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Rapid communication

Improvement of norfloxacin oral bioavailability by EDTA and sodium caprate

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Abstract

The EDTA and sodium caprate (Na caprate) effects on the oral bioavailability of norfloxacin were tested. It was found that absorption kinetic of norfloxacin was markedly accelerated when mixed with EDTA or Na caprate in a ratio of 1:1. When mixed with the absorption enhancers in a ratio of 1:5, only Na caprate improved norfloxacin bioavailability significantly. In vitro dissolution tests demonstrated that EDTA and Na caprate increased norfloxacin dissolution kinetic. However, the correlation between bioavailability and in vitro dissolution improvement was not clearly established. So, we can conclude that the solubilizing property of EDTA and Na caprate did not take a prominent part in norfloxacin absorption.

Keywords: Norfloxacin; EDTA; Sodium caprate; Absorption enhancer; Bioavailability; Dissolution

Many authors have attempted to improve the norfloxacin oral bioavailability by increasing its solubility (Guyot et al., 1995; Fawaz et al., 1996) or by the use of absorption enhancers (Aungst et al., 1996; Robinson and Yang, 1999; Aungst, 2000). On the other hand, norfloxacin is known to interact with divalent and trivalent cations to give insoluble complexes followed by a relatively large decrease in its oral bioavailability (Alkaysi et al., 1992; Wallis et al., 1996). In this study, we have attempted to use chelating and solubilizing properties of two absorption enhancers, disodium ethylenediaminetetraacetate (EDTA) and sodium caprate (Na caprate) in order to increase oral bioavailability of norfloxacin.

The influence of EDTA and Na caprate at different ratios on norfloxacin absorption was evaluated after oral administration of solid preparations to rabbits. Aqueous solution and powder of pure norfloxacin were used as references.

Investigations were carried out on male albinos rabbits, weighing 2–2.5 kg, randomly assigned in six groups ($n = 6$) and fasted 24 h prior to oral administration (water ad libitum). Solution of norfloxacin (Sigma, St. Louis, USA) (10 mg/ml) was obtained by dissolving the drug in water and adjusting the pH to 4.5 with glacial acetic acid. Hard capsules were filled up either with powder of pure norfloxacin or with physical mixtures of norfloxacin with EDTA (Sigma) or with Na caprate (Sigma). Each mixture was used in two molecular ratios, 1:1 and 1:5.

Whatever formulation, a single dose of norfloxacin equivalent to 10 mg/kg was given orally to each animal. After the administration of norfloxacin

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formulations, blood samples (0.5 ml) were taken at defined times (0, 0.5, 0.75, 1, 1.25, 2, 3, 6, 9 and 24 h) from marginal ear vein ponction and collected in heparinized tubes. Plasma was isolated by centrifugation, frozen and stored at −25 °C. Drug concentrations in plasma were determined by employing a HPLC method (Myers and Blumer, 1987). Separation was achieved at room temperature on a Nucleosil C_{18} (250 mm × 4.6 mm) column. The mobile phase was a 78:13:9 (v/v) mixture of 0.02 M dihydrogenpotassium phosphate, methanol, and acetonitrile, containing 0.15% (w/v) of tetrabutylammonium hydroxide. The pH was adjusted to 3 with *o*-phosphoric acid. The flow rate was 1.6 ml/min. Fluorimetric detection was performed with excitation wavelength at 280 nm and emission wavelength at 430 nm.

The t_{max} and C_{max} were extracted from data. $AUC_{0–24\,h}$ was calculated using the linear trapezoïdal rule. The k_e and $t_{1/2}$ were calculated from the slope of the linear regression line in the elimination phase of the semi-logarithmic plot of plasma concentration versus time. Results were expressed by mean ± standard deviation. Statistical analysis was performed using ANOVA analysis. Mean differences were considered to be significant at level $P < 0.05$.

Dissolution studies were performed using USP XXII apparatus (Sotax AT 7). The same hard capsule formulations were tested in 1000 ml of deionized water maintained at a temperature of 37 ± 1 °C. The dissolution medium was stirred with a rotating paddle (75 rpm). The aqueous solutions were filtered and continuously pumped to a flow cell in the UV spectrophotometer and absorbance was monitored automatically at 268 nm. The dissolution tests were carried out over 70 min. All experiments were carried out in triplicate.

