Physical exercise and improvement of liver oxidative metabolism in the elderly

Abstract Drug metabolizing capacity is generally reduced in the elderly, and physical exercise has been reported to increase drug oxidative metabolism. The purpose of this investigation was to study the effects of engagement in a program of regular physical exercise on the clearance and metabolite excretion of antipyrine, a marker of oxidative metabolism, in elderly subjects. The saliva clearance of antipyrine and the production clearances of antipyrine metabolites were studied in 37 elderly women (mean age 66 years). Subjects attended 60-min sessions three times a week for 12 weeks. Each session consisted of both aerobic (training of cardiorespiratory capacity) and nonaerobic (training of muscular strength/endurance and flexibility/coordinating) exercises performed at 50–75% of maximum oxygen uptake. Antipyrine was administered orally and pharmacokinetic parameters were obtained from saliva and urine samples. After 3 months of participation in the exercise program, salivary antipyrine clearance was significantly increased by 17% (SEM) 0.42 (0.02) vs 0.36 (0.02) ml/min/kg; \(P < 0.05\) and the half-life of antipyrine was significantly reduced by 18% (17.9 (1.1) vs 22.3 (1.3) h; \(P < 0.05\)). No significant change with exercise was observed in the renal clearance of antipyrine or in the norantipyrine formation clearance, but significant increases were found for hydroxymethylantipyrine [42 (5) vs 32 (4) \(\mu\)l/kg/min; \(P < 0.05; +31\%\)] and 4-hydroxyantipyrine [243 (18) vs 194 (17) \(\mu\)l/kg/min; \(P < 0.05; +25\%\)] formation clearances. These findings indicate that regular exercise leads to increased disposition of antipyrine in the elderly and that the main metabolic pathways of the compound are changed differentially.

Key words Antipyrine clearance · Elderly · Physical conditioning · Oxidative metabolism

Introduction

The elderly are an important segment of the population from both medical and socio-economic points of view. Elderly patients often exhibit a decline in drug metabolizing capacity which, together with the presence of multiple disease states and multiple drug therapies, increases the likelihood of clinical toxicity and adverse drug reactions (Jorquera et al. 1995). Hepatic biotransformation is a major route of drug detoxification, involving mainly oxidative reactions through the cytochrome-P450-dependent mono-oxygenase system (Ziegler 1994). Antipyrine is a molecule that is metabolized by the cytochrome P450 liver enzymes, and has been used extensively to study the influence of disease and environmental factors on hepatic oxidative metabolism and drug metabolizing capacity (Poulsen and Loft 1988; St Peter and Awni 1991). Antipyrine is metabolized to three major metabolites whose formation is dependent upon one or more selective forms of cytochrome P450. Characterization of the main individual antipyrine metabolite dispositions may therefore provide specific information about liver metabolic pathways (Poulsen and Loft 1988). Antipyrine clearance is not limited by liver blood flow, which declines with age, and constitutes a sensitive marker of hepatic microsomal enzyme activity in the elderly (Jorquera et al. 1998). Lower clearances and longer half-lives of antipyrine have been reported to occur in elderly subjects in a number of studies (e.g. Sotaniemi et al. 1997). Differences in enzyme inducibility that are attributed to smoking, alcohol and other drugs have been proposed to account for these changes (Loft et al. 1988; Vestal and...
Wood 1980), and variables that can predict a low metabolizing capacity, including factors other than age, have been identified (Jorquera et al. 1995).

Despite the potential benefits of exercise, rates of exercise among older adults remain low (Allison and Keller 1997). Participation in regular exercise, however, elicits a number of favourable responses that contribute to healthy aging, and many studies have demonstrated that physical activity can minimize and prevent chronic problems and increase functional activity in the elderly (American College of Sports Medicine 1998). The effects of physical exercise on liver oxidative metabolism are sometimes unclear due to lack of control of environmental factors, or to the use of inappropriate experimental designs and kinetic models (Boel et al. 1984; De Vito et al. 1995; Fabbri et al. 1991; Frenkl et al. 1980; Oriolli et al. 1990). However, we have recently reported the existence of an association between aerobic conditioning and antipyrine clearance that suggests an increased hepatic oxidative metabolism of low-clearance drugs (Villa et al. 1998). Participation in regular exercise programs could thus be proposed as an effective intervention to reduce or prevent the decline of drug metabolism capacity in the elderly.

The purpose of our study was to assess the influence of regular physical exercise on antipyrine clearance and to determine whether the activities of individual cytochrome P450 isoenzymes are differentially affected by exercise in the elderly. Saliva clearance of antipyrine and the production clearances of antipyrine metabolites were measured before and after participation in a 3-month program of exercise.

