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QUARTERLY

Mutagenic activity of quercetin in derivatives of *Escherichia coli* WP2 *uvrA* with increased permeability

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Flavonoids belong to the most common plant metabolites. They occur almost ubiquitously in our diet, in the fruits, vegetables and beverages [1], as well as in several important medicinal plants, e.g. *Anthyllis vulneraria* L., *Pyrola chlorantа L., Erigeron canadensis* L.

The average daily intake of all flavonoids in humans is 1 g per day [2]. This amount is often increased due to the use of medicines that contain plant extracts, or nutriments containing juice from fruits, e.g. citrus fruits. Owing to their importance, flavonoids especially quercetin, have been intensively tested for their genotoxic (mutagenic and carcinogenic) potential [3].

Quercetin 3,3',4',5,7-pentahydroxyflavone (CAS 117-39-5; M_r 302.20) occurs in the plant kingdom either in free form or conjugated with sugars [2].

In human lower intestinal tract, quercetin glycosides are hydrolysed by bacteria. The glycoside derivatives of quercetin are known not to have genotoxic properties, but free quercetin is mutagenic in the Ames test [4, 5]. The presence of S9 rat liver microsome fraction markedly enhanced the mutagenic activity of quercetin in tester strains of *Salmonella typhimurium*.

Quercetin has been shown to be genotoxic in short-term bacterial and mammalian assays [5–8]. However, the mechanism of mutagenic activity of quercetin still remains obscure.

The carcinogenic properties of quercetin have also been studied. Pamucku *et al.* [9] observed an increased frequency of intestine and bladder cancers in rats fed a diet supplemented with 0.1% quercetin. Erturk *et al.* [10] have shown induction of liver and bile duct tumors in rats fed a diet containing 1% and 2% quercetin. To the best of our knowledge, there are no other data available on quercetin carcinogenicity.

The aim of this study was to examine the mutagenic activity of quercetin in derivatives of *Escherichia coli* WP2 *uvrA*.

The strains used were tryptophan-requiring derivatives of *E. coli* WP2 *uvrA*. These strains were isolated by Herrera *et al.* [11] by selecting C21-resistant clones. All *E. coli* WP2 *trpE uvrA* strains and derivative of *E. coli* B strains: IC 2486 *rfa*, SC30-RP2 *rfa*$^+$, IC 2486 *rfa* with pKM101 and SC30-RP2 *rfa*$^+$ with pKM101 were a gift from M. Blanco, Instituto de Investigaciones Citologicas, Valencia, Spain. *S. typhimurium* TA 100 (*hisG46 uvrB rfa*) pKM101 was obtained from B.N. Ames, Biochemistry Department, University of California, Berkeley, CA, U.S.A.

We used lipopolysaccharide-defective *E. coli* B strains with increased permeability to mutagens, and partial permeability to large molecules, e.g. quercetin, polycyclic hydrocarbons. The sensitivity of these strains to flavonoids is probably due to the lipopolysaccharide core of the *E. coli* B cells being incomplete, which confers on them partial permeability to large molecules.

The mutagenic activity of quercetin in the presence and absence of metabolic activation was examined in tester strains *E. coli* B: IC 2486 *uvrA rfa*, SC30-RP2 *uvrA rfa*$^+$, IC 2486 *uvrA rfa* with pKM101, and SC30-RP2 *uvrA rfa*$^+$ with pKM101.

Mutation *rfa* in these strains causes partial loss of the lipopolysaccharide barrier that coats the

