

## No effect of oral N-acetylcysteine on the bioavailability of erythromycin and bacampicillin

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*No effect of oral N-acetylcysteine on the bioavailability of erythromycin and bacampicillin. O. Paulsen, L. Borgström, B. Kägedal, M. Walder.*

**ABSTRACT:** *In vitro* studies with N-acetylcysteine (NAC) solutions used for inhalation treatment have demonstrated inactivation of some antibiotics by NAC. Oral NAC treatment is increasingly common for long-term prophylaxis in chronic bronchitis. During exacerbations, treatment with oral antibiotics will often be given simultaneously. We assessed the effect of simultaneous oral administration of NAC on the bioavailability of two antibiotics in ten healthy volunteers. No effect of NAC was found on the bioavailability of ampicillin, after administration of the prodrug bacampicillin. A slight, but not significant statistical increase in erythromycin serum levels was seen with NAC. Acetylator phenotype did not influence the absorption of NAC, which seemed slightly reduced by bacampicillin, but significantly increased by erythromycin. No decrease of antibacterial activity of sera was found *in vitro* after the addition of NAC or the related thiol glutathione, employing micrococcus luteus and staphylococcus aureus as indicator organisms.

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N-acetylcysteine (NAC) has been used for more than twenty years for inhalation treatment in lung disease complicated by thick mucus secretions. During the last ten years oral NAC has also been effective as a mucolytic agent [7, 10] and extensive trials with continuous medication have shown reduction of the exacerbation rates in patients with chronic bronchitis [2, 12]. During exacerbations, antibiotic treatment is usually instituted.

In earlier studies on inhalation treatment with NAC, inactivation of certain antibiotics occurred when they were added to the NAC solution [6, 9]. No reduction of antibacterial activity occurred when antibiotics were given orally or intramuscularly during inhalation treatment [9, 14].

Interaction studies between oral NAC and antibiotics have demonstrated no NAC influence on the bioavailability of amoxicillin and doxycyclin, reduced absorption of cefalexin, and no decisive effects of erythromycin [5, 11]. Interaction with ampicillin esters has not been studied, although the beta-lactam ring is susceptible to thiol attack and penicillins consequently might be expected to interact with NAC. *In vitro* studies have indicated that an endogenous, related thiol compound, glutathione, may bind to beta-lactam antibiotics, e.g. ampicillin, under physiological conditions [17, 18].

We have investigated the bioavailability after oral, concomitant administration of NAC and erythromycin base or bacampicillin, in healthy volunteers. We also assessed the possible influence of glutathione and

NAC on the antibacterial activity of sera against the indicator organisms, micrococcus luteus and staphylococcus aureus.

### Subjects and methods

The study was approved by the local Ethics Committee and performed in accordance with the Declaration of Helsinki. It was executed as an open comparison of antibiotic and NAC concentrations with simultaneous or separate administration of the drugs.

### Subjects

Ten healthy volunteers, five men and five women, participated in the study. They were 30-45 years old and they weighed 55-91 kg. Physical examinations and laboratory tests were carried out at least one week before the start of the study. No medication beside the test drugs and no alcoholic beverages were allowed during the study or during the preceding week. In all subjects the acetylator phenotype was determined as previously described [13], to evaluate the possible influence of N-acetyltransferase on deacetylation of NAC. Two of the women and four of the men were found to be rapid acetylators.

### Investigational drugs

All drugs were administered in a commercially available form. NAC was given as effervescent tablets

(Mucomyst® 0.2 g), erythromycin as erythromycin base enterocapsules (Ery-Max® 250 mg) and bacampicillin as plain tablets (Pengllobe® 800 mg).

#### Procedure

Initially NAC was given for five days, 600 mg b.i.d., at 08 and 20 h. At each occasion the three NAC tablets were dissolved in 200 ml of tap water. On the 6<sup>th</sup> study day, after overnight fasting, a final dose of NAC, 600 mg, was given and blood for NAC analysis was sampled from an antecubital vein at the following scheduled times: 0, 20, 40 min, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 h after administration. Two hours after drug intake, a standardized breakfast was served [3]. Urine was sampled as one pool 0–12 h for determination of NAC.

At another occasion, after a washout period of at least three days, two tablets of bacampicillin (1600 mg) or four capsules of erythromycin (1000 mg) were administered with 200 ml of tap water in the same way as with NAC and with standardized breakfast and blood sampling for determination of antibiotic concentrations at the same intervals.

