Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2579–2582

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Comparison of the Prevention of Aflatoxin B_1-Induced Genotoxicity by Quercetin and Quercetin Pentaacetate

Ekta Kohli,[a] Hanumantharao G. Raj,[a] Ranju Kumari,[a] Vishwajit Rohil,[a] Narendra K. Kaushik,[b] Ashok K. Prasad[b] and Virinder S. Parmar[b,*]

[a]*Department of Biochemistry, V. P. Chest Institute, University of Delhi, Delhi-110 007, India*
[b]*Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi-110 007, India*

Received 22 October 2001; accepted 20 June 2002

Abstract—Earlier work carried out in our laboratory highlighted the mode of action of acetoxy 4-methylcoumarins in preventing the genotoxicity of aflatoxin B_1 (AFB_1). We have in this report extended the observations to quercetin pentaacetate (QPA), which unlike quercetin (Q) has demonstrated time-dependent inhibition of liver microsome catalysed AFB_1 epoxidation as measured by AFB_1 binding to DNA. The action of QPA is similar to that of the acetoxy 4-methylcoumarins in that they are acted upon by microsomal transacetylase leading to modulation of catalytic activities of certain enzymes (such as P-450 enzymes, NADPH cytochrome C reductase and glutathione *S*-transferase) possibly by way of protein acetylation. In the present work, we have documented the transacetylase-mediated action of QPA in preventing genotoxicity due to AFB_1.

Introduction

Several polyphenolic compounds have demonstrated antimutagenic and anticarcinogenic activities in cell cultures as well as animal models.[1,2] Among polyphenolic compounds, flavonoids have come under serious scrutiny regarding their ability to prevent chemical and radiation induced genotoxicity. Flavonoids in general inhibit cytochrome P-450-mediated formation of reactive intermediates of several classes of chemical carcinogens and mutagens which damage cellular DNA by way of adduct formation.[3–5] Accordingly, quercetin has been shown to inhibit P-450 catalysed activation of several chemical carcinogens including aflatoxin B_1 (AFB_1).[6] Our laboratory has carried out an in-depth study of another class of polyphenolics, namely hydroxy 4-methylcoumarins and their acetyl derivatives on the microsome catalysed epoxidation of AFB_1 and related biological effects.[7–10] The results of these investigations revealed the unique action of the model compound 7,8-diacetoxy-4-methylcoumarin (DAMC) to cause a dramatic irreversible inhibition of microsome-mediated AFB_1–DNA binding, while 7,8-dihydroxy-4-methylcoumarin (DHMC), the deacetylated product of DAMC, failed to elicit the said effects. These results prompted us to postulate the existence of an enzyme in microsomes that can possibly catalyse the transfer of acetyl groups of DAMC to P-450 apoprotein and thereby cause inhibition of microsome catalysed AFB_1–DNA binding both in vivo and in vitro.[7,11] Work carried out by us revealed the action of transacetylase on several acetylated xenobiotics leading to the conclusion that such a transacetylase in general can be termed as acetoxy drug: protein transacetylase.[12] In the present work, we have provided evidences for the action of transacetylase on a flavone acetate, namely quercetin pentaacetate (QPA) and the implications on P-450 catalysed reaction, such as AFB_1 epoxidation and genotoxicity.

Materials and Methods

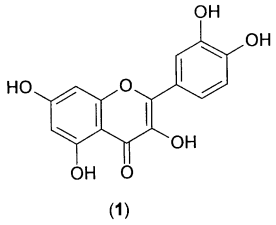
Chemicals

[^{3}H] AFB_1-G was obtained from Moravek Biochemicals (Brea, CA, USA). Quercetin(Q, **1**), NADPH, calf thymus DNA, cytochrome C and AFB_1 were purchased from Sigma Chemical Co, St. Louis, MO (USA). Quercetin was acetylated by the acetic anhydride/pyridine/DMAP method to obtain quercetin pentaacetate (QPA,

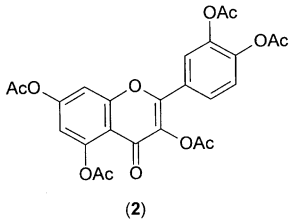
*Corresponding author. Tel.: +91-11-766-6555; fax: +91-11-766-7206; e-mail: virparmar@yahoo.co.in

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00478-X

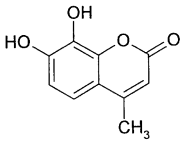
2), which was fully characterised from its spectral data. 7,8-Dihydroxy-4-methylcoumarin (DHMC, **3**) and its diacetyl derivative (DAMC, **4**) were obtained by our earlier published procedure.[7]



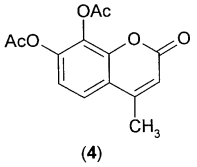
(1)



(2)



(3)



(4)

Animals

Male albino rats of wistar strain weighing around 150–200 g, fed on rat chow supplied by Hindustan Lever Ltd., Mumbai (India) were used.