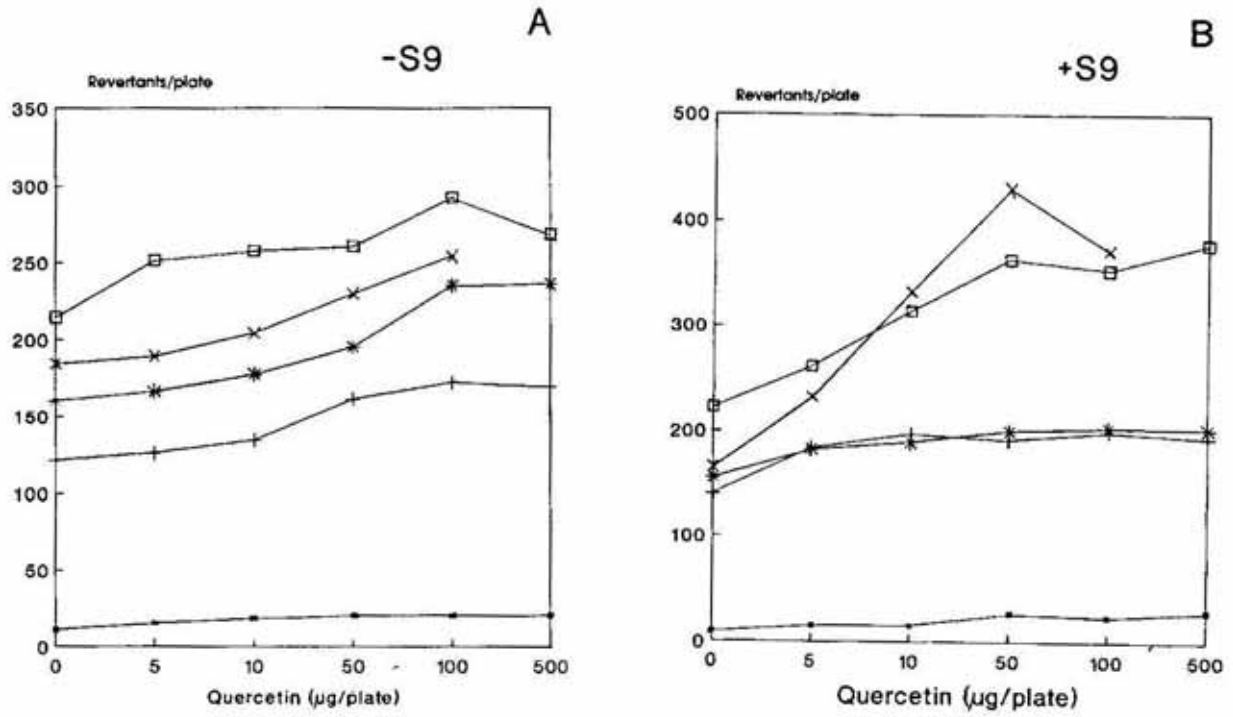

Fig. 1. The induction of Trp^+ and His^+ mutations by quercetin in the absence (A) and presence (B) of metabolic activation (fraction S9).

Revertants were Trp^+ for strains derived from *E. coli* WP2 and His^+ for *S. typhimurium* strain TA100. Quercetin was dissolved in dimethylsulphoxide. The experimental protocol was as follows. To 2 ml of molten top agar at 45°C was added 100 µl of a fresh overnight bacterial culture grown in nutrient broth at 37°C, then 50 or 100 µl of quercetin was added. In experiments with metabolic activation, fraction S9 (50 µl/plate) was added. The mutagenicity assays were carried out in triplicate, and the number of trp^+ and his^+ revertant colonies was scored after incubation at 37°C, in the dark, for 48 h. The number of revertants per plate is the average number from at least 5 separate experiments. *Escherichia coli* B strains: IC 2486 *rfa* (*); IC 2486 *rfa* pKM101 (□); SC30-RP2 rfa^+ (■) SC30-RP2 rfa^+ pKM101 (+). *Salmonella typhimurium* strain TA100 (x).

surface of the bacteria penetration of large molecules through normal cell wall. Plasmid pKM101 increase error-prone DNA repair. Bacteria carrying pKM101 are therefore more mutable and have a higher spontaneous mutation rate. This plasmid also carries an ampicillin resistance gene.

We compared the induction by quercetin of the trp^+ revertants in the presence and absence of metabolic activation in *E. coli* B SC30-RP2 rfa^+, with the induction of trp^+ revertants observed in its *rfa* derivative, IC 2486 (Fig. 1A, B). Studies on the mutagenic activity of quercetin have been performed with its non-toxic concentrations ranging usually from 5 to 500 µg/plate. In experiments with metabolic activation, rat microsomal fraction S9 obtained according to Ames *et al.* [12] and stored at –20°C served as the source of soluble microsomal enzymes. The average concentration of protein in the S9 fraction determined according to Lowry *et al.* [13] was 38 mg/ml (34–42 mg/ml).