After another washout period, medication with NAC effervescent tablets was reinstated with three tablets (600 mg) administered every morning for seven days. On the final day, erythromycin or bacampicillin was administered concomitantly with the last NAC dose, and blood for NAC analysis and antibiotic concentrations was sampled at the time intervals stated above. As previously, urine was collected 0–12 h after drug intake for NAC determination. The differing dosing regimens for the repeated dosing of NAC do not influence the calculated pharmacokinetic values, because of the short elimination half-life of NAC [4].

#### NAC determinations

Plasma samples were analysed by HPLC for concentration of non-protein-bound NAC according to previously described methods [8]. Urine was appropriately diluted and NAC was determined by a procedure similar to that used for plasma NAC determinations.

#### Antibiotic determinations

A microbiological assay, using the agar well system, was deployed using disposable 24 × 24 cm plates with 125 ml DST agar (Oxoid CM 261). Wells with a diameter of 4 mm were made with a regular hole punch machine.

For ampicillin, the indicator organism was *micrococcus luteus* ATCC 9341 in the *in vivo* part of the investigations, and *S. aureus* 6-105 in the *in vitro* part. Antibiotic standards and all samples were run in duplicate. Limits of the assay for ampicillin were 0.05–400 µg·ml<sup>-1</sup>.

For erythromycin, the indicator organism was *micrococcus luteus* ATCC 9341 in both the *in vivo* and the *in vitro* part of the investigation, and the assay

limits were 0.10–100 µg·ml<sup>-1</sup>. The inter- and intra-assay variation was ±10%.

#### Pharmacokinetic calculations

Maximal plasma concentration ( $C_{max}$ ) was defined as the highest experimental plasma concentration obtained, and  $t_{max}$  was the time for  $C_{max}$ . The area under the plasma concentration vs time curve (C,t curve) (AUC), was calculated according to the trapezoidal rule. All AUCs were calculated over the first 12 h. The area under the first moment of the C,t-curve (AUMC) was calculated accordingly. Mean residence time (MRT) is the mean time for drug molecules to transit through the body and was calculated as AUMC/AUC.

Renal clearance, CLR, of NAC was calculated as the amount of NAC excreted in urine, corrected for an endogenously excreted amount, divided by AUC for the 0–12 h interval.

#### Statistical analysis

In the *in vivo* studies, evaluations were made by analysis of variance and two-sided paired Student's *t*-test. Level of significance was set at  $p < 0.05$ .

#### *In vitro* experiment

From a previous study, serum samples had been obtained from six healthy volunteers who had each received 2 g of ampicillin intravenously. Serum samples were taken before infusion, directly after infusion and after 30 min and 1, 1.5 and 2.5 h. In a similar way, sera containing erythromycin after the single-dose administration of this drug were obtained from the present experiment, before antibiotic administration and after 1, 1.5, 2, 3, 4, 6, 8 and 12 h. Each antibiotic-containing sample was divided into four parts. One was assayed for antibiotic concentration and thiol-containing sera were added to the other three parts. To allow for sufficient interaction time, sera were kept for 1 h before bioassay.

#### Thiol sera

The sera were obtained from healthy volunteers. NAC was added in a concentration of 60 µmol·l<sup>-1</sup> and 2 mmol·l<sup>-1</sup> and glutathione in a concentration of 1 mg·ml<sup>-1</sup>. After addition of equal parts of antibiotic-containing sera, the resulting thiol concentrations were chosen to correspond to or exceed levels obtainable *in vivo* in the two principal situations where NAC treatment is instituted, namely for prophylaxis in chronic bronchitis, and as an antidote in paracetamol poisoning. The NAC concentrations after admixture were 30 µmol·l<sup>-1</sup> and 1 mmol·l<sup>-1</sup>, respectively. The glutathione level, 500 µg·ml<sup>-1</sup>, was chosen to correspond to concentrations in whole blood, which greatly exceed those in serum. Thiol sera were tested and found to be without antibacterial activity.

Table I. - Pharmacokinetic parameters for ampicillin and erythromycin after oral dosing with and without simultaneous NAC medication

	$C_{max}$ mg·l	$t_{max}$ h	AUC mg·h·l <sup>-1</sup>	MRT h
Mean ampicillin	14.2	1.18	35.4	2.2
SD	10.3	0.35	18.6	0.2
Mean ampicillin + NAC	11.5	1.00	31.5	2.3
SD	3.9	0.30	9.5	0.1
Mean erythromycin	4.9	3.4	22.1	5.1
SD	2.3	1.2	13.8	0.6
Mean erythromycin + NAC	6.0	3.5	25.1	5.0
SD	4.0	0.5	14.4	0.4

$C_{max}$ : highest experimental plasma concentration;  $t_{max}$ : time for  $C_{max}$ ; AUC: area under the plasma concentration vs time curve; MRT: mean residence time.