Methods

Compounds

Antipyrine (Sigma, St. Louis, Mo., USA), norantipyrine (NORA) and 4-hydroxyantipyrine (OHA) (Aldrich, Milwaukee, Wisc., USA) were obtained from commercial sources and at the highest purity available. 3-Hydroxymethylantipyrine (HMA) was generously donated by Dr. S. Loft (Institute of Pharmacology, Copenhagen, Denmark).

Subjects and procedures

Antipyrine metabolism was studied in 37 elderly women who participated in a program of regular physical exercise. Their mean age was 66 years (range 57–76 years). The study was approved by the Ethics Committee of the University of León. Subjects gave their informed consent before entering the study. Inclusion in the study required that they be medically stable, suffering no hepatic or renal disease and with no hospitalizations within 1 month before the study. All subjects were non-smokers.

Subjects attended 60-min sessions three times a week for 12 weeks. Each session was always initiated with a 15-min warming-up period, finished with a 10-min recovery period, and consisted of both aerobic (training of cardiorespiratory capacity: walking, jogging, trotting, gymnastics – 25 min) and nonaerobic (muscular conditioning: workloads with 10–15 repetitions of combined muscle groups – 20 min; flexibility/coordination: stretching, jumping, throwing – 15 min) exercises at 50–75% of maximal oxygen uptake (VO2max).

Antipyrine kinetics was studied at the beginning and at the end of the exercise program. Antipyrine (1 g) was administered orally following at 10-h fast. Saliva samples were collected at 24 h (Dossing et al. 1982). Urine was collected for 48 h after antipyrine ingestion, in containers with an antioxidant (sodium metabisulfite), and the urine volume was measured. Saliva and urine samples were frozen at −40°C until assay.

VO2max was assessed from a treadmill test using a maximal graded protocol (MacDougall et al. 1991). The test was started at a velocity of 6 km·h−1 and an incline of 3%. Subjects ran with a constant grade and a 1-km·h−1 increase in speed every minute until exhaustion. An oxygen analyser was used for measuring oxygen consumption (VO2) and other respiratory/metabolic variables. Heart rate was recorded continuously during exercise. The criteria used for attaining VO2max included a plateau of VO2 at increasing work rate and/or a maximum heart rate within 5 beats·min−1 of the age-predicted maximum.

The Ruffler-Dickson index (RI) was calculated at the beginning and at the end of the exercise program by the equation:

\[ RI = \frac{(P_0 - P_1) + 2(P_2 - P_3)}{10} \]

where \( P_0 \), \( P_1 \), and \( P_2 \) correspond to the heart rate before, at the end, and 60 s after, respectively completing a test in which 30 complete flexions were performed in 45 s (Barthelemy et al. 1985).

Analytical methods

Percent body fat was estimated from the sum of four skinfolds (Jackson and Pollock 1978). Antipyrine concentration in the saliva was determined using a high-performance liquid chromatography (HPLC) technique (Danhof et al. 1979) as follows: to 1.0 ml of saliva, 100 µl of a solution containing 400 µg phenacetin/ml ethanol and 100µl 2 N NaOH was added. After extraction with 5 ml dichloromethane-n-octane (1:1, v/v) on a whirllmixer for 15 s, the organic layer was collected and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 100µl eluent, of which 25 µl was injected into the HPLC system.

Levels of antipyrine and its three main metabolites in urine were measured (Mitaki et al. 1988) as follows: to 1.0 ml of urine, 50 mg of sodium metabisulfite, 1 ml of 1.0 M acetic buffer pH 5.0 and 50 µl of glusulase were added. Samples were incubated in a shaker bath at 37°C for 3 h. Then, 0.5 ml of the buffer and 20 µg of phenacetin were added to each tube, and the samples were subsequently extracted with 5 ml of ethyl acetate by shaking them on a horizontal mixer for 10 min at 90 cycles/min. After centrifugation at 400 g for 10 min, the organic layer was collected and evaporated to dryness. The residue was dissolved in 100 µl eluent, of which 25 µl was injected into the HPLC system.

The HPLC system consisted of a SP8000 pump (Spectra Physics, San José, Calif., USA), a Spheri-10 RP-18 10-µm column (Brownlee Columns, San José, Calif., USA), and a Spectra Chrom 200 detector (Spectra Physics) set at 254 nm. Column temperature was maintained at 40°C by a water circulator. The mobile phase, consisting of 0.1 M sodium acetate, 7.5% acetonitrile and 0.5% triethylamine, pH = 6.6, was delivered at a flow rate of 3.5 ml/min.

Linear calibration with correlation coefficients better than 0.990 was obtained for all assay procedures. The limit of quantitation for levels of antipyrine in saliva was 0.1 µg/ml. The limit of quantitation in urine was 2 µg/ml for antipyrine, HMA and NORA, and 5 µg/ml for OHA.

Pharmacokinetic analysis

Salivary antipyrine clearance and half-life were calculated by the equations (Dossing et al. 1982):

\[ Cl_{AP} = \frac{\ln(D/V_d) - \ln C_t}{t} \times V_d \]