Quercetin in the presence and absence of metabolic activation increased the number of trp^+ revertants in all tester strains of *E. coli* B. The yield of mutants was higher in the *rfa* strain than in the rfa^+ parent, both in the presence and absence of plasmid pKM101 (Fig. 1A, B). However, we observed also a small increase by quercetin in the number of trp^+ mutants in the absence of pKM101.

It is known that quercetin is a strong mutagen for *S. typhimurium* strains containing plasmid pKM101 (TA98, TA97, TA100, TA102) and the presence of rat liver S9 fraction increases the mutagenicity of quercetin for tester strains [4, 5]. In this study we used *S. typhimurium* TA100 to detect base substitution mutations induced by quercetin and its metabolites.

The dose-response curve of quercetin for strain *E. coli* B IC2486 *rfa* pKM101 was similar to that found for the *rfa* bearing strain of *S. typhimurium* TA100, which also contains plasmid pKM101 (Fig. 1A, B). The mutagenic activ-

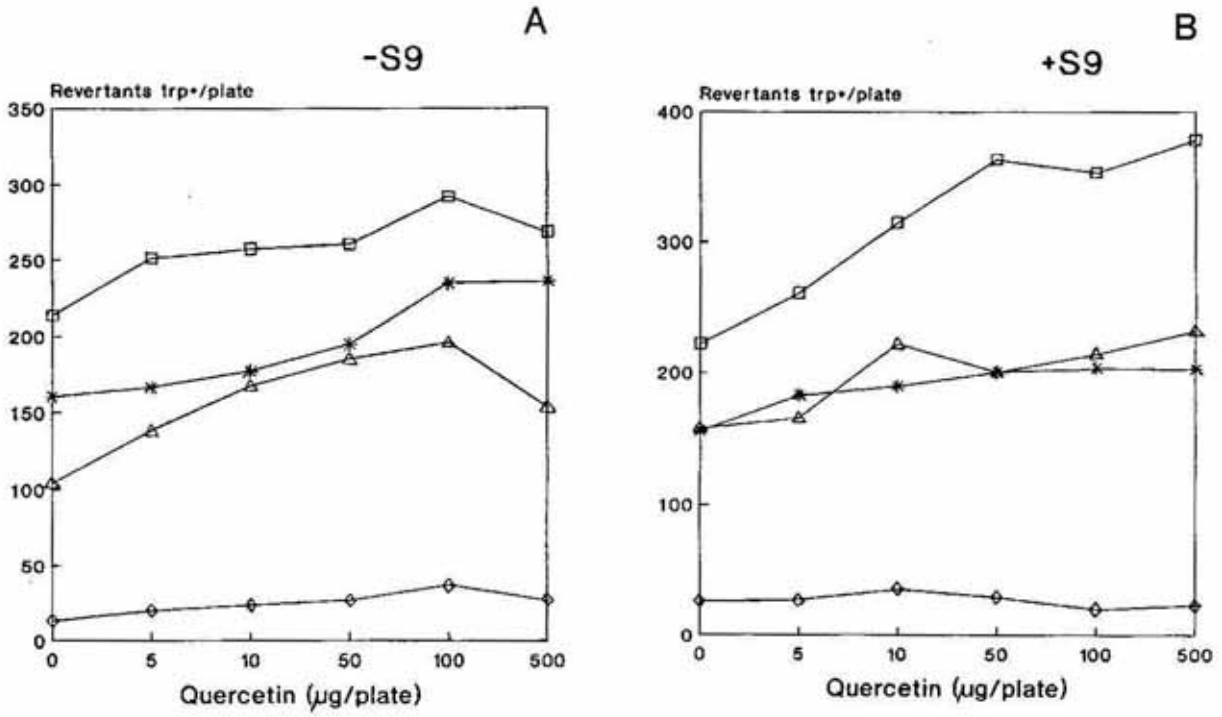

Fig. 2. The induction of trp$^+$ mutations by quercetin in the absence (A) and presence (B) of metabolic activation (fraction S9) in E. coli WP2 uvrA and in the rfa derivatives of the WP2 strain.
E. coli B strains: WP2 *uvrA* (◊); WP2 *uvrA* pKM101 (Δ); IC2486 *uvrA* (✳); IC2486 *uvrA* pKM101 (□). The experimental protocol as in Fig. 1.

