J Diarrhoeal Dis Res 1993 Jun;11(2):97 - 100
0253 - 8768/93 $ 1.00 + 0.10

Comparative Effects of Nicotinic Acid and Nicotinamide on Cholera Toxin-induced Secretion in Rabbit Ileum

André Briend,[1,2] Samir Kumar Nath,[1,3] Martine Heyman,[1] Jehan François Desjeux,[1]

[1]Unité INSERM 290, Hôpital Saint Lazare, 107 rue du Faubourg Saint Denis 75010 Paris, France; [2]Seconded by ORSTOM, Institut Français de la Recherche Scientifique pour le Développement en Coopération; [3]Present address: International Centre for Diarrhoeal Disease Research, GPO Box 128, Dhaka 1000, Bangladesh.

ABSTRACT

Nicotinic acid reduces the cholera-toxin induced fluid secretion in experimental animals but its toxicity at high doses prevent its therapeutic use in patients suffering from cholera. This study aimed to determine whether nicotinamide, the non toxic amide derivative of nicotinic acid, is as effective as nicotinic acid in inhibiting cholera toxin induced intestinal secretion *in vivo*. Four intestinal loops, with their blood supply intact, were isolated in 30 rabbits and injected with either (i) 30 mM mannitol, (ii) 30 mM mannitol + 10 µg cholera toxin, (iii) 30 mM glucose, or (iv) 30 mM glucose + 10 µg cholera toxin. These rabbits were then randomly assigned to three groups receiving intraluminally either 100 mg/kg of nicotinic acid, 100 mg/kg of nicotinamide, or 10 ml/kg of Ringer solution. Measurement of intestinal fluid accumulation showed that nicotinic acid, but not nicotinamide, significantly reduced cholera toxin induced intestinal secretion.

Key words: Cholera toxin; Nicotinic acid; Nicotinamide; Rabbit ileal loop.

INTRODUCTION

Nicotinamide is the amide derivative of nicotinic acid or vitamin PP. Both compounds exhibit the same anti-pellagrous vitaminic activities but their pharmacological action at high doses is quite different (1,2). High doses of nicotinic acid induce vaso-dilatation, lower cholesterol levels, and prevent the rise in tissue of cAMP (cyclic adenosine 3',5'-monophosphate) elicited by a variety of stimuli such as adrenaline, ACTH, and glucagon (2,3). High doses of nicotinic acid also have side effects and may induce nausea, vomiting, and peripheral vascular dilation or "flushing". Hepatic toxicity of nicotinic acid has also been reported (2). Nicotinamide has none of the pharmacological effects nor the side effects of nicotinic acid: it does not induce a vasodilatation, has no effect on cholesterol levels, and has no known toxicity (2).

In rabbits, low doses (5 mg/kg) of nicotinic acid prevent cholera toxin-induced intestinal secretion and higher doses (100 mg/kg) reverse the established cholera intestinal fluid secretion (4). There is evidence that nicotinic acid inhibits the formation of cAMP and restores sodium fluxes to normal levels (4-5). A therapeutic trial in Bangladesh using 50 to 60 mg/kg of nicotinic acid suggested that this drug slightly reduces stool output in cholera patients (6). These doses are lower than those shown to be effective in rabbits to reverse cholera induced intestinal secretion, but use of higher doses is prevented by the existence of side effects.

Nicotinamide may also interfere with the mechanism of action of the cholera toxin (7,8). Cholera toxin induces its pathological effects via ADP-ribolysation of the stimulatory GTP-binding protein G_s following a reaction which produces nicotinamide:

$$NAD + G_s = ADP\text{-ribosylated-}G_s + \text{nicotinamide} + H^+$$

There is evidence that this reaction is reversible by the law of mass action: in isolated rabbit ileum, high doses of nicotinamide has been shown to inhibit and reverse the cholera toxin-induced secretion (7-8). This suggests that high doses of nicotinamide may inhibit or reverse the ADP ribosylation occurring after fixation of the cholera toxin on the enterocyte.

Correspondence and reprint requests should be addressed to: Dr. André Briend.

The purpose of this study was to compare the effects of high doses of nicotinic acid and nicotinamide on cholera-induced intestinal fluid secretion in rabbits *in vivo* to determine whether high doses of nicotinamide could be considered for therapeutic use in cholera patients instead of high doses of nicotinic acid.

After the surgical procedure, the loops were returned to the abdominal cavity and the incision sealed. After 3 hours, rabbits were killed by pentobarbital overdose and the isolated loops removed.