The in vivo results are shown in Fig. 1 and Table 1. The norfloxacin mixtures with EDTA or Na caprate in the molecular ratio of 1:1 exhibited very similar bioavailability profiles with a t_{max} value nearly closed to that obtained from norfloxacin solution. When Na caprate proportion was increased, all the evaluated pharmacokinetic parameter values were improved. Thus, in comparison with powder of pure norfloxacin, there was a 1.7-fold increase of C_{max} ($P = 0.013$) and a 2-fold decrease of t_{max} ($P = 0.009$). Last but not least, the inter-animal variability was considerably reduced and so the standard error values of C_{max} and $AUC_{0–24\,h}$ were divided by 4 as compared with those obtained from pure norfloxacin. Surprisingly, when

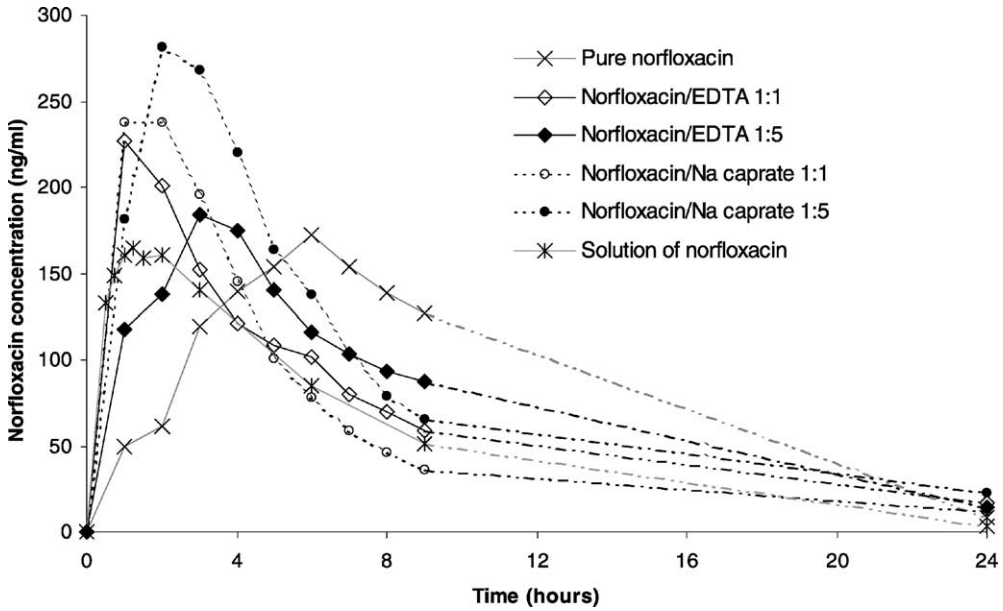

Fig. 1. Mean plasma concentration–time profiles of norfloxacin after oral administration to rabbits of different norfloxacin investigated preparations at a dose of 10 mg of drug per kilogram.

Table 1
Pharmacokinetic parameters after oral administration to rabbits of a dose equivalent to 10 mg/kg of different investigated norfloxacin preparations

Parameters	Solution of norfloxacin	Powder of norfloxacin	Norfloxacin:EDTA (1:1)	Norfloxacin:EDTA (1:5)	Norfloxacin:Na caprate (1:1)	Norfloxacin:Na caprate (1:5)
t_{max} (h)	1.31 ± 0.41 (31.2%)	5.6 ± 1.85 (33.1%)	1.4 ± 0.49[a] (35.0%)	3.8 ± 2.79 (73.4%)	1.80 ± 0.75[a] (41.6%)	2.6 ± 0.80[a] (30.8%)
C_{max} (ng/ml)	178 ± 34 (19%)	181 ± 86 (48%)	258 ± 64 (25%)	214 ± 101 (47%)	276 ± 59 (21%)	313 ± 38[a] (12.1%)
$t_{1/2}$ (h)	3.68 ± 1.83 (49.7%)	4.24 ± 1.16 (27.3%)	7.56 ± 1.03[a] (13.6%)	5.71 ± 1.55 (27.1%)	6.68 ± 1.40[a] (21.0%)	7.54 ± 0.75[a] (9.9%)
k_e (h^{-1})	0.265 ± 0.117 (44.1%)	0.174 ± 0.038 (21.8%)	0.094 ± 0.015[a] (16.0%)	0.129 ± 0.030 (23.2%)	0.110 ± 0.029 (26.4%)	0.093 ± 0.009[a] (9.7%)
$AUC_{0-24\,h}$ (ng h/ml)	1584 ± 189 (12%)	2060 ± 853 (41%)	1655 ± 471 (28%)	1871 ± 377 (20%)	1478 ± 532 (36%)	2132 ± 241 (11%)

