Polish Journal of Environmental Studies Vol. 9, No. 3 (2000), 157-161

The Influence of Quercetin on Some Biochemical Parameters in Rats Exposed to Environmental Contamination with Fluorine Compounds

B. Czerny, A. Put, Z. Myśliwiec, Z. Juzyszyn

Chair of Pharmacology and Toxicology; Pomeranian Medical University, 70-111 Szczecin, Powstancow Wlkp. 72, Poland

Received 23 September, 1999
Accepted 5 November, 1999

Abstract

Male Wistar rats were exposed for 3 and 6 months to NH_4F in a toxicological chamber. Some animals received sodium salts of quercetin sulfonic acids at a dose of 5 or 20 mg/kg body weight. It was found that quercetin alleviates biochemical changes in the liver caused by ammonium fluoride, in particular concerning enzyme activities and lipid metabolism.

Keywords: Ammonium fluoride; quercetin; fluoride intoxication

Introduction

Emissions of fluorine compounds present a serious threat to the environment in many industrial areas. As the quantity of fluoride emitted continues to rise so does the concentration, reaching in some places alarming levels and acting harmfully on living organisms. Large doses of fluorine inhibit tissue respiration and carbohydrate and lipid metabolism, attributed on the cellular level to disorders in the metabolism of calcium and magnesium and modification of enzymatic processes [12, 18].

The aim of this work was to study the influence of chronic exposure of rats to ammonium fluoride in air on some biochemical parameters in serum and in liver homogenate. The liver was selected due to its central role in detoxication. Quercetin, an aglucon of the ubiquitous flavonoid - rutin, was administered to some rats in order to check its antitoxic properties.

Flavonoids are polyphenol compounds present in large quantities in plants. Pharmacological studies have confirmed the multifarious properties of flavonoids, among them rutin and its aglucon - quercetin [1, 9, 15]. Quercetin is present in large concentrations i.a. in apples, tea and onion [27]. It acts as coenzyme in carbohydrate metabolism of the myocardium and has a beneficial influence in arterial hypertension. Quercetin retards atherosclerosis and protects the liver against harmful effects of various factors [23, 25, 26].

Material and Methods

Experimental Conditions

Salts of quercetin 8,5-disulfonic acid (Na_2QDSA) - NaQSA-5 and NaQSA-8, were synthesized at the Department of Inorganic Chemistry, Technical University of Rzeszow, and a 1:1 mixture was prepared. Preliminary studies to determine LD_{50} and antiinflammatory properties were performed at the Department of Pharmacology, Medical University in Wroclaw. Bactericidal activity was checked at the Experimental Laboratory of the POLFA Cracow Pharmaceutical Company.

Experiments were performed in inbred male Wistar rats. 12 groups of 10 animals each were formed.

- **Groups I-VI** were studied for 3 months and **groups VII-XII** for 6 months.
- **Groups I and VII** served as controls, were given standard chow and water and were kept in the animal room
- **Groups II and VIII** received quercetin 1 at a dose of 5 mg/kg body weight/24 h

- **Groups III and IX** received quercetin 2 at a dose of 20 mg/kg body weight/24 h
- **Groups IV and X** were exposed to NH_4F 2 mg/m^3 in a toxicological chamber with controlled air flow, hu midity and temperature.
- **Groups V and XI** were exposed to NH_4F 2 mg/m^3 in a toxicological chamber and received 5 mg/kg b.w./24 h quercetin
- **Groups VI and XII** were exposed to NH_4F 2 mg/m^3 in a toxicological chamber and received 20 mg/kg b.w./24 h quercetin

Air flow (10 m^3/h), humidity and temperature in the toxicological chamber were kept constant. Humidity and temperature were maintained at levels in the animal room.

Exposure continued for 3 or 6 months, 6 hours per day and five days per week. Ammonium fluoride in the form of aerosol was introduced at 2 mg/m^3 air. The concentration of fluorine in the toxicological chamber was monitored using an ion-selective electrode according to Polish norm (PN 83/z-04093,07). Mortality, weight gain and peripheral blood cell count were recorded. Urinalysis was performed on two occasions: before the experiment and one day before autopsy.

After 3 or 6 months the animals were sacrificed by decapitation, livers were excised and homogenized.

Biochemical Tests

Serum: activities of cholinesterase (ChE) [29], aspartate (AspAT) and alanine (A1AT) transaminases [16], alkaline phosphatase (AP) [28] and concentrations of bilirubin [14], total lipids [5], cholesterol [2] and triglycerides [5] were measured. Liver homogenate: concentrations of total cholesterol [24] and triglycerides [5] were determined.

