Full Length Research Paper

Effect of iron on the proliferation of lung adenocarcinoma cells *in vitro*

Zhe Cheng[1], Yongna Song[1], Jie Xia[1], Yan Kang[1], Ling-ling Dai[1] and Yufeng Liu[2]*

[1]Department of Respiratory Medicine, the First Affiliated Hospital of Zhengzhou University, Institute of Clinical Medical Research of Henan Universities, Zhengzhou 450052, China.
[2]Department of Pediatrics, the First Affiliated Hospital of Zhengzhou University, Institute of Clinical Medical Research of Henan Universities, Zhengzhou 450052, China.

To investigate the effect of iron on the cell proliferation of lung adenocarcinoma cell, A549 cells were respectively treated with chloride ferric ($FeCl_3$) and/or deferoxamine (DFO) at different concentrations, the viable cell number and cell viability were analyzed in each group at 6, 12, 24 and 48 h, respectively. After A549 cells were respectively treated with $FeCl_3$ at different concentrations, the viable cell number in each $FeCl_3$ subgroup gradually increased with the prolongation of culture time, likewise that in the control group. However, all the numbers of the $FeCl_3$ subgroup were higher than those in the control group (except for the number in 150 µmol/L subgroup at 24 h), exhibiting statistically significant differences (P<0.05). Among different $FeCl_3$ subgroups, the viable cell number in 100 µmol/L subgroup was the highest at any detected time point (P<0.05). And cell viability of $FeCl_3$ subgroups were all higher than those of the control group during culture time. On the contrary, after A549 cell being treated with DFO at different concentrations, the viable number and cell vitality in each subgroup were lower than those in the control group, and all the differences were of statistical significance (P<0.05). In the control group, viable number increased with the extension of culture time with cell viability keeping over 95% while those in DFO subgroups decreased. And comparisons among different DFO subgroups showed that the higher the concentration, the more apparent the decline becomes. In DFO subgroups, A549 cells under light microscope looked smaller and viable numbers were less compared with those in the control group, and such inhibitory effect became even more apparent in subgroups with high concentrations of DFO. Our finding suggests that $FeCl_3$ can promote the growth of A549 cells while DFO can exert an anti-proliferative effect on A549 cells.

Key words: Chloride ferric, A549 cells, proliferation, deferoxamine.

INTRODUCTION

Iron is one of the essential basic elements of human life (Arredondo and Nunez, 2005). All cells need to take up iron to keep their proliferation during the growth and development periods, in which tumor cells, naturally, are included. Such dependence on iron can be illustrated in some key reactions catalyzed by iron-containing protease, in which iron plays a key catalytic roles in the processes, from oxidative respiration to energy metabolism, from folic acid metabolism to DNA synthesis and repair, etc (Le and Richardson, 2002). Cañete et al.

(2010) present a morphological approach in observing the interaction of cationic magnetic nanoparticles with human lung adenocarcinoma A-549 cells. Lactoferrin (Lf), an iron-binding protein present in mammalian secretions, binds IGBP1 and promotes the acceleration of cellular apoptosis (Li et al., 2011).

Lung carcinoma is one of the most common malignant tumors with high incidence, which has posed a big threat for human health. Traditionally, transfusion of whole blood or blood component could enhance the immune functions of patients suffering from lung carcinoma, especially for those complicated with mild anaemia. However, transfusion can cause a change from an iron-deficient state of a patient with anaemia to an iron-rich state,

---
*Corresponding author. E-mail: lyf6012@126.com.

and according to recent studies, it is an iron-rich state rather than an iron-deficient state which is more favorable to the proliferation of tumor cells. Thus, the study on the effect of iron on the development of lung carcinoma and its mechanism may be important in theoretical and clinical values.

In this study, human lung adenocarcinoma cell line A549 was chosen as the study object, and deferoxamine (DFO) and/or chloride ferric ($FeCl_3$) at different concentrations were separately applied to A549 cells to observe cell growth and proliferation, and investigated the effect of iron on lung carcinoma cells and its effective mechanism.