t_{max}, time to reach C_{max}; C_{max}, maximum plasma concentration; $t_{1/2}$, terminal half-life; k_e, constant of elimination rate; $AUC_{0-24\,h}$, area under the plasma level curve between time 0 and 24 h; values in parentheses indicate the inter-subject coefficients of variation (CV%).
[a] Statistically significant difference in comparison with powder of pure norfloxacin.

the ratio was increased to 1:5, a back action on the pharmacokinetic parameters (except for $AUC_{0-24\,h}$) was obtained from the norfloxacin:EDTA mixture. Differences between the mean calculated $AUC_{0-24\,h}$ from all norfloxacin preparations as compared to norfloxacin solution were not statistically significant. However, it must be emphasized that intra-individual variability from the pure powder of norfloxacin was large. As shown in Fig. 1, in the norfloxacin preparations, except powder pure product, plasma concentrations decreased quickly after C_{max} was attained. On the other hand, physical mixtures of norfloxacin either with EDTA or Na caprate improved t_{max} as compared to powder of pure drug.

In order to know if the dissolution kinetics of norfloxacin preparations were correlated with the promoting effect of EDTA and Na caprate, dissolution tests were carried out. As shown in Fig. 2, both absorption enhancers improved dissolution kinetics. Nevertheless, Na caprate was more effective than EDTA and dissolution kinetic increased as the concentration of absorption enhancer increased. This result is in agreement with those previously reported by Fawaz et al. (1996) for norfloxacin solid dispersion and cyclodextrin inclusion complexes. However, surprisingly, the best in vivo absorption kinetic increase was obtained from norfloxacin:EDTA (1:1) whereas in vitro dissolution was faster with the 1:5 mixture. Moreover, Na caprate is a better solubilizing agent than EDTA and known as a non-ionic surfactant. So, this could explain its solubilizing effect on norfloxacin. On the other hand, Na caprate solubility in gastric medium being poor, its in vivo effect on norfloxacin absorption would be delayed.

All these results suggested that Na caprate, acting by increasing intra-cellular calcium level, was more effective on norfloxacin absorption than EDTA, which acts by chelating extra-cellular Ca^{2+} and opening up tight junction. These findings are in agreement with those reported by Tomita et al. (1996). Morishita et al. (1993) had shown that EDTA causes more cellular damage to the mucosa than Na caprate and so EDTA may exhibit the best promoting effect at once in the small intestine and in the colon. The region in the gastro-intestinal tract where EDTA does act on the mucosa was likely not the most useful one for norfloxacin absorption. Thus, results from EDTA were finally less interesting than from Na caprate. More

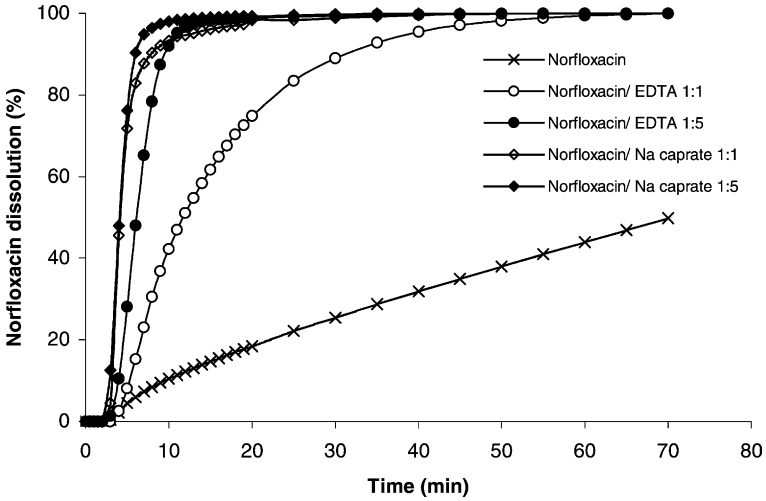

Fig. 2. Dissolution profiles of pure norfloxacin and norfloxacin mixtures with EDTA and Na caprate.