Preparation of liver microsomes

Male rats were sacrificed, liver excised and microsomes prepared as described earlier.[13]

Effect of test compounds on liver microsome catalysed AFB_1–DNA binding

Rat liver microsomes (1 mg protein) were mixed separately with the test compound (DAMC/DHMC/QPA/Q, 100 μM) and phosphate buffer (100 mM, pH 7.4) and water to make the volume 0.8 mL. The contents (scaled up as per requirement) were preincubated at 37 °C in a shaking water bath. The aliquots (0.5 mL portion) were removed periodically into another set of tubes containing [^{3}H]AFB_1 (250 μCi/μM) in 20 μL DMSO, 0.1 mg calf thymus DNA, 2 mM NADPH and made to a final volume of 1 mL and incubated for 30 min at 37 °C. At the end of incubation, 2 mL of the extraction mixture (phenol/chloroform/isoamyl alcohol 50:50:1 v/v/v) and 0.9 mg calf thymus DNA as the carrier were added. The DNA was isolated by the procedure of Wang and Cerutti[14] and the recovery of DNA was 50–70%. Isolated DNA was dissolved in 0.1 M NaCl, one aliquot (0.2 mL) was added to a vial containing 8 mL of Bray's scintillation fluid for determination of radioactivity using a Liquid Scintillation Counter (Beckman Model LS 6000).[15] Results of [^{3}H] AFB_1 binding to DNA were corrected for DNA recovery and expressed as [^{3}H] AFB_1 bound/mgDNA/30 min. In this assay procedure, samples with DMSO substituting for the test compound served as the control.

AFB_1-induced micronuclei formation in bone marrow cells

Treatment. Male albino rats of wistar strain (150–200 g) were administered, separately, the test compound (300 mg/kg b wt dissolved in 0.1 mL DMSO) intraperitoneally (ip), followed by a second dose of the test compound along with a dose of AFB_1 (4 mg/kg b wt in DMSO). A group of rats were injected with AFB_1 alone, while control animals received DMSO alone. The animals were sacrificed 26 h after the last injection.

Isolation of bone marrow, preparation of smear and staining

Bone marrow cells were isolated by flushing femora of rats with 2 mL of Hanks' Balanced Salt Solution (HBSS). For preparation of smear and staining, the method of Schimd[16] was followed as adapted by Raj et al.[17]

Assay of liver microsome catalysed activation of NADPH–cytochrome C reductase by quercetin and quercetin pentaacetate

The method consisted of preincubation of the test compound with microsomes, followed by the addition of substrates for the reductase assay (cytochrome C and NADPH) as described earlier.[8] Rat liver microsomes (25 μg protein) were separately mixed with a fixed concentration of the test compound (DAMC/DHMC/QPA/Q added in 50 μL of DMSO), phosphate buffer (0.05 M, pH 7.7) and water to make up to 0.8 mL. The contents (scaled up as per requirement) were preincubated at 37 °C in a shaking water bath. The aliquots (0.5 mL portion) were removed periodically into the spectrophotometer cuvette (1 cm light path) containing 0.1 mM EDTA, 36 mM cytochrome C and 1 mM NADPH in a total volume of 1 mL, the progress of the reaction was monitored at 550 nm.

Table 1. Influence of quercetin (Q) and quercetin pentaacetate (QPA) on liver microsome catalyzed AFB_1–DNA binding in vitro: comparison with hydroxy- and acetoxy-4-methylcoumarins

Test compd	Pre-incubation time (min)	AFB_1–DNA binding (% inhibition)
QPA	10	6.70
	20	12.80
	30	18.00
Q	10	5.70
	20	5.20
	30	6.20
DAMC	10	19.00
	20	37.50
	30	56.80
DHMC	10	8.50
	20	8.20
	30	8.90

The concentration of the test compound included in the incubation was 100 μM. The values are an average of three observations with variation <5%. The details are given under Materials and Methods.