Groups were compared using Student's t-test for unpaired results and taking $p < 0.05$ as the level of significance.

Liver Histochemistry

Unfixed, frozen samples were sliced to 10 fm thickness in a cryostat [7]. Succinate dehydrogenase [20], acid and alkaline phosphatase [21] reactions were studied. Reaction intensity was scaled as follows: + very weak, + + weak, + + + moderate, + + + + strong and + + + + + very strong.

Results

General Findings

No significant differences between groups as to mortality, weight gain, peripheral blood cell count and urinalysis were revealed.

Biochemical Tests

(a) Influence of ammonium fluoride and quercetin on enzyme activities in serum (Figs. 1 and 2)

Cholinesterase. Cholinesterase activity fell after 3 and 6 months of exposure to ammonium fluoride by 22%, as compared to non-exposed animals. The differences were statistically significant. Administration of 5 mg/kg/24 h quercetin for 3 months resulted in normal activities of the enzyme, while 20 mg/kg/24 h significantly increased the activity. Exposed animals receiving quercetin at either dose for 6 months had ChE activities similar to non-exposed groups.

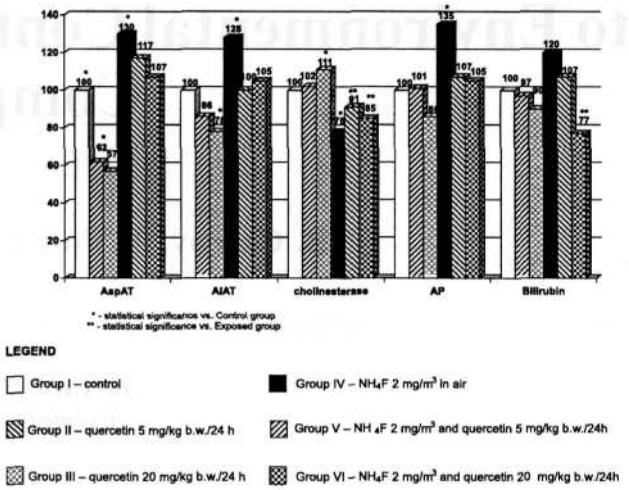

Fig. 1. Influence of quercetin on some biochemical parameters in serum of rats chronically exposed to ammonium fluoride vapours for 3 months.

Transaminases. AspAT activity was higher after 3 or 6 months of exposure to NH_4F. Quercetin at either dose was effective in reducing the activity rise, more evidently at a dose of 20 mg/kg/24 h. A similar pattern was observed for A1AT.

Alkaline phosphatase. NH_4F increased alkaline phosphatase activity by 35% after 3 and 23% after 6 months of exposure. The differences were statistically significant. Quercetin at either dose reduced activity.

Bilirubin concentration. Bilirubin concentrations in serum rose proportionally to duration of exposure. Levels were 20% higher after 3 and 49% after 6 months of inhaling NH_4F. Animals on either dose of quercetin had bilirubin levels comparable with controls.

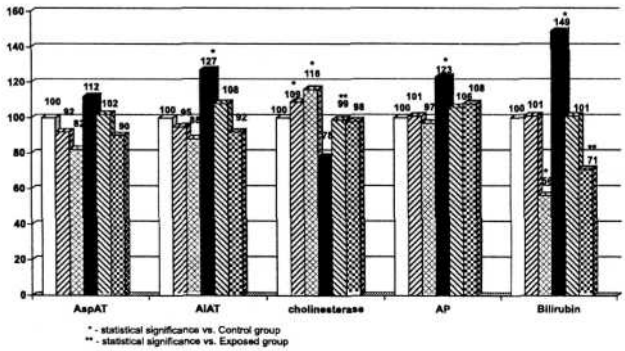

Fig. 2. Influence of quercetin on some biochemical parameters in serum of rats chronically exposed to ammonium fluoride vapours for 6 months.

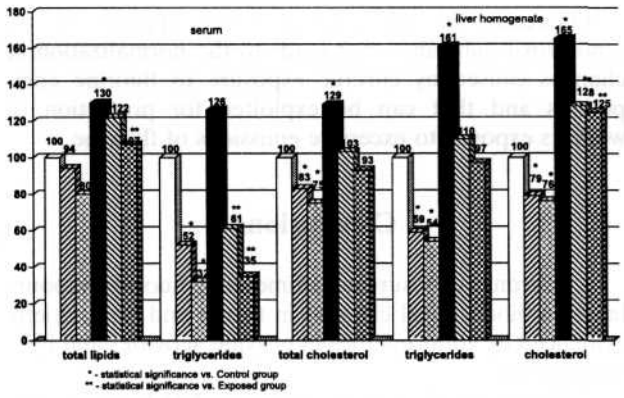

Fig. 3. Influence of quercetin on some lipid fractions in rats chronically exposed to ammonium fluoride vapours for 3 months.

