Induction of the SOS gene (*umuC*) by 4-quinolone antibacterial drugs

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Summary. Induction by the 4-quinolone group of antibacterial drugs of the *umuC* gene, the SOS function most involved in error-prone DNA repair (together with *umuD*), was assessed in a strain of *Escherichia coli* harbouring a *umuC::lacZ* gene fusion. All 4-quinolones tested induced this *umuC::lacZ* fusion, with maximum induction at 4-quinolone concentrations close to their minimum inhibitory concentrations for this strain, and the SOS Inducing Potential (SOSIP) was closely related to antibacterial activity. Mitomycin C, a known mutagen, was a slightly better inducer (in terms of SOSIP) than any of the quinolones. In contrast, induction by 4-quinolones of the *sfiA* (*sulA*) gene, an SOS function involved in cell division inhibition, was better than induction by mitomycin C in an *E. coli* strain harbouring an *sfiA::lacZ* gene fusion. The *umuC* gene fusion was induced at lower concentrations of 4-quinolone than was the *sfiA* gene fusion.

Introduction

The ability of the 4-quinolone antibacterial drugs to induce the SOS response in *Escherichia coli* is well documented.^{1–4} Investigations have concentrated mainly on the expression of the *sulA* (*sfiA*) gene, which codes for a cell division inhibitor.^{1–3} Since SOS processing of DNA is error-prone,^{5,6} it has been suggested that quinolones may be (weakly) mutagenic.^{1,3,7} However, insufficient attention has been paid to the fact that the products of the *umuDC* genes are a pre-requisite for SOS-mediated mutagenesis.^{5,6,8–10} Furthermore, according to current theory, some SOS genes such as *sulA* are repressed less strongly than others such as *umuDC*.⁶ Therefore, a weak inducing signal will lead to derepression and subsequent expression of *sulA* but not *umuDC*. We have tried to establish whether quinolones are capable of producing a sufficiently strong inducing signal to permit expression of the *umuC* gene as measured by the synthesis of β -galactosidase in an *E. coli* strain harbouring a *umuC::lacZ* gene fusion,¹¹ and have compared *umuC* induction with that of *sulA*.

Materials and methods

Bacterial strains

Strains used in this study are listed in table I. Strain GW1104 is temperature-sensitive, so all experiments were performed at 30°C.

Antibacterial agents

The following quinolones were used in this study: ciprofloxacin (Bayer), sparfloxacin, pefloxacin (Rhône Poulenc), ofloxacin (Hoechst), tosufloxacin (Lederle), difloxacin, temafloxacin (Abbott), fleroxacin (Roche), acrosoxacin, nalidixic acid (Sterling Winthrop) and enoxacin (Warner Lambert). The compounds were initially dissolved in 0.01N NaOH and diluted to the required concentrations in sterile distilled water. Mitomycin C (Sigma) was dissolved and diluted in sterile distilled water.

MIC determinations

From overnight cultures of the strains, 10- μ l were transferred into 2.5 ml of brain heart infusion (BHI) broth (to give 10⁴–10⁵ cfu/ml) containing appropriate concentrations of the quinolones. The mixtures were incubated for 16 h at 30°C and the tubes were examined for growth. The MIC of each quinolone was taken to be the lowest concentration at which there was no visible growth.

Table I. Bacterial strains used in this study

Strains	Characteristics
<i>Escherichia coli</i> K12 GC4415 ¹²	<i>thr leu his pyrD trp::MuC⁺ lac malB galK rpsL srl-300::Tn10 sfiA::Mud1(Ap lac)</i>
GW1104 ⁸	<i>his-4 tif-1 sfiA11 lac U169 malE::Tn5 uvrA⁺ umuC::Mud(Ap lac)</i> (temp ^s Amp ^r Kan ^r)

Received 22 Feb. 1991; accepted 29 April 1991.

Induction of SOS genes

Buffers and reagents for SOS induction. B buffer was Na_2HPO_4 16.1 g, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 5.5 g, KCl 0.75 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.25 g, sodium dodecyl sulphate (SDS) 1 g, β -mercaptoethanol 2.7 ml, distilled H_2O to 1L, adjusted to pH 7. ONPG solution (5 mg/ml) was ONPG 500 mg/100 ml in phosphate buffer, pH 7. Phosphate buffer, pH 7 was 0.1 M $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ 61 ml and 0.1 M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 39 ml.

The test strains were grown overnight in BHI broth at 30°C and 0.1-ml volumes were then diluted in 5 ml of BHI broth and incubated at their respective temperatures in a shaking waterbath until the cultures reached a density of 2×10^8 cfu/ml (c. 3 h at 30°C). At this time, ranges of concentrations of 4-quinolones or mitomycin C were mixed with the cultures and incubated for 3 h at 30°C. The β -galactosidase activity was then measured.