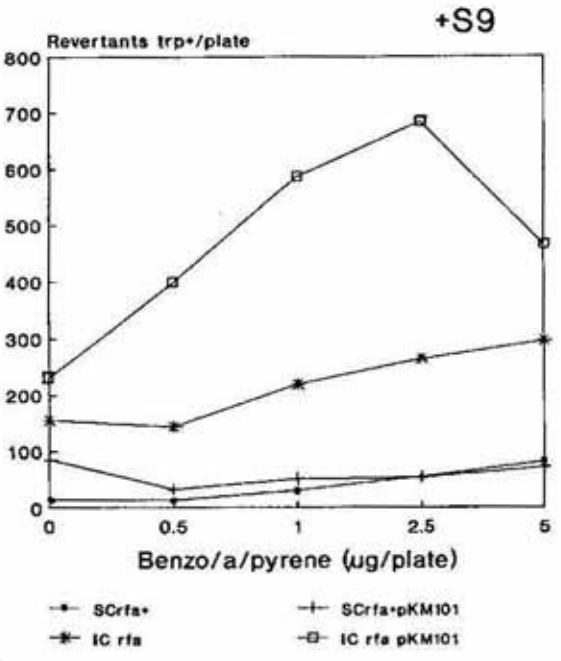

Fig. 3. Dose-response curves of trp$^+$ mutation in strains E. coli B at various amounts of benzo[a]pyrene (B[a]P) in the presence of metabolic activation (fraction S9).
Abbreviations and experimental protocol as in Fig. 1. B/a/P was dissolved in dimethylsulphoxide.

ity of quercetin on TA100 was similar to the results for IC2486 *rfa* pKM101. Metabolic activation distinctly increased the mutagenic activity of quercetin for strain *S. typhimurium* TA100 and for strain *E. coli* B IC2486 *rfa* pKM101 (Fig. 1B).

The induction of *trp*$^+$ and *his*$^+$ mutations by quercetin in the presence of metabolic activation in *E. coli* and *S. typhimurium* strains was similar (Fig. 1B). MacGregor [3, 7] suggested that the increase in the mutagenic activity of quercetin in the presence of metabolic activation (S9) was caused by introduction of hydroxyl groups in the B ring and oxidation to "quinone" derivatives, and then to unknown reactive ultimate metabolites.

We also analyzed the mutagenesis induced by quercetin in *E. coli* WP2 *uvrA* strains without and with the plasmid pKM101, which are currently used in mutagenicity assays (Fig. 2A, B).

Weaker induction by quercetin of *trp*$^+$ mutations in the presence and absence of metabolic activation, in tester strains WP2s *uvrA* and WP2 *uvrA* pKM101, may be due to the difficulty the large molecule of quercetin has in entering into the bacterial cells.

However, the higher mutagenic activity of quercetin in *E. coli* B strains with increased permeability: IC 2486 *rfa*, IC 2486 *rfa* pKM101, seems to result from their defective lipopolysaccharide core. This increased permeability is known to enhance the mutagenic action of large molecules of different mutagens belonging to various classes of chemicals.

As positive control in our experiments was used benzo/a/pyrene (B/a/P), a known strong mutagen. Its mutagenicity in the presence of metabolic activation for tester strains IC 2486 and strains SC30-RP2 is shown in Fig. 3. The induction of trp^+ revertants was higher in the *rfa* than in its rfa^+ parent strain.

These results indicate that application of *E. coli* strains with increased permeability could be very useful in studies on the mechanism of chemical mutagenesis.

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