## Results

### Pharmacokinetics of antibiotics

After a single dose of antibiotic, none of the pharmacokinetic parameters for the antibiotics observed differed from those obtained with concomitant NAC administration (table I, figs 1 and 2).

Mean serum levels of ampicillin were slightly lower with NAC co-medication. One individual demonstrated extremely high  $C_{max}$  for ampicillin after single dose medication, with a correspondingly high AUC. With concomitant NAC,  $C_{max}$  in this subject was reduced from 42.8 to 21.4 mg·l<sup>-1</sup>, and AUC decreased from 87.3 to 53.0 mg·h·l<sup>-1</sup>. When this subject was excluded, mean  $C_{max}$  in the remaining nine subjects was 11.0 mg·l<sup>-1</sup> without, and 10.4 mg·l<sup>-1</sup> with NAC co-medication. Mean AUC was the same, 29.6 and 29.1 mg·h·l<sup>-1</sup>. Thus, NAC did not seem to influence the pharmacokinetics of bacampicillin (table I, fig. 2).

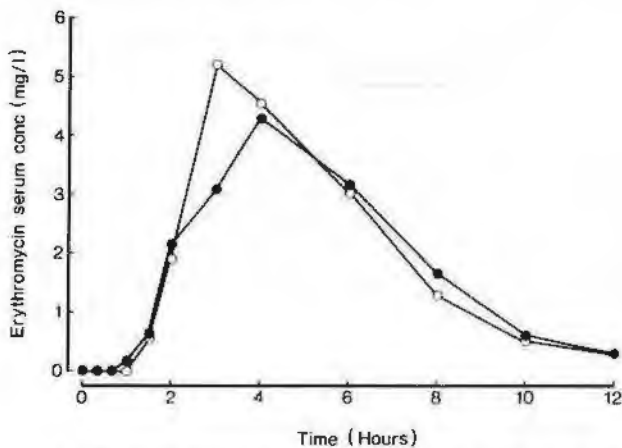


Fig. 1. ●: mean serum levels of erythromycin given as single drug; ○: mean serum levels of erythromycin with simultaneously administered NAC. The differences are not statistically significant with respect to AUC,  $C_{max}$ ,  $t_{max}$  and MRT.

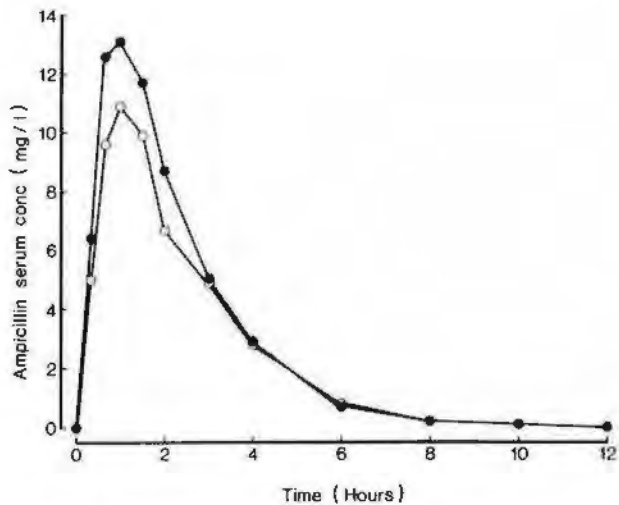


Fig. 2. ●: mean serum levels of ampicillin after administration of bacampicillin; ○: mean serum levels of ampicillin after simultaneous administration of bacampicillin and NAC. The differences are not statistically significant with respect to AUC,  $C_{max}$ ,  $t_{max}$  and MRT.

Erythromycin absorption showed a large interindividual variation with a  $C_{max}$  ranging from 2.1 to 8.5 (mean 4.9) mg·l<sup>-1</sup> after erythromycin single dose administration. With concomitant NAC, the range was 2.2 to 15.1 (mean 6.0) mg·l<sup>-1</sup>. Similar  $t_{max}$  values were obtained for erythromycin with and without concomitant NAC, 3.4–3.5 h. MRT was fairly constant, 4.0–5.5 h (mean 5.1) without and 4.3–5.6 h (mean 5.0) with NAC co-medication. AUC for erythromycin increased in seven out of ten subjects, from mean 22.1 to 25.1 mg·h·l<sup>-1</sup>.  $C_{max}$  of erythromycin was higher in seven out of ten subjects with NAC, and also in two of the three subjects without increased AUC, but the difference was not statistically significant.