The assay procedure for β -galactosidase was similar to that described previously.¹² Samples (0.3 ml) of the reaction mixtures were added to 2.7 ml of B buffer¹² and incubated at 37°C for 10 min for the temperature to equilibrate. The β -galactosidase assay was then initiated by adding 0.6 ml of ONPG solution. The assay was terminated after an appropriate time for colour development (10–90 min) by adding 2 ml of 1 M Na_2CO_3 solution. The absorbance at 420 nm (A_{420}) was read against a colorimeter blank in which the bacterial culture was replaced by BHI broth. Time of colour development was adjusted so that the final A_{420} was approximately 0.1–0.4. Units of enzyme activities were calculated according to the formula used by Miller:¹³ Miller Units of β -galactosidase activity =

$$1000 \left[\frac{A_{420} - (1.75 \times A_{550})}{t \times v \times A_{600}} \right]$$

where, A_{420} and A_{550} were the absorbance values at the relevant wavelength after t min, and v was the reaction volume. A_{600} was the initial absorbance of the culture at 600 nm.

The SOS Inducing Potential (SOSIP)¹² for each compound is the slope of the linear region of the dose-response curve. It is a single parameter that represents the number of β -galactosidase units induced/ μmol of compound tested.

Results

The induction of the *umuC::lacZ* fusion by 4-quinolones is shown in figs. 1–4. Mitomycin C was included as an example of a known mutagen. All the 4-quinolones tested induced *umuC*, but differences were evident in the levels of induction and the ranges of concentrations over which the various quinolones induced *umuC*. These differences are reflected in their SOSIPs (table II). Mitomycin C produced twice as much β -galactosidase (fig. 4) as the most active quinolone, pefloxacin (fig. 3), at their optimum concentrations. The concentration of quinolone at which

Fig. 1. Induction of *umuC::lacZ* by quinolones in *E. coli* strain GW1104 after 3 h at 30°C: ciprofloxacin ○—○; sparfloxacin △—△; tosufloxacin ●—●.

Fig. 2. Induction of *umuC::lacZ* by quinolones in *E. coli* strain GW1104 after 3 h at 30°C: ofloxacin ○—○; norfloxacin △—△; temafloxacin ●—●.

Table II. SOS inducing potential (SOSIP) and maximum inducing concentration for 4-quinolones in *E. coli* strain GW1104 (*umuC::lacZ*)

4-Quinolone	SOSIP (Miller units/ μmol)	Maximum inducing concentration (μM)
Mitomycin C*	1800 (200)	0.599
Ciprofloxacin*	1600 (5800)	0.027
Sparfloxacin	833	0.051
Tosufloxacin	575	0.074
Ofloxacin	300	0.278
Difloxacin	280	0.150
Temafloxacin	225	0.144
Fleroxacin	143	0.136
Norfloxacin	136	0.235
Pefloxacin	109	0.450
Acrosoxacin	105	0.220
Enoxacin	63	0.313
Nalidixic acid	2	7.466

* Values in brackets are SOSIPs measured for *sulA* in *E. coli* strain GC4415.

Fig. 3. Induction of *umuC::lacZ* by quinolones in *E. coli* strain GW1104 after 3 h at 30°C: enoxacin ○—○; pefloxacin △—△; difloxacin ●—●.

Fig. 4. Induction of *umuC::lacZ* by quinolones and mitomycin C in *E. coli* strain GW1104 after 3 h at 30°C: mitomycin C ○—○; nalidixic acid △—△; fleroxacin ●—●; acroloxacin □—□.

maximum induction occurred correlated well with, and was close to, the MIC of the respective drug against this strain (GW1104) (fig. 5). The SOSIP of each quinolone correlated even better than the maximum inducing concentration with the respective MIC (fig. 6).

A comparison of the induction of *umuC* and *sulA* by ciprofloxacin and mitomycin C is shown in fig. 7. The concentration of ciprofloxacin or mitomycin C required to induce *umuC* was lower than that required to induce *sulA*; ciprofloxacin induced approximately the same level of *sulA* as mitomycin C, whereas mitomycin C induced greater levels of *umuC* than ciprofloxacin at their optimum concentrations as measured by the respective *lacZ* fusions. The level of *sulA* induction was at least one order of magnitude higher than the level of *umuC* induction. It should be noted also that lower concentrations of ciprofloxacin than of mitomycin C are capable of inducing *sulA* whereas both compounds induce *umuC* at similar concentrations. This is reflected by the SOSIPs of these compounds for both fusions (table II).

Fig. 5. Correlation between maximum inducing concentration and MIC of quinolones in *E. coli* strain GW1104 (*umuC::lacZ*); r (correlation coefficient) = 0.954.

