ANTOXID INCREASES FERRIC REDUCING ANTIOXIDANT POWER (FRAP) EVEN STRONGER THAN VITAMIN C

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Antoxid (AX) is a new herbal free radical scavenger obtained from Radix Scutellariae baicalensis Georgi. The water-alcoholic extract of radix obtained according to described procedure (1) is very rich in flavonoids with baicalin as a main one (approximately 72%). The pharmacological activity possesses also product of hydrolysis aglicon—baicalein (Figure 1). Antoxid is the main component of BAICADENT-GEL used in stomatology. There are several parameters reflecting the antioxidative properties of agents in blood. One of them is FRAP (Ferric Reducing Ability of Plasma) (2, 3). However the extract from Scutellariae baicalensis, standardized to baicalin, was a subject of various investigations, particularly evaluating the antioxidative properties (4, 5), there were no reports on its influence on FRAP.

The aim of study was to determine the influence of Antoxid on ferric reducing ability of plasma (FRAP) and the comparison of the results with vitamin C influence on FRAP.

EXPERIMENTAL

The material was blood plasma from patients of Surgery Department, Medical University Clinic. The blood was taken on anticoagulant. The patients did not receive any drugs.

FRAP was evaluated by the measurements of Fe²⁺/TPTZ-complex by colorimetric method with spectrophotometer (6). The Antoxid was dissolved in methanol/water and used at concentrations as follows: 5.0; 10.0; 20.0; 30.0 and 50.0 µg/mL. Vitamin C (AC) was dissolved in water and used in concentrations the same as above. The results were evaluated with Student’s t-test.

General procedure of FRAP measurement

To 450 µL of plasma the methanol/water solutions of Antoxid or vitamin C in various concentrations were added. The mixture was incubated for 30 min. at 37°C. 20 µL of mixture was added to coupling agents (TPTZ/FeCl₃) and incubated for 4 min. at 37°C, then centrifuged (4000 × g) for 10 min. The absorbance was measured at λ = 593 nm and compared with the control. Every experiment was repeated 10 times (n = 10).

RESULTS AND DISCUSSION

The comparison of Antoxid ferric reducing capacity with plasma ferric reducing capacity showed, in general, lower activity of Antoxid than plasma. However, the data of FRAP for plasma and Antoxid at concentrations 20-50 µg/mL were close to each other. The Antoxid at lower concentration (5-10 µg/mL) had much lower ferric reducing ability (FRAP) than plasma (Table 1).

The examination of Antoxid influence on plasma ferric reducing capacity showed strong antioxidative ability of the extract. Antoxid at concentrations 10-50 µg/mL statistically significantly (p = 0.000016) increased FRAP. The strongest effect was obtained with 30 µg/mL concentration of Antoxid.

The next step was to compare the activity of Antoxid with vitamin C in their influence on FRAP. The investigation of vitamin C on ferric reducing ability of plasma showed an increase of FRAP dependent on vitamin C concentration (Figure 2).

The highest FRAP value was reached for 50 µg/mL vitamin C concentration, but it was lower than that obtained after Antoxid treatment. The results point that Antoxid in doses 30-50 µg/mL has stronger antioxidative ability than vitamin C measured as FRAP. The activity of Antoxid at concentration 30 µg/mL was 18.5% higher than that of vitamin C at the same concentration.

The antioxidative properties of various flavonoids are well known. They are good chelators of metals ions, particularly iron or copper, the Fenton reaction catalyst. The antioxidative properties of baicalin, the main flavonoid of Antoxid, are connected with xanthine oxi-
dase inhibition (7). Flavonoids influence also on radical generation by chelating transition metal ions, which catalyze the reaction. It was reported that high flavonoid intake showed significant effect on the liver, but not on the brain. It is probably because the liver is the main metabolic organ for flavonoids. Many flavonoids have hepatoprotective effect when the liver is under pathological condition and may play a chemopreventive role by reducing oxidative stress in living system (8-10).

Recently, Firuzi (11) examined in vitro the influence of various flavonoids on FRAP, using the artificial model of blood (TPTZ/FeCl3). The most active was baicalein. It looks that it plays more important role in FRAP influence than flavonoid baicalin. Thus, it seems that the Antioxid’s ferric reducing ability is determined mainly by baicalein with the participation of baicalin. Both baicalin and baicalein had antioxidant activity, but their antioxidant effects were derived from different pathways. The major reaction of baicalein was on scavenging superoxide free radical whereas the inhibitory effect on xanthine oxidase was the minor reaction. Baicalein had better antioxidant effect on inhibiting xanthine oxidase but the superoxide free radical scavenging activity was not as high as that of baicalin (12, 13).

**CONCLUSIONS**

1. Antioxid in concentration > 5 µg/mL increases ferric reducing ability of human plasma.
2. Antioxid in concentration 30 µg/mL is more active than vitamin C in influence on FRAP.

