Life Sciences, Vol. 65, Nos. 18/19, pp. 2011-2014, 1999

0024-3205/99/$-see front matter

ELSEVIER

PII S0024-3205(99)00467-1

PLACENTAL TRANSFER OF STOBADINE IN RABBITS

E. Ujházy, M. Dubovický, L. Šoltés, [1]V. Faberová, [2]M. Zemánek, A. Gajdošík

Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, [1]Drug Research Institute, Modra, [2]Slovakofarma JSC, Hlohovec, Slovak Republic

Summary

Stobadine, a pyridoindole antioxidant, was investigated for its placental transfer and distribution in New Zealand white rabbits on the 27th day of gestation. The concentrations of stobadine were determined in maternal and foetal organs (plasma, brain, heart) at 30, 60, 120, and 360 minutes after oral administration of the drug in a dose of 5 mg/kg. The results obtained proved that after oral stobadine intake by rabbits at the stage of advanced pregnancy both maternal and foetal organs were under a certain drug level which could act protectively against oxidative stress - frequently occurring during late organogenesis, foetal stages and delivery, as well as during early postnatal development.

Key Words: antioxidant, stobadine, placental transfer, rabbit

Oxidative stress represents an important risk factor for tissue and organ injuries not only in mothers, but especially in the developing foetus which is insufficiently protected by its immature antioxidative enzyme systems (1). The aim of the study was to investigate the extent of placental transfer of a novel antioxidant, a cerebro - and cardioprotective agent - stobadine (2) - in rabbits.

Methods

Drugs. ^{3}H-stobadine (dihydrochloride salt; specific activity 495 GBq/mmol, radiochemical purity > 95% (3)) was synthetised in the Institute for Research, Production, and Use of Radioisotopes, Prague, Czech Republic. Cold, unlabelled dihydrochloride of stobadine, developed at the Institute of Experimental Pharmacology Slovak Academy of Sciences, Bratislava, was prepared in Slovakofarma JSC, Hlohovec, in cooperation with the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic.

Address for correspondence:

Dr. Eduard Ujházy, Institute of Experimental Pharmacology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 16 Bratislava, Slovak Republic Fax: +421-7-375 928

Animals. Seventeen timed-pregnant New Zealand white rabbits were used. The animals were kept under 18±2°C, relative air humidity 55±10%, and natural light. The pelleted diet and water were available *ad libitum*. At least 3 weeks before the beginning of the experiment the animals were acclimatised to the above conditions. For mating each female was placed together with one male until copulation was observed. After 6 - 7 hours the mating was repeated with another male. The day of copulation was designated day 0 of pregnancy. The body weight of animals was 4.43±0.17 kg (means±s.e.m.).

Treatment. An aqueous solution of drugs (stobadine + ^{3}H-stobadine) was administered to rabbits in a dose of 5 mg/kg (sample volume of 1 ml/kg body weight) orally on day 27 of pregnancy. In time intervals of 30, 60, 120, and 360 minutes blood samples were withdrawn from the *vena auricularis magnae*. Plasma was obtained by centrifugation at 3,000 rpm for 10 minutes in a refrigerated centrifuge (Janetzki T23, Leipzig, Germany) and stored at -18°C until analysis. The animals (4 -5/time point) were killed by cervical dislocation at 30, 60, 120, and 360 minutes after drug administration. Tissues were quickly removed, weighed and immediately frozen and kept at -18°C until analyzed. Tissue specimens were homogenised (1/4, w/v) in 0.9% saline. The stobadine concentrations were determined by a liquid scintillation counting method (4) in blood plasma, brain and heart of mothers and foetuses. Due to a low volume of foetal plasma/organs, one sample represented a pool from 3-4 foetuses.

Results

The plasma concentrations of stobadine in both the does and foetuses are represented in Figure 1. The significance of the results at the probability level of $p < 0.02$ and $p < 0.001$ are indicated by asterixes.

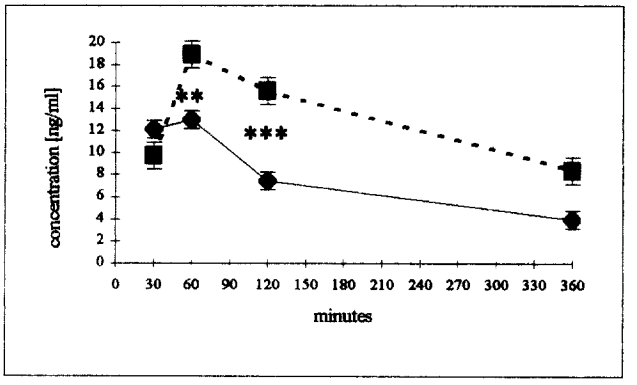

Fig. 1
Stobadine concentration in plasma in mothers (—) and foetuses (- - -). (**p < 0.02, ***p < 0.001.)

The stobadine time curves in the heart and brain in mothers and foetuses are depicted in Figure 2, panels A and B.