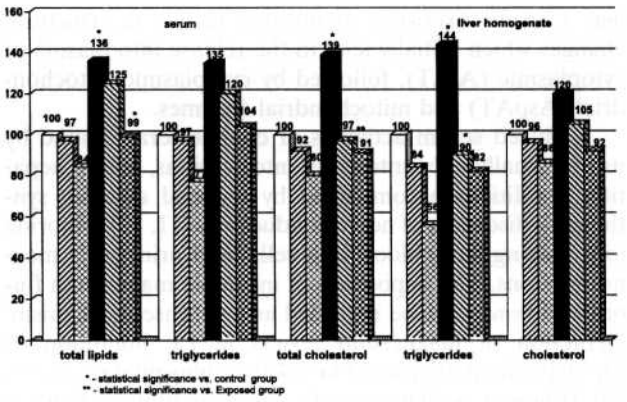

Fig. 4. Influence of quercetin on some lipid fractions in rats chronically exposed to ammonium fluoride vapours for 6 months.

(b) Influence of ammonium compounds and quercetin on lipids (Figs. 3 and 4)

Total lipids. Total lipids in serum of rats exposed to ammonium fluoride for 3 or 6 months rose by 37%, as compared with controls. There was no change in the group receiving 5 mg/kg/24 h quercetin for 3 months. A reduction in lipid level was noted when quercetin was administered at the higher dose for 6 months.

Cholesterol. Cholesterol levels in serum of exposed animals rose by 29% after 3 and 39% after 6 months. Quercetin was effective in reducing cholesterol levels to those found in the non-exposed group.

Triglycerides. The concentration of triglycerides was higher by 26% after 3 and 35% after 6 months of exposure to NH_4F. In the 3-month experiment, rats protected with quercetin had markedly lower levels. Triglyceride levels approached normal after 6 months of administration of 20 mg/kg/24 h quercetin.

(c) Biochemical tests in liver homogenates (Figs. 3 and 4)
Cholesterol. After 3 months of exposure the cholesterol content in liver exceeded control values by 65% and after 6 months by 20%. The administration of quercetin resulted in cholesterol content approaching normal values.

Triglycerides. The triglyceride content in liver of exposed animals rose by 61% after 3 months and 44% after 6 months. Quercetin at either dose was effective in lowering triglyceride levels to control values.

(d) Liver histochemistry (Table 1)

The activity of succinate dehydrogenase in livers of control animals was moderate. After 3 or 6 months of exposure to NH_4F the activity diminished. Activities remained at control level in rats receiving quercetin. No significant changes in acid and alkaline phosphatase activities were noted.

Discussion

Fluorine compounds, like many other toxic substances, lead to changes in enzyme activities. The underlying pathomechanism may differ, possibly involving:

- inhibition of enzymes
- disorders in mechanisms regulating enzymatic reac tions in cells
- release of enzymes from damaged cells into blood
- abnormalities of cellular metabolism

According to Henkc [13], a marked increase in aminotransferase activity is a sign of hepatocellular dam-

Table 1. Influence of NH_4F and sulfonic derivatives of quercetin on histochemical reactions in rat liver.

Enzymes	3-month exposure				6-month exposure			
	Control	NH_4F	NH_4F + sulfonic derivatives of quercetin		Control	NH_4F	NH_4F + sulfonic derivatives of quercetin	
			5 mg/kg body weight	20 mg/kg body weight			5 mg/kg body weight	20 mg/kg body weight
Succinate dehydrogenase	+++	++	+++	+++	+++	++	+++	+++
Alkaline phosphatase	+	+++	+	+	+	+	+	+
Acid phosphatase	++++	++	++++	++++	++++	++	+++	++++

Reaction intensity: + very weak; ++ weak; +++ moderate; ++++ strong; +++++ very strong

age. Chronic exposure to fluorine results in structural changes which initially lead to the release into plasma of cytoplasmic (A1AT), followed by cytoplasmic-mitochondrial (AspAT) and mitochondrial enzymes.