MATERIALS AND METHODS

Cell culture

A549 cells were purchased from Shanghai Institutes for Biological Sciences, China. Frozen A549 cells were thawed out in a thermostat-controlled water-bath at 37°C, added into F12K medium (GIBCO,USA) supplemented with 10% deactivated FBS (Hyclone,USA), and then cultured in a humidified incubator at 37°C with 5% CO_2. Cells were harvested every 3 to 4 days. Cells in exponential phase of growth were taken for experimental use and cell viability was measured by Trypan blue staining.

Experimental groups

Four groups were assigned: $FeCl_3$ (Sigma) group, DFO (deferoxamine, sigma) group, $FeCl_3$+DFO group and the blank control group. $FeCl_3$ group and DFO group were subgrouped according to different concentrations (10, 50, 100 and 150 μmol/L, respectively). $FeCl_3$+DFO subgroups were obtained by mixing equal concentrations of $FeCl_3$ and DFO (10, 50, 100 and 150μmol/L, respectively) while the control group was obtained by adding the same amount of saline. The proliferation of A549 cells was respectively measured at 6, 12, 24 and 48 h in each subgroup mentioned above and the control group.

Typan blue staining

From the control group and each subgroup, cells at different time points were taken respectively. Adequate amount of them was added into 0.1% trypanblau solution. Then a drop of mixed solution was trickled into the cell numbering plate and observed under the light microscope. A cell with blue stained endochylema and a swollen body was numbered as a dead cell while a cell with a round body was numbered as a viable cell. Dead cells and viable cells in four big squares were respectively numbered based on the following formula:

Viable numbers in four squares/4 × 10^4× dilution multiple × cell suspension volume

And cell viability was figured out as follows:

Cell viability= viable number/total cell numbers × 100%

Statistical analysis

Statistical analysis was carried out by SPSS13.0 software, and data were presented by the means ± standard error of means ($\bar{x} \pm s$). Variance analysis was used to compare the measurement data between groups and t-test was used for comparison within groups. P<0.05 was considered as statistically significant.

RESULTS

Effect of $FeCl_3$ on the proliferation of A549 cells

After A549 cells being separately cultured with different concentrations of $FeCl_3$, the results at different detected time points showed that the viable numbers in both $FeCl_3$ group and the control group increased with the prolongation of culture time. Comparison between $FeCl_3$ group and the control group at the same time point showed that the viable numbers in each $FeCl_3$ subgroup was higher than those in the control group except for that in 150 μmol/L subgroup at 24 h, and all the differences between each subgroup and the control group were of statistical significance (P<0.05) with except for that between 5 μmol/L subgroup and the control group (P>0.05). Among the different $FeCl_3$ subgroups, the viable number in 100 μmol/L subgroup showed the highest proportion at different time points (Figure 1). Cell viability tests showed the proportions taken by viable cells in all $FeCl_3$ subgroups and the control group increased with the extension of culture time, and so did cell viability (Figure 3). All cell viability curves of $FeCl_3$ group was higher than that of the control group without big deflection, when compared to the curves of DFO group, the tendencies indicated by the curves of $FeCl_3$ group and those of DFO group stood in great contrast.

Effect of DFO on the proliferation of A549

After A549 cell being respectively cultured with DFO at different concentrations, the results displayed that both viable cell number and cell viability in each DFO subgroup was less or lower than those of the control group at the same time point, which had statistical significance (P<0.05), and as the concentration of DFO became higher, the viable cell number and cell viability were fewer. Moreover, the viable cell number in the control group increased with the prolongation of culture time and cell viability kept constantly above 95%. In contrast, the viable cell number in each DFO subgroup decreased with the prolongation of time, and such effect was even more apparent with the increasing of DFO concentration (Figures 2 and 4).