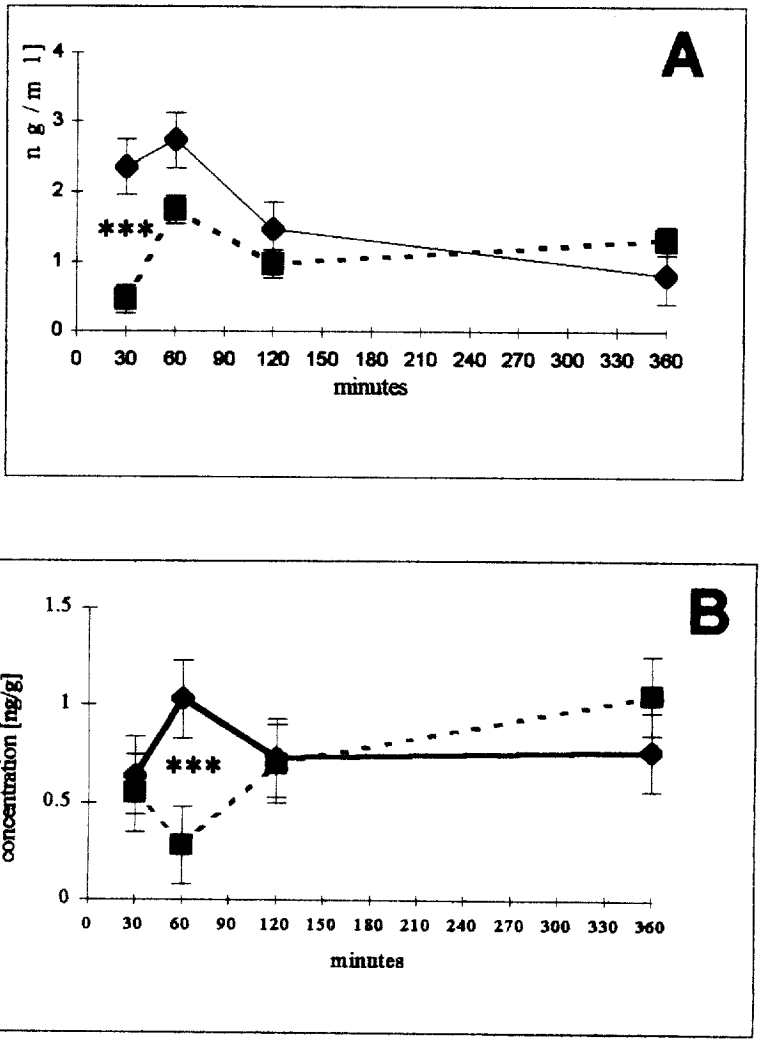

Fig. 2
Stobadine concentration in heart (panel A), and brain (panel B) in mothers (—) and foetuses (- -). (***$p < 0.001$.)

Discussion

After oral administration of stobadine in the dose of 5 mg/kg, the drug was rapidly resorbed from the gastrointestinal tract of pregnant dams and 60 minutes after intake the plasma concentration of stobadine reached its peak with the value of approx. 13 ng/ml. Within the same time interval, the plasma value of stobadine in foetuses exceeded 19 ng/ml and over the studied period of 60 to 360 minutes (elimination phase) the plasma concentration of the drug was found to be significantly higher in foetuses as compared to dams (Fig. 1). As a consequence, the value of the area under the curve is substantially higher in foetuses than in dams, i.e. the bioavailability of stobadine in foetuses exceeded that in dams. This somewhat unusual finding should be considered in the light of the fact that the concentration of the drug was investigated in tissues that were homologous, yet not identical. Namely, if the distribution of the parent drug molecules between blood elements and plasma is different in

samples taken from the mother and foetus, then at the same stobadine concentration in maternal and foetal blood, the values in the corresponding plasma samples will be different. Differences in the maturation of foetal blood elements compared to the elements in maternal blood and differences in the content of plasma proteins in the blood of the mother and of the foetus (e.g. α-foetoprotein) account for the above mentioned findings. This assumption is partially supported also by the finding that the stobadine concentration in the heart - i.e. an organ directly supplied by blood - was higher in the mothers than in foetuses (Fig. 2, panel A). This applied practically to the whole period studied. The nonsignificantly increased stobadine concentration in the foetal heart compared to the maternal one at 360 minutes suggests the existence of a certain affinity of the drug molecules to the foetal heart tissue.

This probability is documented by the results given in Fig. 2, panel B. While the curve representing stobadine concentration in the brain of dams exhibits the trend of drug elimination from the tissue, stobadine concentration in the foetal brain tissue keeps increasing over the same time period. The great difference between blood-brain barrier permeability of dams and of their foetuses as well as differences in the content of some tissue constituents, such as myelin and lipids (5) should be taken into account.

In conclusion, it can be stated that after oral stobadine intake by rabbit dams at the stage of advanced pregnancy both maternal and foetal organs will be under a certain drug level, as demonstrated in the organs examined, i.e. the heart and brain. The antioxidative/free-radical scavenging property of stobadine may thus exert an important preventive function in injurious situations such as hypoxia-reperfusion, which under conditions of inadequate supply of appropriate antioxidants to the heart, brain, etc., may result in irreversible tissue/organ damage induced by reactive oxygen species.

Acknowledgement

This work was supported by grants No. 134, 1021, and 5305 from the Slovak Grant Agency for Science, Bratislava, Slovak Republic. We thank Mr. J. Janšak for his technical assistance.

References

1. A.G. FANTEL, Teratology **53** 196-217 (1996).
2. S. ŠTOLC, R. VLKOLINSKÝ, and J. PAVLÁSEK, Brain Res. Bull. **42** 335-340 (1997).
3. L. ŠOLTÉS, T. TRNOVEC, Pharmazie **42** 863-864 (1987).
4. V. ŠČASNÁR, M. ŠTEFEK, J. Radioanal. Nuclear. Chem. **111** 117-122 (1987).
5. T. YAGINUMA, Asian Med. J. **29** 173-180 (1986).