recently, according to a study on Caco-2 cell, Quan et al. (1998) had reported that there is a good relationship between the enhancement permeability of cells and the toxic effects of EDTA and Na caprate. This finding could explain why EDTA being less cytotoxic, is less effective than Na caprate on norfloxacin absorption. However, all these results do not explain why the promoting effect of EDTA was lower with 1:5 norfloxacin:EDTA mixture than with the 1:1 mixture.

In conclusion, EDTA and Na caprate improved norfloxacin oral absorption kinetic. This result is likely due, at least in part, to their solublizing effect on norfloxacin. The mechanisms by which both absorption enhancers promote absorption are more complex and different for each one. EDTA and Na caprate had a weak effect on absorption as compared with norfloxacin pure powder so they appeared as poor absorption enhancers for this drug. However, they allowed to obtain blood concentration profiles after administration of solid dosage forms similar to those obtained from the drug solution. Finally, Na caprate was more effective than EDTA because it improved at once the rate and the extent of norfloxacin absorption.

References

Alkaysi, H.N., Abdel-Hay, M.H., Sheikh Salem, M., Gharaibeh, A.M., Na'was, T.E., 1992. Chemical and microbiological investigations of metal ion interaction with norfloxacin. Int. J. Pharm. 87, 73–77.

Aungst, B.J., 2000. Intestinal permeation enhancers. J. Pharm. Sci. 89, 429–442.

Aungst, B.J., Saitoh, H., Burcham, D.L., Huang, S.M., Mousa, S.A., Hussain, M.A., 1996. Enhancement of intestinal absorption of peptides and non-peptides. J. Contr. Rel. 41, 19–31.

Fawaz, F., Bonini, F., Guyot, M., Bildet, J., Maury, M., Lagueny, A.M., 1996. Bioavailability of norfloxacin from PEG 6000 solid dispersion and cyclodextrin inclusion complexes in rabbits. Int. J. Pharm. 132, 271–275.

Guyot, M., Fawaz, F., Bildet, J., Bonini, F., Lagueny, A.M., 1995. Physicochemical characterization and dissolution of norfloxacin/cylodextrin inclusion compounds and PEG solid dispersions. Int. J. Pharm. 123, 53–63.

Morishita, M., Morishita, I., Takayama, K., Machida, Y., Nagai, T., 1993. Site-dependent effect of aprotinin, sodium caprate, Na_2 EDTA and sodium glycholate on intestinal absorption of insulin. Biol. Pharm. Bull. 16, 68–72.

Myers, C.M., Blumer, J.L., 1987. High-performance liquid chromatography of ciprofloxacin and its metabolites in serum, urine and sputum. J. Chromatogr. 422, 153–164.

Quan, Y.S., Hattori, K., Lundborg, E., Fujita, T., Murakami, M., Muranishi, S., Yamamoto, A., 1998. Effectiveness and toxicity screening of various absorption enhancers using caco-2 cells monolayers. Biol. Pharm. Bull. 21, 615–620.

Robinson, J.R., Yang, X., 1999. Absorption enhancers. Encyclopedia of Pharmaceutical Technology, vol. 18. Dekker, New York.

Tomita, M., Hayashi, M., Awazu, S., 1996. Absorption-enhancing mechanism of EDTA, caprate, and decanoylcarnitine in caco-2 cells. J. Pharm. Sci. 85, 608–611.

Wallis, S.C., Charles, B.G., Gahan, L.R., Filippich, L.J., Bredhauer, M.G., Duckworth, P.A., 1996. Interaction of norfloxacin with divalent and trivalent pharmaceutical cations. In vitro complexation and in vivo pharmacokinetic studies in the dog. J. Pharm. Sci. 85, 803–809.