Previous *in vivo* studies examined the effect of nicotinamide and nicotinic acid on cholera toxin-induced fluid secretion after 3 h (4) or 4 h (5). By that time, the net fluid absorption observed in control loops was clearly reversed to a net fluid secretion in the presence of cholera toxin. The lag phase following cholera toxin intraluminal administration during which no fluid secretion is seen usually lasts about 15 min (9) and maximal secretion is reached after 3 h. It was assumed that 3 h was an adequate time lag to compare the effects of nicotinic acid and nicotinamide on cholera toxin-induced fluid secretion.

After removal, loops were weighed, before and after being emptied, by a large incision, to estimate by difference of volume of fluid remaining in the loop. Surface area of the open loops was also measured. Water flux was estimated with the formula:

Table I – Description of different treatment groups allocation

	Treatment					
	Nicotinic acid		Nicotinamide		Ringer	
Number of rabbits	10		10		10	
Number of loops	40		35		40	
	with toxin	without toxin	with toxin	without toxin	with toxin	without toxin
with glucose	10	10	9	9	10	10
with mannitol	10	10	17	9	10	10
Total	20	20	17	18	20	20

MATERIALS AND METHODS

After an overnight fast, 30 white male rabbits weighing on average 2 kg (range: 1500, 2600 g) were anaesthetised with intravenous pentobarbital (2 mg/kg), given via a marginal ear vein. Four ileal loops about 10 cm long proximal to the appendix were emptied manually and then isolated keeping their blood supply intact. At least 2 to 3 cm were left between each loop and each had a different blood supply. Each loop, in random order, was then filled with 5 ml of Ringer solution containing either: (i) 30 mM mannitol, (ii) 30mM mannitol + 10µg cholera toxin, (iii) 30mM glucose, or (iv) 30mM glucose + 10 µg cholera toxin.

This design was used to compare the effect of nicotinic acid and nicotinamide on cholera toxin-induced secretion when the glucose-sodium co-transporter was activated or not. Mannitol, not actively transported by the glucose-sodium co-transporter, was given at the same molar concentration as glucose in loops without glucose to control for the effects of intraluminal osmotic pressure.

Immediately after the loops were isolated, each rabbit was given an injection of 10 ml/kg of a solution taken from a vial identified with only a code number and containing either (i) Ringer, (ii) Ringer + 10 mg/ml nicotinic acid, or (iii) Ringer + 10 mg/ml nicotinamide. This injection was made in the ileum, about 5 cm proximally from the isolated loops. For the last two groups, the nicotinamide and nicotinic acid were given as an 80 mM solution and the total dosage injected was 100 mg/kg or 0.8 mmol/kg. This dose corresponded to the maximal dose which could be used in a therapeutic trial without inducing side effects. No attempt was made to use a lower dose or to establish a dose response curve: it was assumed that an absence of effect with the highest dose would suggest that a lower dose is also ineffective.

Table II. Effect of nicotinic acid and nicotinamide on water flux of rabbit ileum in vivo

a) loops treated with cholera toxin	Mean ($^{*}\mu l/cm^2.h$)	Standard deviation
Controls (n = 20)	−48.4	18.7
Nicotinic acid (n = 20)	−29.5	29.0
Nicotinamide (n = 17)	−48.6	17.5
F = 4.27, 2 degrees of freedom, $p<0.05$		

b) loops without cholera toxin	Mean ($\mu l/cm^2.h$)	Standard deviation
Controls (n = 20)	24.7	34.2
Nicotinic acid (n = 20)	17.9	19.1
Nicotinamide (n = 18)	28.6	22.9
F = 0.81, ns.		

$$J(ml/cm^2.hour) = \frac{\text{injected volume(ml)} - \text{final volume(ml)}}{\text{surface area } (cm^2) \times 3 \text{ hours}}$$

Water flux was regarded as negative when water accumulated in the lumen indicating a fluid secretion.

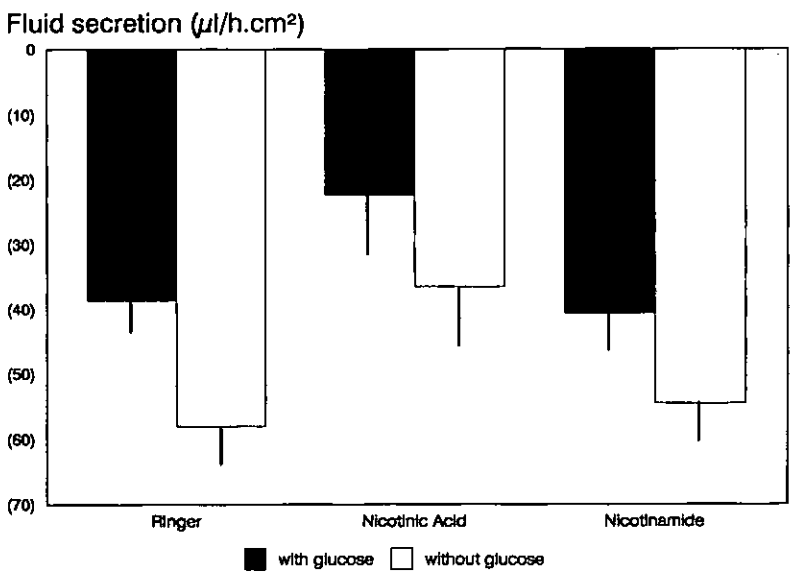
One rabbit died before the measurement of fluid secretion. Another rabbit had a haemorrhagic loop which was not included in the analysis. Altogether, 115 loops were available for analysis (Table I).