Table II. - Pharmacokinetic parameters for oral NAC given with and without oral antibiotic medication

	$C_{max}$ $\mu\text{mol}\cdot\text{l}^{-1}$	$t_{max}$ h	AUC $\mu\text{mol}\cdot\text{h}\cdot\text{l}^{-1}$	MRT h	CLR $\text{l}\cdot\text{h}\cdot\text{kg}^{-1}$
Mean NAC	13.62	0.68	23.03	2.31	0.053
SD	6.0	0.36	6.25	0.39	0.011
Mean NAC + Ampicillin	10.93	0.72	20.95	2.65	0.057
SD	6.07	0.37	6.80	0.39	0.019
Mean NAC + Erythromycin	18.92	0.63	27.73	2.46	0.066
SD	13.22	0.51	11.74	0.43	0.023

$C_{max}$ : highest experimental plasma concentration;  $t_{max}$ : time for  $C_{max}$ ; AUC: area under the plasma concentration vs time curve; MRT: mean residence time; CLR: renal clearance

### NAC disposition

No correlation was found between acetylator phenotype and plasma levels or the bioavailability of NAC.

NAC was rapidly absorbed; mean  $t_{max}$  0.68 h (table II, fig. 3). The absorption was equally rapid with simultaneous administration of antibiotics,  $t_{max}$  being 0.72 h with ampicillin and 0.63 h with erythromycin. MRT was the same with and without antibiotics. Mean  $C_{max}$  for NAC given as a single drug was  $13.6 \mu\text{mol}\cdot\text{l}^{-1}$ . The levels were higher, but not significantly, when NAC was given together with erythromycin, mean  $18.9 \mu\text{mol}\cdot\text{l}^{-1}$ . However, AUC was higher in nine out of ten subjects, and mean AUC increased significantly from 23.0 to  $27.7 \mu\text{mol}\cdot\text{h}\cdot\text{l}^{-1}$  ( $p < 0.05$ ).

The moderate decrease of  $C_{max}$  ( $10.93 \mu\text{mol}\cdot\text{l}^{-1}$ )

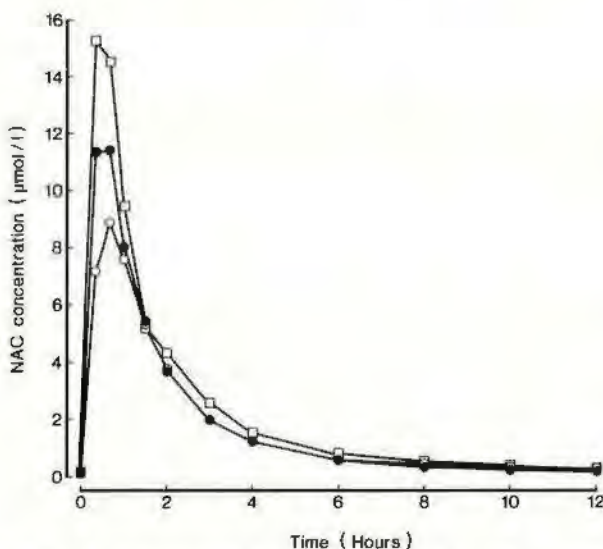


Fig. 3. ●: mean plasma levels of NAC as single drug; □: mean plasma levels of NAC with simultaneous erythromycin administration; ○: mean plasma levels of NAC with simultaneous bacampicillin administration. The differences between □ and ○ are statistically significant with respect to AUC and  $C_{max}$ , but not to  $t_{max}$  and MRT.

and AUC ( $20.95 \mu\text{mol}\cdot\text{h}\cdot\text{l}^{-1}$ ) of NAC with bacampicillin co-medication was found to be highly significant when compared with levels of NAC given with erythromycin ( $p < 0.01$ ). CLR of NAC was low, and not influenced by co-medication with either antibiotic.

### Adverse drug experiences

No serious adverse drug experiences (ADE) occurred during any of the five experimental periods and the 22 cases of ADE were evenly distributed. In seven cases stomach pain was reported, in five headache. Other ADE were nausea (4), cold (2), epigastralgia (1), dysmenorrhoea (1), and sore throat (1).

### In vitro experiments

Since thiol sera were added in equal parts to the sera with known antibiotic concentrations, the resulting mixture could be expected to contain half of the antibiotic concentration of the corresponding undiluted serum, provided no inactivation of antibacterial activity by thiols had taken place. As can be seen in table III, the expected antibacterial activity was found with both antibiotics. The antibacterial activity was not reduced, either by the addition of NAC, or the addition of glutathione.