Effect of $FeCl_3$+ DFO on the proliferation of A549 cells

The viable cell number in each $FeCl_3$+ DFO subgroup was very close to that in the control group, and the difference had no statistical significance (P>0.05).
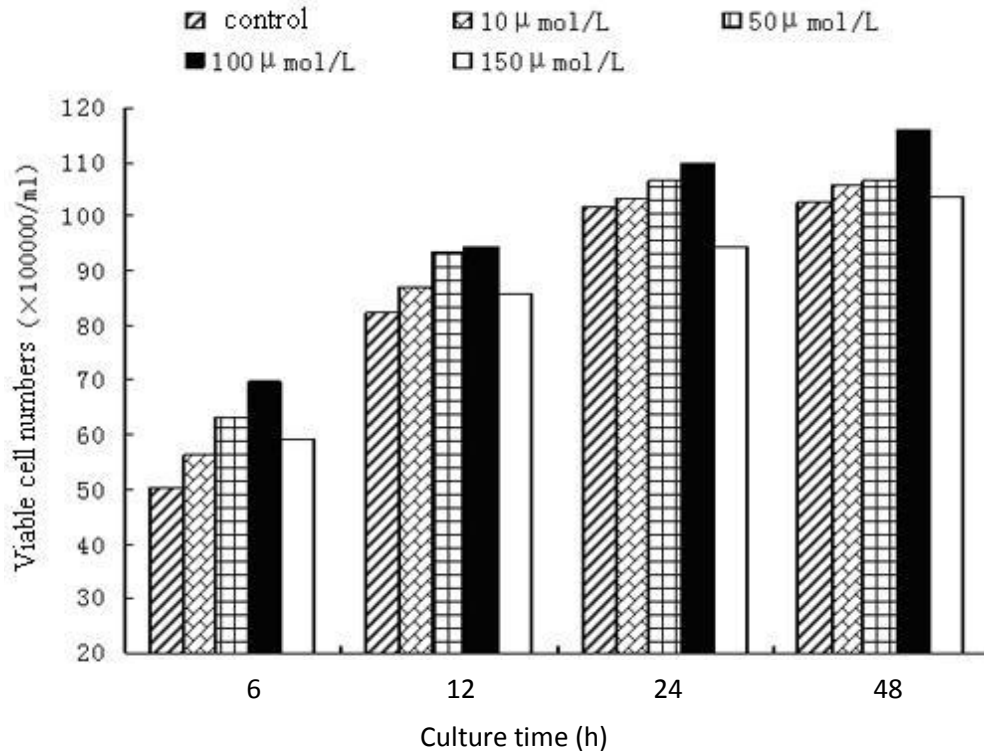
Figure 1. Effect of FeCl₃ at different concentrations on the proliferation of A549 cells.
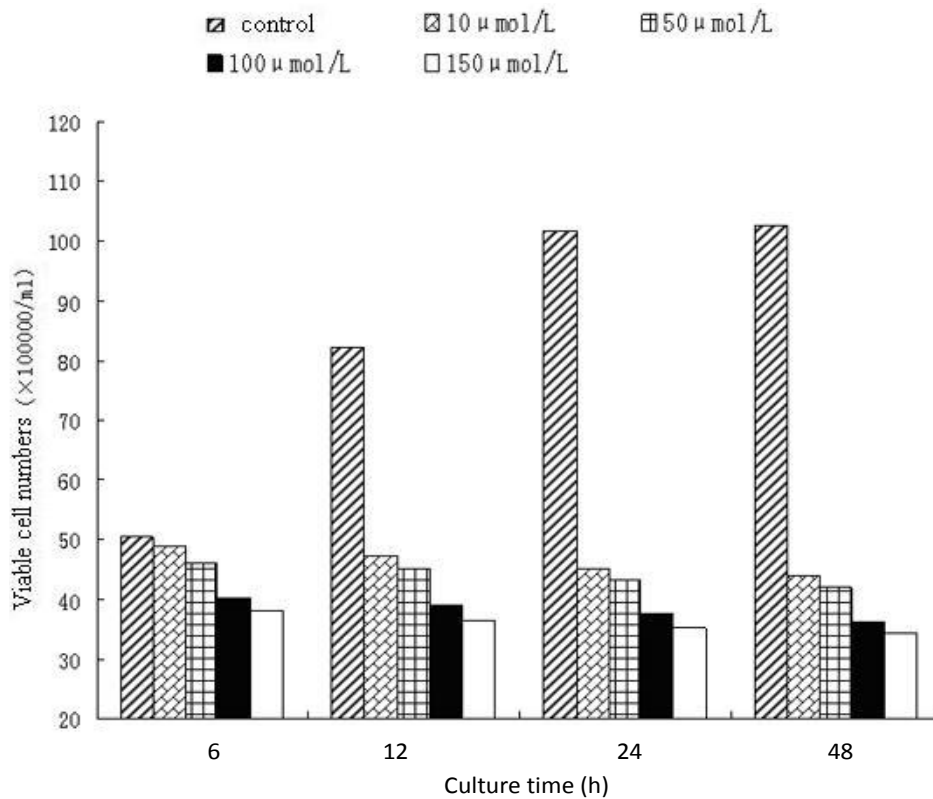

