decrease of SIC, whereas serum iron parameters and haemoglobin levels were not different from those of non phlebotomized mice. In *iron overloaded mice*, at D0 we found an increase of serum iron, HIC and SIC as compared to control mice. At D22 : i) in the absence of phlebotomies, SIC significantly increased compared to D0 whereas a decrease of both serum iron and haemoglobin levels was found. ii) phlebotomies strongly decreased serum iron and haemoglobin levels, whereas both LIC and SIC were similar to D0 values. In addition, iron overload induced : i) an hepatic increase of hepcidin1 mRNA level, partially corrected by phlebotomies, and a decrease of hepatic TfRc mRNA level which was not further modulated by phlebotomies, ii) a splenic increase of ferroportin mRNA level which was not modulated by venesection therapy, and a TfRc increase at D0, remaining significant in the phlebotomized group. Conclusions. Our data show that, in our model, the first iron source to compensate iron needs is the spleen. Furthermore, they suggest that hepatic hepcidin induction produced in this secondary iron overload model leads to a decrease in iron bioavailability, mainly related to splenic iron sequestration, which ends up with a decrease of haemoglobin levels. The liver seems to be resistant to this "hepcidin effect". These results should be kept in mind when discussing venesection therapy in iron overloaded patients without abnormal hepcidin downregulation.

P-09

BIOCHEMICAL CHARACTERISATION OF FERROPORTIN IN XENOPUS OOCYTES REVEALS NICKEL TRANSPORT ACTIVITY

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The nature of iron transport via the iron efflux protein ferroportin was examined by expressing the human form in Xenopus oocytes and measuring iron efflux after microinjection of radiolabelled ^{59}Fe solutions. A time course revealed iron efflux increased linearly with time reaching a plateau after 8 hours. Iron efflux displayed a relatively linear increase over a concentration range 60-150¼M indicating a 1st order process. Efflux of iron via ferroportin was temperature dependent; however there was no evidence of either pH or sodium dependence. When ^{59}Fe as ferric iron was injected into ferroportin-expressing oocytes iron efflux was 10-fold less when compared to ^{59}Fe ferrous iron injection, indicating ferrous is the preferred substrate for ferroportin. Studies in rat have suggested that nickel may share the same absorption pathway as iron(Muller-Fassbender et al., 2003; Tallkvist and Tjalve, 1994). In addition a plant homologue of ferroportin has been shown to play a role in nickel detoxification in plants (Schaaf et al., 2006). To test whether the human form of ferroportin can transport nickel, oocytes were injected with ^{63}Ni. We found a significant 2 fold increase in nickel efflux compared to control oocytes, indicating that ferroportin can transport nickel and that nickel can compete with iron for transport via ferroportin. We found that exposure of ferroportin expressing oocytes injected with ^{59}Fe to increasing concentrations of hepcidin 25 had no effect on iron efflux. This indicates that hepcidin binding to ferroportin per se is not sufficient to inhibit iron transport and that additional proteins not present in oocytes are required for the internalisation and degradation of ferroportin by hepcidin. Further data on the transport of other divalent metals via ferroportin and the effect of the copper oxidase ceruloplasmin on iron efflux will be presented.

