS controls were: PGE 43·3±6·9; TXB_2 36·7±6·0; 6-keto-$PGF_{1\alpha}$ 46·8±8·7; LTC_4/LTD_4 1·82±0·41.

mucosa was 1·70±0·51 ng (g wet tissue)$^{-1}$/30 min. This showed no significant change with tamrabhasma (0·1–10 $\mu g\ mL^{-1}$, Table 3).

Colon mucosa; effect of $CuCl_2$. In separate specimens, the amounts of prostanoids accumulating in the control incubates of human colonic mucosal pieces (ng (g wet tissue)$^{-1}$/30 min) were: PGE 43·3±6·9; TXB_2 36·7±6·0, 6-keto-$PGF_{1\alpha}$ 46·8±8·7 (n = 6). $CuCl_2$ (10 $\mu g\ mL^{-1}$) increased the amounts of PGE (27%, $P < 0.05$) and 6-keto-$PGF_{1\alpha}$ (64%, $P < 0.05$). The mean amount of TXB_2 was 26% higher ($P < 0.1$; Table 4).

LTC_4/LTD_4 accumulation in control colonic mucosal incubates was 1·82±0·41 ng (g wet tissue)$^{-1}$/30 min, and with $CuCl_2$ there was no significant change.

The CuO in tamrabhasma probably yields about 10 ng mL dissolved Cu^{2+} (Hayward et al 1967) and it therefore seems unlikely that dissolved Cu^{2+} can explain the increase of prostanoids with tamrabhasma.

In the colon, $CuCl_2$ 0·1 or 1 $\mu g\ mL^{-1}$ had no effect, but 10 and 50 $\mu g\ mL^{-1}$ increased PGE and PGI_2 levels, whereas 250 $\mu g\ mL^{-1}$ gave results similar to the controls. This bell-shaped response was also observed in gastric tissues for 6-keto-$PGF_{1\alpha}$ production.

In contrast to our findings with human tissues, Maddox (1973) obtained a reduced PGE_2 formation with 0·1 mM Cu^{2+} in sheep vesicular homogenates. Boyle et al (1976) also showed a reduction in PGE_2 formation in bovine seminal vesicle microsomes, although the Cu^{2+} concentrations used were much higher. The difference may therefore be due to species, tissue type, or position on the concentration-effect curve.

Since endogenous prostaglandins may contribute to ulcer healing by their various actions on mucosal protective factors (Scratcherd 1987), and may have anti-inflammatory effects (Zurier 1988; Hoult & Moore 1978), the ability of tamrabhasma to increase gastric mucosal PGE may explain, at least in part, the anti-ulcer effect of the compound. The even greater effect on colonic mucosal prostaglandin formation raises the importance of studying tamrabhasma as a protective agent in inflammatory bowel disease; the possibility of a prostaglandin anti-inflammatory effect in this disorder is strengthened by the finding that nonsteroidal anti-inflammatory drugs can worsen the condition (Rampton & Sladen 1981). Although an increase of thromboxane formation would presumably be a disadvantage, this occurred only with 100 times more tamrabhasma than that which increased PGE and 6-keto-$PGF_{1\alpha}$. The leukotrienes, which have been implicated as mediators of gastric and colonic mucosal damage (Boughton-Smith et al 1988; Boughton-Smith & Whittle 1988), are not increased by tamrabhasma.

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