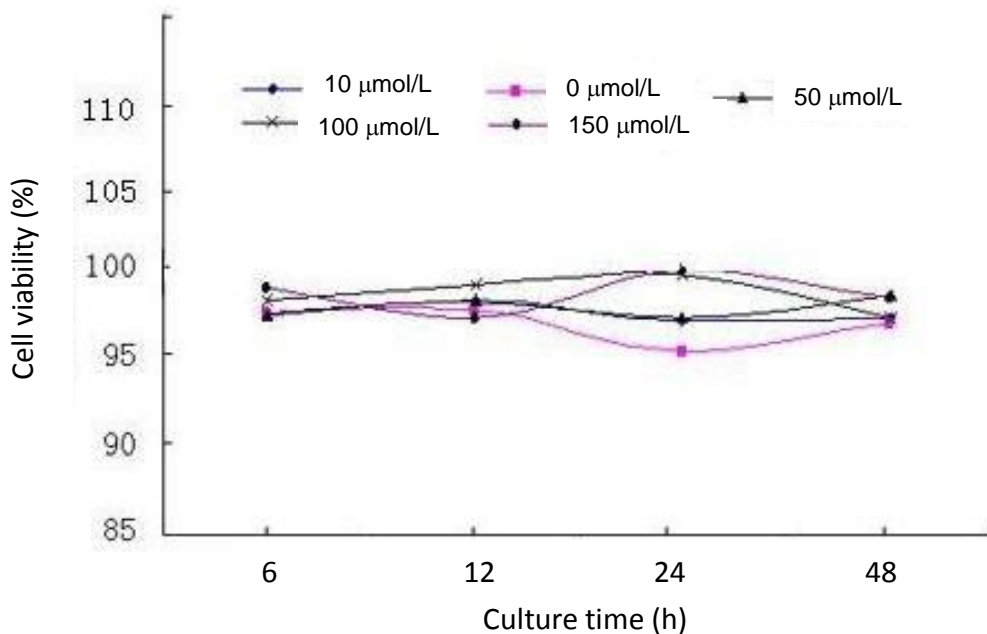**Figure 2.** Effect of DFO at different concentrations on the proliferation of A549 cells.

Figure 3. Effect of FeCl3 at different concentrations on cell viability of A549 cells.
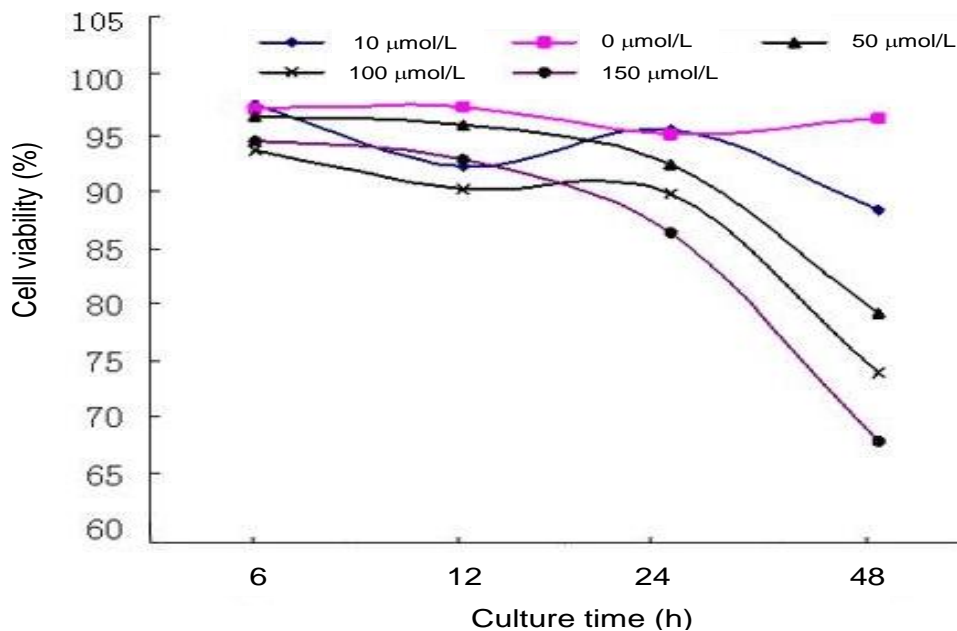