Reduced serum activities of cholinesterase found by us are usually encountered in intoxications, severe hepatitis, conditions accompanied by reduced albumin synthesis or disorders of nerve conductivity [11,12]. Fluoride ions passing from blood into cells bind principally magnesium ions. The deposition of insoluble magnesium fluoride in bones can be regarded as a defense mechanism. Depletion of magnesium results in i.a. inhibition of Mg-dependent enzymes, like alkaline phosphatase [8, 19, 22]. However, we have found an increase in the activity of this enzyme. Elevated activity of secretory enzymes, including alkaline phosphatase, is pathognomonic of liver damage.

Chronic exposure of rats to vapours of ammonium fluoride resulted in the rise in serum total lipids, triglycerides and cholesterol content. By analogy, the amount of triglycerides and cholesterol in liver homogenate increased. One of the chief causes for increased triglyceride and cholesterol content in serum are abnormalities of enzyme activities. This is most probably true of triglyceride lipase, some non-specific esterases and pyrophosphatase. All these enzymes are inhibited by fluoride [19, 30].

Opinions as to the influence of fluorine on the concentration of cholesterol are conflicting. Many authors have not observed any change in cholesterol levels during exposure to fluorine [3, 4], while others have confirmed the hypercholesterolemic properties of fluorine compounds [6, 10, 30].

The introduction in recent years of pure compounds isolated from plants represents a significant step forward in phytotherapy. Galenic preparations, like infusions, fail to recover up to 40% of the active substance. Quercetin is a component of plant preparations successfully used for the dilatation of renal vessels and improvement of renal perfusion. It also inhibits resorption in renal tubules and in this way accelerates the elimination of toxins [17].

Results of the present biochemical and histochemical experiments demonstrate the beneficial influence of quercetin in chronic intoxication with ammonium fluoride in rats. This was evidenced by milder changes in enzyme activities, parameters of lipid metabolism and histochemistry. The activities of aminotransferases and cholinesterase in animals receiving quercetin were lower. In non-exposed animals the activities remained in the normal range, while in rats exposed to ammonium fluoride for 6 months the differences were small as compared with controls.

The administration of quercetin had a positive effect on lipid metabolism. A decrease in the serum content of cholesterol, triglycerides and total lipids was confirmed in exposed and non-exposed rats alike. Preventive use of quercetin led to the normalization of cholesterol and triglyceride content in liver homogenates.

The present results in animals may be useful for the prevention of fluorine toxicity in humans. Taking into account similarities in homeostatic mechanisms of rats and humans it can be envisaged that the pathomechanism of fluorine intoxication in both cases is similar.

Quercetin in the present study appears to be a non-toxic substance that leads to the normalization of changes caused by chronic exposure to fluorine compounds and that can be exploited for protection of workers exposed to excessive emissions of fluorine.

Conclusions

1. Chronic exposure to ammonium fluoride vapours leads to biochemical changes in serum and liver, chiefly evidenced by abnormalities in enzyme activities and lipid metabolism.
2. Biochemical changes caused by NH_4F are accom panied by liver damage.
3. Quercetin effectively inhibits changes caused by flu orine compounds and could be of use for protecting workers exposed to excessive emissions of fluorine com pounds.

References

1. VAN ACKER S.A., DE GROOT M.J., VAN DEN BERG D.J., et al. A quantum chemical explanation of the anti oxidant activity of flavonoids. Chem. Res. Toxicol. 9 (8), 1305, **1996.**
2. BLASZCZYSZYN M. Accurate and simple determination of total cholesterol in the serum. Wiad. Lek. **23** (16), 1413, **1970.**
3. CHINOY N.J., NARAYANA M.V., SEQUEIRA E., et al. Studies on effects of fluoride in 36 villages on Mensana Dis trict, North Gujarat. Fluoride **25** (3), 101, **1992.**
4. CHINOY N.J., SHARMA M., AHMEDABAD M.M. Bene ficial effects of ascorbic acid and calcium on reversal of flu oride toxicity in male rats. Fluoride **26** (1), 45, **1993.**
5. CHROMY V., HORNAKOVA M., BREINEK P., VROB-LOVSKY P. Extractive photometric determination of serum triglycerides (in Czech). Bioch. Clin. Bohemoslov. **6,** 167, **1977.**
6. DOUSSET J.C., RIOUFOL C, PHILIBERT C, BOUR BON P. Effects of inhaled HF on cholesterol, carbohydrate and tricarboxylic acid metabolism in guinea pigs. Fluoride **20** (3), 137, **1987.**
7. ELLIOTT K.A. Tissue slice technique. W: Methods in Enzymology. Red. S.P. Colowick N.O. Kaplan. Academic Press, New York 3, 3, **1955.**
8. FARLEY J. et al. Monofluorophosphate is hydrolyzed by alkaline phosphatase and mimics the actions of NaF on skel etal tissues in vitro. Calcif. Tissue Int. 40 (1), 35, Jan. 1987.
9. FORMICA J.V., REGELSON W. Review of the biology of Quercetin and related bioflavonoids. Food Chem. Toxicol. **33,** 1061, **1995.**
10. FRADA G.D., DI BIASI S., PINTACUDA S. La fluorosis. Minerva Med. **67,** 75, **1976.**
11. GRABOWSKA M., GRUMINSKA M. Wplyw fluorku sodu na ATP-azy aktywowane magnezem z bton erytrocytow ludzkich. Folia Med. Cracov. **26,** 29, **1985.**
12. GUMINSKA M. Biochemiczne mechanizmy dzialania fluoru na zywy organizm. Folia Med. Cracov. **23,** 306, **1981.**
13. HENKE J. Wartosc diagnostyczna okreslenia aktywnosci enzymow surowicy w zatruciach ostrych. Med. Pracy, **18,** 61, **1967.**