Table 2. Effect of quercetin (Q) and quercetin pentaacetate (QPA) on AFB_1-induced micronuclei formation in rat bone marrow cells

Group	Group description	*N*	Micronucleated cells/1000 cells	
			Mean	95% Confidence limits
1	Control	4	1.75	0.6666–2.8334
2	AFB_1alone	4	7.25	6.6842–7.8158
3	Q	4	2.00	1.0760–2.9240
4	QPA	4	2.25	1.6842–2.8158
5	$Q + AFB_1$	4	6.00 (22.72%)	5.0760–6.9239
6	$QPA + AFB_1$	4	4.75 (45.45%)	4.1842–5.3158
7	DAMC	4	2.25	1.6842–2.8158
8	$DAMC + AFB_1$	4	3.75 (63.63%)	2.8118–4.6882

N, Number of animals in each group; $P < 0.001$: (Group 2 vs Group 1; Group 3 vs Group 2; Group 4 vs Group 2; Group 6 vs Group 2; Group 6 vs Group 4; Group 5 vs Group 3; Group 5 vs Group 1; Group 8 vs Group 2; Group 7 vs Group 2); $P < 0.005$: (Group 6 vs Group 1); $P < 0.05$: (Group 5 vs Group 2; Group 6 vs Group 5; Group 8 vs Group 7; Group 8 vs Group 1; Group 8 vs Group 5); NS (not significant): (Group 5 vs Group 1; Group 4 vs Group 1; Group 4 vs Group 3; Group 7 vs Group 1; Group 8 vs Group 6).

Results and Discussion

The relative activities of Q and QPA to prevent AFB_1-induced genotoxicity are reported here. The preincubation of rat liver microsomes with QPA resulted in the time-dependent inhibition of AFB_1 binding to calf thymus DNA in vitro (Table 1). It is evident from the results (Table 1) that the polyphenols (Q and DHMC) failed to produce time-dependent inhibition of liver microsome catalyzed AFB_1-epoxidation (measured as AFB_1–DNA binding) unlike their corresponding acetoxy derivatives. The results documented in Table 2 highlight a comparative account of Q and QPA to prevent the clastogenic action of AFB_1 in bone marrow cells. The pretreatment of rats with QPA and DAMC caused considerably higher reduction of AFB_1-induced micronuclei incidence in bone marrow cells (Table 2). DHMC was found ineffective in significantly reducing the micronuclei induction in bone marrow cells by AFB_1 (results not shown here) although Q proved a little better (Table 2). The role of tranacetylase in the action of QPA and DAMC is further confirmed by the results described in Table 3. We have established in our previous publications[8] that DAMC through the action of TAase drastically activates the catalytic activity of NADPH cytochrome C reductase. Q, like DHMC is ineffective in causing the time dependent activation of the reductase, while QPA is effective in producing irreversible activation of the reductase (Table 3). Several reports have cited the potential of Q to inhibit P-450-linked MFO activities, such as epoxidation of AFB_1 and benzo(a)pyrene.[5,6] Our results (Table 2) denote an important observation that QPA, like DAMC is an irreversible inhibitor of AFB_1-epoxidation catalyzed by liver microsomal P-450. We have earlier reported acetylation of recombinant glutathione S-transferase isoform 3-3 by DAMC catalyzed by hepatic microsomal transacetylase.[18] Accordingly our earlier observation that the P-450 activities were inhibited by DAMC could possibly be due to the acetylation of P-450 apoprotein.[19] These results explain that QPA and DAMC are superior to their hydroxy analogues in preventing the induction of genotoxicity due to AFB_1 (Table 2).These observations indicate that the action of Q and QPA is not only confined to the inhibition of P-450 activities, but they also modulate many other cellular activities.[20,21] These results further confirm our hypothesis that acetylated polyphenols (DAMC, QPA, etc.) are acted upon by the microsome membrane bound transacetylase resulting in the possible acetyl CoA-independent acetylation of enzyme proteins with altered catalytic activity as highlighted in this report.

Table 3. Influence of quercetin pentaacetate (QPA) and quercetin (Q) by transacetylase catalyzed time dependent activation of NADPH cytochrome C reductase

Test compd	Preincubation time (min)	NADPH cytochrome C reductase (% activation)
QPA (5 μM)	5	7.10
	10	13.30
	20	25.00
	30	37.00
Q (5 μM)	5	3.70
	10	3.30
	20	5.40
	30	5.70
DAMC (2 μM)	5	10.20
	10	18.90
	20	39.90
	30	57.60
DHMC (5 μM)	5	2.80
	10	2.50
	20	3.10
	30	3.50

Values are an average of four observations with variation <2%. The numbers in the parentheses indicate the concentration of the test compound in the incubation mixture.

Acknowledgements

We thank the Danish International Development Agency (DANIDA) and the Council for Scientific and Industrial Research (CSIR, New Delhi) for financial assistance.