Fig. 6. Correlation between SOS inducing potential and MIC of quinolones in *E. coli* strain GW1104 (*umuC::lacZ*); r (correlation coefficient) = 0.974.

Fig. 7. Comparison of *umuC::lacZ* (*E. coli* GW1104) and *sulA::lacZ* (*E. coli* GC4415) induction by ciprofloxacin and mitomycin C after 3 h at 30°C: —, *sulA::lacZ* induction; ---, *umuC::lacZ* induction; upper dotted line (.....) indicates basal expression of *sulA::lacZ*, lower dotted line represents basal expression of *umuC::lacZ*; ciprofloxacin ○; mitomycin C △.

Discussion

An *E. coli* *sulA* fusion strain has been proposed as a screen for potential mutagenicity.^{12,14,15} However, measurement of *sulA* expression alone will give no indication of the extent of induction of the various SOS genes nor of the mutagenic potential of the inducing treatment. Our results clearly show that the 4-quinolones are capable of inducing the *umuC* gene an SOS function involved in error-prone repair and mutagenesis.^{6,9} *UmuD* shares a common promoter and operator with *umuC*;⁶ hence, induction of the *umuC* fusion is effectively also that of *umuD*. According to current understanding, LexA repressor is strongly bound to the *umuC* promoter region and requires a strong SOS inducing signal for derepression and subsequent expression of the gene.⁶ Clearly, 4-quinolones are capable of generating such a signal.

Induction of the *umuC* gene is probably a better indicator of mutagenic potential than is induction of *sulA*. This argument is supported by the fact that mitomycin C exhibits a lower SOSIP than ciprofloxacin with *sulA*, but a higher SOSIP with *umuC* (table II). Such a reversal in induction potency has been observed also with ultraviolet light and nalidixic acid.¹⁶ Nevertheless, problems may arise in the use of the *umuC::lacZ* fusion as a screen for mutagenic potential. One disadvantage is that induced levels of *umuC* are relatively low (at least in a *lacZ* fusion) and the fusion may not be sufficiently sensitive to differentiate mutagenic and non-mutagenic compounds accurately.

An interesting paradox is evident on close examination of fig. 7. If *umuC* is repressed more strongly than *sulA*,⁶ low concentrations of ciprofloxacin (or mitomycin C) would be expected to induce *sulA* but not *umuC*. However, *umuC* is induced at concentrations about 10-fold lower than those which are capable of inducing *sulA*. This implies that some other mechanism must regulate the expression of these SOS genes, possibly some form of attenuation. Strain differences are unlikely as strains GC4415 and GW1104 are equally sensitive to ciprofloxacin (MIC 0.01 mg/L). *SulA* induction is maximal at quinolone concentrations approximately $10 \times \text{MIC}$,¹ whereas

umuC induction is maximal at the MIC. The implications of this finding are at present unclear.

The measurement of SOSIP was an attempt to classify the different quinolones in order of potential ability to induce *umuC*, because interpretation of induction curves was not easy. For example, enoxacin maximally induced *umuC* at a lower concentration than pefloxacin, yet pefloxacin induced approximately 2.5 times as much β -galactosidase (fig. 3). Therefore, it was difficult, if not impossible, to decide which of these parameters was more indicative of relative *umuC* inducing ability. The SOSIP takes both factors into account. Previous investigations of SOS induction by assessing expression of *sfiA*^{1,3} have ranked the 4-quinolones in the order of SOS inducing potential ciprofloxacin > fleroxacin = pefloxacin > nalidixic acid. Our SOSIP data (table II) broadly agree with this classification.

The SOSIP for *umuC* (table II) may correlate with the potential mutagenicity of the 4-quinolones in bacteria with a functional SOS response, as proposed for *sulA*.¹² A previous report on mutagenicity of 4-quinolones found that the order was ciprofloxacin > norfloxacin > nalidixic acid.¹ If SOSIP were to correlate with mutagenicity, sparfloxacin, for example, would be expected to exhibit a stronger mutagenic effect than pefloxacin because it has a higher SOSIP value (table II). Alternatively, SOS-mediated mutagenesis may require only a certain threshold level of *umuC* gene product, induction above this threshold resulting in mutagenesis. In this case, all 4-quinolones capable of achieving this level of induction might be expected to be approximately equally mutagenic, assuming that all are capable of mutagenesis only via SOS-processed DNA damage and do not cause other forms of direct DNA damage. It has been suggested by others that it is not valid to predict potential mutagenicity from SOS induction alone.³ Preliminary experiments in our laboratory suggest that there is no correlation between SOSIP and mutagenicity at least for 4-quinolones.

We thank K. Hiom for the gift of strain GW1104.

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