14. HINSBERG K., MERTEN R. Chemische Bestimmungsmethoden im Klinischen Laboratorium. Urban-Schwarzenberg, Miinchen, 3, 1952.
15. HOLLMAN P.C.H., KATAN M.B. Absorption, metabolism and health effects of dietary flavonoids in man. Biomed. Pharmacother. **51** (8), 305, **1977.**
16. KOKOT F. Metody badan laboratoryjnych stosowanych w klinice. PZWL, Warszawa, **1969.**
17. KUHNAU J. The flavonoids. A class of semi-essential food components, their role in human nutrition. World Review of Nutrition and Dietetitcs, **24,** 117, **1976.**
18. MACHOY Z. Biochemiczne mechanizmy dzialania zwiazkow fluoru. Folia Med. Cracov. **28,** 61, **1987.**
19. MACHOY-MOKRZYNSKA A., PUT A., CEGLECKA M., et al. Influence of essential phospholipids (EPL) on selected biochemical parameters of lipid metabolism in rats chroni cally exposed to ammonium fluoride vapours. Fluoride **27** (4), 201, **1994.**
20. PEARSE A.G.E. Histochemistry. Theoretical and applied. Churchill Livingstone, Edinburgh, **1968.**
21. PEARSE A.G.E. Histochemistry. Theoretical and applied. Churchill Livingstone, Edinburgh **1972.**
22. PILLAI K., MATHAI A., DESHMUKH P.B. Effect of sub strate dosage of fluoride on male mice. Toxicol. Lett, **44,** 1, **1988.**
23. ROBAK J., et al. The effect of some flavonoids on non-enzy matic lipid oxidation and enzymatic oxidation of arachidonic acid. Pol. J. Pharmacol., **38,** 483, **1986.**
24. RUDEL **L.L.,** MORRIS M.D. Determination of cholesterol using o-phtalaldehyde. J. Lipid Res., **14,** 364, **1973.**
25. SIESS M.H., et al. Time course of induction of rat hepatic drug-metabolizing enzyme activities following dietary ad ministration of flavonoids. J.Toxicol.Environ. Health, **49,** 481, **1996.**
26. STEINBERG D., et al. Beyond cholesterol modifications of low density lipoprotein that increase its atherogenicity. N. Engl. **J.** Med. **320,** 915, **1989.**
27. DE VRIES **J.H,** JANSSEN P.L, HOLLMAN P.C., VAN STAVEREN W.A., KATAN M.B. Consumption of quercetin and kaempferol in free-living subjects eating a variety of diets. Cancer Lett. **114,** 141, **1997.**
28. WEBER **H.,** WEGMANN T. Atlas der klinischen Enzymologie. Methoden und Arbeitsvorschriften. G. Thieme Verlag, Stuttgart **9,** 13, **1968.**
29. WESTSTONE H.J., TENNANT R., WHITE B.V. Studies on cholinesterase activity. I. Serum cholinesterase. Methods and normal values. Gastroenterology, **33,** 41, **1957.**
30. ZAKRZEWSKA H., OROWICZ W. Wplyw emisji przemyslowych zawierajacych zwiazki fluoru na profil lipidow osocza u owiec. Bromat. Chem. Toksykol. **30,** 1, 49, **1997.**