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P-10

OVEREXPRESSION OF MITOCHONDRIAL FERRITIN SENSITIZES CULTURED CELLS TO OXIDATIVE STRESS VIA AN IRON-MEDIATED MECHANISM

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Cytosolic ferritin is well known for its ability to sequester and store iron, consequently exerting protective roles against iron-mediated free radical damage. Mitochondrial ferritin (MtF) is a newly identified ferritin H chain-like protein, which is expressed only in mitochondria. However, the function of MtF is unknown. Mitochondria are the location of heme and iron-sulphur cluster synthesis and, therefore, mitochondrial iron levels must be well regulated. Excess iron in mitochondria will promote the generation of harmful reactive oxygen species (ROS) that are produced as a side reaction of mitochondrial electron transport. In previous studies, we have shown that, by sequestering intracellular iron, MtF overexpression markedly affects intracellular iron homeostasis in mammalian cells and also inhibits tumor growth. To asses how MtF overexpression affects the response of cells to oxidative stress, we took advantage of a well established cell line that stably overexpresses mouse MtF under the control of tetracycline. These cells were treated with tert butyl-hydrogen peroxide (tBHP) and examined for the effects of MtF expression on cell viability, redox status and other stress parameters. Somewhat surprisingly, we consistently observed that MtF expression sensitizes cells to oxidative stress. The expression of MtF caused a dose dependent decrease in cell viability that was associated with decreases in mitochondrial metabolic activity and mitochondrial membrane potential, with a concomitant increase in ROS production and apoptosis as well as a decrease in the reduced form of glutathione. Since our previous studies have demonstrated that MtF expression significantly elevated transferrin receptor (TfR) levels, decreased cytosolic ferritin levels and increased cellular iron uptake, we speculate that the elevated uptake of transferrin-iron, accompanied by decreased cytosolic ferritin levels, may be responsible for the damaging effects of MtF expression under oxidative stress. This hypothesis is supported by our finding that MtF-expressing cells treated with tBHP had elevated TfR levels (both protein and message) as well as a significant increase in iron uptake. MtF expression also increased pro-apoptotic Bax protein levels and decreased anti-apoptotic Bcl-2 protein levels. Moreover, the iron chelator salicylaldehyde isonicotinoyl hydrazone (SIH) was able to prevent cell damage induced by tBHP, further supporting our idea that iron is responsible for the tBHP-induced cell death. In conclusion, our study revealed that MtF affects cellular iron homeostasis and redox status. This study, together with our previous results, indicates that, although iron accumulated in MtF may be unavailable for metabolic use and Fenton chemistry, the newly acquired iron from the extracellular environment may be responsible for the increased sensitivity to oxidative stress in MtF-overexpressing cells.

P-11

ANALYSIS OF IRP1/IRP2 CHIMERAS

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Iron regulatory proteins 1 and 2 (IRP1 and IRP2) are iron-regulated RNA-binding proteins that control the expression of proteins required for iron homeostasis (i.e. ferritin and transferrin receptor-1). Although IRP1 and IRP2 are ~60% identical, they are differentially regulated by intracellular iron. Iron-replete conditions stimulate the formation of a [4Fe-4S] cluster in IRP1 converting it into a cytosolic aconitase while simultaneously preventing its RNA-binding function. In contrast, iron stimulates the proteasomal degradation of IRP2. Conserved cysteines within a unique 73-amino acid region of IRP2 were previously thought to be required for iron-dependent degradation. However, three independent studies have shown that mutation of these cysteines, or removal of the entire 73-amino acid domain, does not affect iron-mediated IRP2 degradation (*Bourdon et al, BCMD, 2003; Hanson et al, JBC, 2003; Wang et al, MCB, 2004*). The region(s) of IRP2 that is required for iron-mediated degradation, as well as any potential role that RNA-binding may play in this process, is not known. To identify a region(s) of IRP2 that is required for iron-dependent degradation, we used secondary and tertiary structure predictions to divide IRP1 and IRP2 into four homologous domains, and then used domain swapping to generate a series of chimeric IRP1/IRP2 molecules. The RNA-binding activity and iron-dependent degradation of the chimeras were analyzed in stable tetracycline-inducible HEK293 cell lines. After overnight induction, all chimeric molecules were expressed at similar levels and had a half-life of >8 h. Only three of the seven chimeras bound RNA, suggesting that the overall structure of the chimeras may be altered compared to wild type IRP2. Iron-dependent degradation was observed in chimeras containing three domains of IRP2. One chimera did not bind RNA but still demonstrated iron-mediated degradation. To confirm that RNA-binding is not required for iron-degradation, we generated a single amino-acid substitution in IRP2 (R856Q) that abolished RNA-binding activity and showed that this mutant was degraded by iron. We conclude that although RNA-binding is not required for iron-degradation it is