Table III. - Mean antibacterial activity of sera ( $\mu\text{g}\cdot\text{ml}^{-1}$ ) with known content of erythromycin or ampicillin before and after addition of equal amounts of thiol-containing sera (concentrations of antibiotics in sera containing NAC and glutathione can be multiplied with a factor 2 to correct for dilution of sera).

	(n=30)		(n=17)
Ampicillin	80.9	Erythromycin	2.06
+ NAC $2 \text{ mmol}\cdot\text{l}^{-1}$	45.2	+ NAC $2 \text{ mmol}\cdot\text{l}^{-1}$	0.99
+ NAC $60 \mu\text{mol}\cdot\text{l}^{-1}$	44.4	+ NAC $60 \mu\text{mol}\cdot\text{l}^{-1}$	0.96
+ Glutathion $1 \text{ mg}\cdot\text{ml}^{-1}$	42.9	+ Glutathion	0.96

### Discussion

In the present investigation of an *in vitro* model we could not confirm the earlier reported binding between glutathione and ampicillin [17], and no effect of NAC was seen at either high or low concentrations. Additionally, no reduction of the anti-bacterial activity of erythromycin from thiols was found.

Accordingly, the *in vivo* results demonstrated a lack of adverse effects of NAC towards ampicillin, the absorption of which was unaffected. However, significantly less NAC was absorbed with ampicillin co-medication than with erythromycin. An absorption interaction between two drugs might be expected to affect the availability of both. However, if both drugs utilize the same transport mechanism, but have different affinity constants, only one of the drugs might be affected within a certain concentration interval. Recent studies indicate a dose-dependent absorption of bacampicillin and the existence of a capacity-limited transport system in the human intestine for aminopenicillins [15, 16]. The natural substrate for a specialized transport process is an endogenous substance, and drugs utilizing such processes should be similar in chemical structure. The penicillin nucleus is thought to be derived from L-cysteine and valine, and aminopenicillins may have di-peptide-like structures.

A recent review article has discussed transport systems for the precursor amino acids of the NAC-related thiol, glutathione [1], and such amino acids can be formed from NAC. The absorption of NAC is rapid, but the oral availability is only about 10% [4]. Since the oral availability of ampicillin esters is much higher, absorption of NAC could be reduced if NAC has to compete with ampicillin for the same transport system.

In the case of erythromycin, no decrease of serum levels was found with NAC, but rather a tendency to increased concentrations that was not statistically significant.

On the other hand, there was an increased availability of NAC when given together with erythromycin as compared with that found with ampicillin co-medication. The antibiotics may differ in their effects on bacteria that can effect deacetylation of drugs in the gut before absorption. Alternatively, the fraction of first pass metabolism of NAC that occurs in the liver through oxidative pathways might marginally be inhibited by erythromycin, but it is unlikely to be influenced by ampicillin.

Thus, the present investigation does not indicate any need for a change in dosage or of choice of antibiotic when treatment with erythromycin or bacampicillin is considered in patients with ongoing treatment with NAC. Also, since addition of these antibiotics does not reduce the bioavailability of NAC, no change of dosage of NAC seems indicated.

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RÉSUMÉ: Des études *in vitro* avec des solutions de N-acétylcystéine utilisées pour inhalation, ont démontré l'inactivation de certains antibiotiques par la N-acétylcystéine. Les traitements oraux à base de N-acétylcystéine sont de plus en plus courants pour la prophylaxie à long terme dans la bronchite chronique. Au cours des exacerbations, le traitement avec des antibiotiques oraux sera souvent donné concomitamment. Dans cette étude, nous avons étudié, chez dix volontaires sains, l'effet sur la biodisponibilité de l'administration orale simultanée de deux antibiotiques et de la N-acétylcystéine. L'on n'a trouvé aucun effet de la N-acétylcystéine sur la biodisponibilité de l'ampicilline, après administration de la 'prodrug' bacampicilline. L'on a observé une augmentation légère, mais statistiquement non significative, des niveaux sériques d'érythromycine avec la N-acétylcystéine. Le phénotype d'acétylation n'influence pas l'absorption de la N-acétylcystéine, qui semblait légèrement réduite par la bacampicilline, mais par contre significativement augmentée par l'érythromycine. Si l'on emploie *Micrococcus luteus* et *Staphylococcus aureus* comme organismes indicateurs, l'on n'a pas trouvé de diminution de l'activité antibactérienne du sérum *in vitro* après administration de N-acétylcystéine ou du thiol parent, la glutathione.