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It become evident that Ca^{2+}-dependent release of arachidonic acid (AA) and subsequent formation of bioactive lipid mediators such as prostaglandins and leukotrienes in red blood cells (RBCs) can mofify physiological functions of neighboring RBCs and platelets, and thus influence hemostssis and thrombosis. Here we identified a novel type of cytosolic PLA_2 in bovine and human RBCs and purified it to apparent homogeneity with a 14,000-fold purification. The purified enzyme, termed $rPLA_2$, had a molecular mass of 42 kDa and revealed biochemical properties similar to group IV $cPLA_2$, but showed different profiles from $cPLA_2$ in several column chromatographies . Moreover, $rPLA_2$ did not react with any of anti-$cPLA_2$ and $sPLA_2$ antibodies and examined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometric analysis. Divalent metal ions tested exhibited similar effects between $rPLA_2$ and $cPLA_2$, whereas mercurials inhibited $cPLA_2$ but had no effect on $rPLA_2$. Antibody against the 42 kDa protein not only precipitated the $rPLA_2$ activity, but also reacted with the 42 kDa protein from bovine and human RBC in immunoblot analysis. The 42 kDa protein band was selectively detected in murine fetal liver cells as a type of progenitor cells of RBCs. Finally, we found that EA4, a derivative of quinone newly developed as an inhibitor for $rPLA_2$ in vitro, inhibited a Ca^{2+} ionophore- and mechanical stress-induced AA release from bovine and human RBCs. Furthermore, Ca^{2+} ionophore- and exogenous AA-induced AA release inhibited by PD 98059, MAP kinase inhibitor. Our results altogether demonstrate that the 42 kDa $rPLA_2$ is identified as a novel form of Ca^{2+}-dependent PLA_2 and responsible for the Ca^{2+}-dependent AA release from human and bovine RBCs and modulated by MAP kinase at least in part.

[PA4-10] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Protective effects of paeonol on cultured rat hepatocytes exposed to Br-A23187

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Br-A23187 (Ca2+ ionophore) is frequently used as a model of Ca2+-dependent cell killing as Br-A23187 induces both necrotic and apoptotic cell death. The aim of this study was to evaluate the protective effects of paeonol isolated from Paeoina Moutan on cultured rat hepatocytes exposed to Br-A2187. Cell killing was assessed propidium iodide fluorometry. Br-A23187 caused dose-dependent cell killing. Br-A23187 (10μM)-induced cell killing was decreased in the presence of paeonol (20, 50 and 100 μM). On the other hand, Br-A23187-induced [Ca+2]i level was increased dose-dependently. However, paeonol decreased Br-A23187-induced [Ca+2]i level in dose-dependent manner. Additional measurements of enzyme activities were made to know whether paeonol inhibits those enzyme activities such as LDH, S-GOT and S-GPT on cultured hepatocytes.
Therefore, the present results indicate that paeonol has protective effects against Br-A23187-induced hepatocytotoxicity in rats, indicating paeonol decreases [Ca+2]i level and inhibits enzyme activities related to hepatotoxicity.

[PA4-11] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

The Screening of Toxicity from the Processed Aconiti Tuber in vitro and in vivo

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Aconiti Tuber is the root of *Aconitum* sp(Ranunclaceae) which has been considered as one of the most important herbal medicine having diuretic and analgesic effect. But it has been well known that A. Tuber contained toxic compounds, aconitine alkaloids so that only processed A. tubers have been used as herbal drug traditionally.
For the investigation of Aconiti Tuber toxicity on Liver, Kidney cell lines, the commercial processed Aconiti Tuber which is called as Yumbuja was more processed into four kinds of processing methods after Chinese Pharmacopoeia and other traditional medicine literatures and these five processed drugs were extracted with hot water.
Processing methods
①. Blanch the commercial processed Aconiti Tuber -Yumbuja- with water, 2-3 times a day until all salt is rinsed out. Boil together with Radix Glycyrrhizae, black beans and water until the centre of the cut surface is devoid of white core and cut slice is numbless to the tongue. remove Radix Glycyrrhizae, black beans, cut the drug into slices, and dry in the sun. (To each 100kg of Ryumbuja add 5kg of Radix Glycyrrhizae and 10kg of black beans)
②. Blanch the commercial processed *Aconiti Tuber* -Yumbuja- with water, 2-3 times a day until all salt is rinsed out. Boil together with water until the centre of the cut surface is devoid of white core and cut slice is numbless to the tongue. cut the drug into slices, dry in the sun.
③. Blanch the commercial processed Aconiti Tuber -Yumbuja- with water, 2-3 times a day until all salt is rinsed out. Treat it on 120℃, for 40 minutes in dry oven, and cut into slices and dry in the sun.
④. Treat the commercial processed Aconiti Tuber -Yumbuja- on 120℃, for 40 minutes in vacuum oven, cut into slices and dry in the sun.
In Vitro test(MTT assay on Vero 76 and NCTC clone 1468)
The toxicities of hot H2O extracts of five processed drugs were evaluated on the two kinds of 2 x 105 cell line(Vero 76: kidney cell, NCTC clone 1469: liver cell) by MTT assay. The vero 76 cells showed no or very weak toxicities of all processed drugs in concentration of 0.18 - 0.5mg/200㎕, but the NCTC clone cell showed IC50 in concentration of 12-20㎍/200㎕. Especially, IC of Yumbuja was the lowest of all (12㎍/200㎕). From this data, it suggested that the prossesed Aconiti Tuber could be decreased toxicity in kidney cell, but these processing methods may not help to decrease of liver toxicity.
In Vivo test(Acute toxicity in mice)
For the investigation of A. tuber toxicity, hot water extracts from five kinds of processed A. Tuber were tested in mice one time oral administration in 5 different dosis(2.0g/㎏, 2.6g/㎏, 3.2g/㎏, 4.0g/㎏, 5.0g/㎏). Their LD50 values were above 4-5g/㎏.

[PA4-12] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Antiaging effect of CS in ovariectomy induced rat liver

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The ovarian hormone deficiency induced ovariectomy rat is widely used as aged model due to its practicality, convenience, and cost effectiveness. The surgically ovariectomized rat induces aging by reactive oxygen species(ROS) generation.
Reduced cartilage Chondroitin Sulfate(CS), a component of articular cartilage proteoglycan, levels may be a risk factor involved in articular cartilage in elderly people.
To investigate the deaging effects of intraperitoneally injected CS on various antioxidative enzyme activity (Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), reduced-Glutathione (GSH), oxidized-Glutathione (GSSG), Glutathione Peroxidase (GPx)) and histopathology of liver tissue, ovariectomized rats were used.
The antioxidative effects of CS(100㎎/㎏ and 200㎎/㎏ body weight) were investigated by the antioxidative enzyme activities of liver homogenate fractions (liver total homogenate, mitochondrial, and microsomal fractions). In addition, the ovariectomized rat liver was histologically examined.
Intraperitoneally injected CS, dependent on dosage, indicated a protective effect against ovariectomy-