References and Notes

1. Guengerich, F. P.; Kim, D. H. *Carcinogenesis* **1990**, *11*, 2275.
2. Newmark, H. C. *Dietary Phytochemicals in Cancer Prevention and Treatment: Advances in Experimental Medicine and Biology*; American Institute for Cancer Research; Plenum: New York, 1996; Vol. 401.
3. Wang, Z. Y.; Huang, M. T.; Ferraro, T.; Wong, C. Q.; Lou, Y. R.; Reuhl, K.; Iatropoulos, M. C.; Yang, S.; Conney, A. H. *Cancer Res.* **1970**, *52*, 1162.
4. Wang, Z. Y.; Wang, L. D.; Lee, M. J.; Ho, C. T.; Huang, M. T.; Canney, A. H.; Yang, C. S. *Carcinogenesis* **1995**, *16*, 2143.
5. Anderson, D.; Dobzynska, M. M.; Basavan, N.; Basavan, A.; Yu, T. W. *Mutat. Res.* **1998**, *402*, 269.
6. Smith, T. J.; Yang, C. S. In *Food Phytochemicals for Cancer Prevention*; Huang, M. T.; Osawa, T.; Ho, C. T.; Rosen, R. T.

Eds.; American Chemical Society Symposium Series; Washington, DC, 1994; Vol. 546.
7. Raj, H. G.; Parmar, V. S.; Jain, S. C.; Goel, S.; Singh, A.; Gupta, K.; Rohil, V.; Tyagi, Y. K.; Jha, H. N.; Olsen, C. E.; Wengel, J. *Bioorg. Med. Chem.* **1998**, *6*, 1895.
8. Raj, H. G.; Parmar, V. S.; Jain, S. C.; Goel, S.; Singh, A.; Tyagi, Y. K.; Jha, H. N.; Olsen, C. E.; Wengel, J. *Bioorg. Med. Chem.* **1999**, *7*, 369.
9. Raj, H. G.; Malik, S.; Parmar, V. S.; Kohli, E.; Tyagi, Y. K.; Rohil, V.; Dwarakanath, B. S.; Adhikari, J. S.; Bose, M.; Jain, S. C.; Olsen, C. E. *Teratogen. Carcinogen. Mutagen.* **2001**, *21*, 181.
10. Raj, H. G.; Kohli, E.; Rohil, V.; Dwarakanath, B. S.; Parmar, V. S.; Malik, S.; Adhikari, J. S.; Tyagi, Y. K.; Goel, S.; Gupta, K.; Bose, M.; Olsen, C. E. *Mutat. Res.* **2001**, *494*, 31.
11. Raj, H. G.; Parmar, V. S.; Jain, S. C.; Kohli, E.; Ahmed, N.; Goel, S.; Tyagi, Y. K.; Sharma, S. K.; Wengel, J.; Olsen, C. E. *Bioorg. Med. Chem.* **2000**, *8*, 1707.
12. Raj, H. G.; Parmar, V. S.; Kohli, E.; Tyagi, Y. K.; Jain, S. C.; Olsen, C. E. *FASEB J.* **2000**, *14*, 1445.
13. Allameh, A.; Saxena, M.; Raj, H. G. *Cancer Lett.* **1988**, *40*, 49.
14. Wang, T. V.; Cerutti, P. *A. Cancer Res.* **1979**, *39*, 5165.
15. Burton, K. *Biochem. J.* **1956**, *62*, 315.
16. Schmid, W. T. *Mutat. Res.* **1975**, *31*, 9.
17. Raj, H. G.; Gupta, K.; Rohil, V.; Bose, M.; Biswas, G.; Singh, S. K.; Jain, S.C,; Parmar, V.S.; Olsen, C.E.; Wengel, J. *Teratogen. Carcinogen. Mutagen.* **1998**, *18*, 249.
18. Kohli, E.; Kumari, R.; Gupta, G.; Raj, H. G.; Parmar, V. S.; Ahmad, N.; Jain, S. C.; Olsen, C. E. *Drug Metab. Rev.* **2001**, *33* (Suppl. 1), 39.
19. Singh. I.; Kohli E.; Raj, H. G.; Gyanda, K. K.; Jain, S. K.; Tyagi, Y. K.; Gupta, G.; Kumari, R.; Kumar, A.; Pal, G.; Prasad, A. K.; Rastogi, R. C.; Olsen, C. E.; Jain, S. C.; Parmar, V. S. *Bioorg. Med. Chem.* In press.
20. Alliangana, D. M. *East. Afr. Med. J.* **1996**, *73*, 752.
21. Stefansen, M.; Mattache, C.; Oma, A.; Szegli, G. *Roum. Arch. Microbiol. Immunol.* **1997**, *56*, 3.