**REFERENCES**

There are many reports on antioxidative properties of flavonoids from roots of *Scutellaria baicalensis* Georgi, a widely used Oriental medicinal plant (1-3). The most active are baicalin and baicalein (4). There are also some investigations on antioxidative properties of Antioxid, the water-alcoholic extract obtained from *Scutellariae radix* in crystalline form, standardized to 65-70% baicalin (5). There are no reports on Antioxid efficiency in alleviation of oxidative stress caused by environmental chemicals, e.g. aromatic hydrocarbons.

The aim of the study was to investigate the influence of Antioxid on lipid peroxidation in mitochondria stimulated by tert-butyl hydroperoxide (*t*-BOOH) or xylene.

**EXPERIMENTAL**

The study was performed in vitro on human placental mitochondria. Mitochondria were isolated by the Radi method (6) from mature placenta obtained after physiological delivery at the Medical University Obstetric-Gynecological Clinic. The proteins in mitochondria were measured by Lowry method (7). Antioxid was dissolved in the mitochondrial buffer (TRIS-HCl ñ pH 7.4) and used in following concentrations: 1.5; 3.0; 6.0; 12.0 and 30 µg/mL. The antioxidative properties of Antioxid were examined at mitochondria stimulated with 1% *t*-BOOH or 17.64 µg/mL xylene. In the xylene treatment, Antioxid was applied 30 min. before, simultaneously or 30 min. after xylene addition.

The general principles of MDA measurement

The lipid peroxidation was evaluated by MDA (malondialdehyde) level measured spectrophotometrically with the thiobarbituric acid method (TBARS) (8). The results were compared to ascorbic acid as a positive control.

**RESULTS AND DISCUSSION**

*t*-BOOH stimulation

It was observed that Antioxid inhibits lipid peroxidation at three highest from five concentrations tested: 6.0; 12.0 and 30 µg/mL, but not in doses of 1.5 and 3.0 µg/mL (Figure 1).

It means that Antioxid in doses higher than 6 µg/mL is able to reduce lipid peroxidation, resulting in the dose dependent (p < 0.001) MDA level decrease.

Xylene treatment

The aim of this experiment was to examine the ability of Antioxid to alleviate oxidative stress stimulated by aromatic hydrocarbon – xylene. Our previous study (9) showed that xylene in concentration higher than 17.64 µg/mL increased lipid peroxidation in mitochondria expressed as MDA level.

We also wanted to check whether simultaneous exposition to xylene and Antioxid does not give harmful interaction in the free radicals peroxidation cascade. The results show that simultaneous mitochondria treatment with xylene and Antioxid at concentrations of 6.0 and 12.0 µg/mL leads to statistically significant (p < 0.001) decrease in MDA level in comparison to the control without Antioxid (Figure 2). The obtained results indicate high effectiveness of Antioxid in the reduction of lipid peroxidation stimulated by the aromatic hydrocarbon – xylene.

It was also interesting to explain whether the effectiveness of Antioxid will be modified depending on the sequence of treatment. In other words, it would suggest whether Antioxid is more useful as preventing (giving before the exposition to hydrocarbon) or repairing (giving after the exposition to hydrocarbon) agent. If added after the xylene exposition only, the higher dose (12.0 µg/mL) was able to reduce MDA level significantly (p < 0.001) (Figure 2).

Different results were obtained when Antioxid was given 30 min before exposition to xylene. Preincubation of mitochondria with Antioxid at concentrations 6.0 or 12.0 µg/mL efficiently prevents MDA level increase at both concentrations.

In summary, there are differences in effectiveness of AX on lipids peroxidation caused by xylene depending on order of exposition. Antioxid is more effective given simultaneously or before toxic agent, so more in prevention than in reparation of lipid peroxidation elicited by xylene.
Malondialdehyde formed from polyunsaturated acids is a useful parameter for determining extends of lipid peroxidation. It was shown that baicalin at concentration of 1 mM inhibits MDA formation in the brain and kidney homogenates and at concentrations of 1.5-6 µM reduced phospholipid liposomes oxidation (4, 10). The mechanism is not yet explained, although it has been described for other flavonoids, e.g. rutin and quercetin (11). They inhibit Fe(II)- or NADPH-dependent lipid peroxidation by inhibiting superoxide formation, hydroxyl radical formation in the Fenton reaction and peroxyl radical generation. The mechanism of xylene influence on lipids peroxidation is unknown. It can be expected that similarly to toluene in biotransformation pathway to benzoic acid with cytochrome P enzymes superoxide anion radical is generated (12). It was proved that flavonoids from S. baicalensis are able to scavenge free radicals (3). This could be the inhibition mechanism of lipid peroxidation caused by Antoxid.

CONCLUSIONS

1. Antoxid at concentrations at least 6.0 µg/mL significantly inhibited lipid peroxidation caused by t-BOOH or xylene.
2. The effectiveness of Antoxid depends on applied doses as well as on timing of treatment.
3. Antoxid has preventing and repairing activity towards lipid peroxidation caused by xylene, but acts stronger as preventive agent.

REFERENCES