For the statistical analysis, loops were considered individually and their fluid accumulation was compared by one-way or two-way analysis of variance.

RESULTS

On average, in rabbits who were given nicotinic acid, fluid accumulation in cholera toxin-stimulated loops was lower than in those who received either nicotinamide or Ringer solution (Table II). There was no significant difference in fluid accumulation in loops without toxin (Table II).

Cholera toxin-stimulated loops which received glucose accumulated less fluid than loops without glucose. This effect was present in the three treatment groups (Figure).



Comparative effect of treatment with nicotinamide and nicotinic acid on water flux (mean +/- sem) in ileal loop with and without glucose.

Two-way analysis of variance: Treatment effect: F = 4.76, 3 df, p = 0.013
Glucose effect: F = 7.72, 1 df, p = 0.008
No significant interaction.

DISCUSSION

This study confirms the anti-secretory effect of nicotinic acid on cholera toxin-induced intestinal secretion, (4,5) although in this study the inhibition of secretion was only partial.

It also confirmed that glucose reduced the cholera induced net fluid secretion, as expected from the activation of the glucose sodium co-transport observed when glucose and sodium are present in the lumen (10).

In addition, this study suggests that nicotinamide is not effective in inhibiting cholera toxin-induced fluid secretion in rabbit ilium *in vivo* . This result contrasts with those obtained on isolated loops *in vitro* (5,6). This discrepancy may be due to differences in local concentration of nicotinamide. In this experiment, nicotinamide was not injected into the loops themselves but proximally from them and reached them through general circulation and then the local blood supply. Since a systemic dose of 0.8 mmol/kg was given, local concentration of nicotinamide may have been lower than the 10 mM concentration in which the loops where bathed *in vitro* and may be not high enough to reverse the ADP-ribosylation of the G_s protein. In any case, this study suggests that high doses of nicotinamide cannot be used instead of nicotinic acid in cholera patients to decrease stool output.

ACKNOWLEDGEMENTS

We thank Mrs. Céline Candalh for her skilful technical assistance.

REFERENCES

1. McCormick DB. Niacin. *In*: Shils ME, Young VR, eds. Modern nutrition in health and disease. 7th ed. Philadelphia: Lea and Fibiger, 1988:370-5.
2. Roe DA. Diet, nutrition and drug reactions. *In*: Shils ME, Young VR, eds. Modern nutrition in health and disease. 7th ed. Philadelphia: Lea and Fibiger, 1988:630-45.
3. Butcher RW, Baird CE, Sutherland EW. Effects of lipolytic and antilipolytic substances on Adenosine 3',5'-monophosphate levels in isolated fat cells. *J Biol Chem* 1968;243:1705-12.
4. Turjman N, Gotterer GS, Hendrix TR. Prevention and reversal of cholera enterotoxin effects in rabbit jejunum by nicotinic acid. *J Clin Invest* 1978;61:1155-60.
5. Turjman N, Cardamone A, Gotterer GS, Hendrix TR. Effect of nicotinic acid on cholera-induced fluid movement and unidirectional sodium fluxes in rabbit jejunum. *Johns Hopkins Med J* 1980;147: 209-11.
6. Rabbani GH, Butler T, Bardhan PK, Islam A. Reduction of fluid-loss in cholera by nicotinic acid. *Lancet* 1983;2:1439-42.
7. Marshall AT, Prenton E, Shiells RA. Nicotinamide reverses and blocks the secretory actin of cholera toxin on isolated rabbit ileum. *J Physiol* (London) 1989;415:97.
8. Falk G, Freeman M, Marshall AT, Prenton E, Shiells RA, Slack I. Nicotinamide reverses and blocks the secretory action of cholera toxin on isolated rabbit ileum. *J Physiol* (London) 1990;421:399-409.

9. Carpenter CCJ. Clinical and pathophysiological features of diarrhea caused by *Vibrio cholerae* and *Escherichia coli*. *In:* Field M, Fordtran JS, Schultz SG, eds. Secretory Diarrhea. Bethesda, MD: American Physiological Society, 1980:67 – 83.

10. Greenough WB III, Khin – Maung – U. Oral rehydration therapy. *In:* Field M, ed. Diarrheal diseases. New York: Elsevier, 1991:485 – 99.