Figure 4. Effect of DFO at different concentrations on cell viability of A549 cells.

DISCUSSION

Iron is necessary to support life, which exists in the body in two forms: ferrous iron and ferric iron (Fairweather-Tait, 2004). Ferrous iron and ferric iron can mutually transform between each other through oxidation-reduction reaction, but the accomplishment of oxidation-reduction reaction has to resort to electron transfer. It is just because of electron transfer that iron has been endowed with various biological functions. However, these biological functions in themselves are greatly different from each other, in that some functions can be beneficial for organizations while some can cause injury, or even cells canceration (Eisenstein, 2000; Huang,

2003). Whether iron has effects on the proliferation of lung carcinoma cells, and if it does, what is the effectively mechanism like? Reports concerning these questions are rare until now, to our knowledge. Based on these above factors, this study investigated the effect of iron on the proliferation of lung adenocarcinoma A549 cells.

In this study, A549 cells were respectively treated with $FeCl_3$ at four different concentrations. The results showed the viable cell numbers in $FeCl_3$ subgroups and the control group increased with the extension of culture time, and comparisons among different $FeCl_3$ subgroups at the same time point showed that the viable cell number in subgroup of 100 µmol/L was the highest of the four, displaying some time-and-dose dependent manner. However, when the concentration of $FeCl_3$ reached 150 µmol/L, the viable cell number showed a decline, which might be explained by the toxic effect of iron. Regardless of the extension of culture time, all the cell viability curves of $FeCl_3$ subgroups constantly located above that of the control group, with that of 100 µmol/L subgroup highest. These above results indicated certain amount of $FeCl_3$ can promote the growth of A549 cells and inhibit the apoptosis of A549 cells, and its mechanism may be related to the reason that iron can improve DNA synthesis of A549 cells by activating the RNA reductase.

According to some updated reports, iron consumption could inhibit the progress of hepatic fibrosis of hepatopaths and reduce the incidence of canceration (Fargion et al., 2011). And some studies reported that iron played an important role in the development of non-alcoholic liver disease to hepatic cellular cancer (Starley et al., 2010). In addition, studies on breast carcinoma showed that controlling the effect of iron on proteins could inhibit the development of breast carcinoma (Jiang et al., 2010). Our study also proved that certain amount of iron can promote the growth of tumor cells. Meanwhile, some studies indicated that transferrin, no matter in the process of exo- or endogenous iron metabolism, could promote the proliferation of tumor cells *in vivo* or *in vitro* (Brookes et al., 2006; Park et al., 2009). All the results suggested that clinically, iatrogenic iron supplement for patients suffering from tumors or those from the regions with the high incidence of tumors should be carried out with cautions, and if the hemoglobin concentration is low but without declining to such a degree of life-threatening, blood-transfusion should be tried to avoid or the volume should be reduced to as small volume as possible, to inhibit the initiation and development of tumors or to avoid adverse effects on chemotherapy for tumors.

Some studies indicated that iron consumption could inhibit the proliferation of colon carcinoma cell line Caco-2 and induce its apoptosis (Sun et al., 2009). In our study, DFO at four different concentrations was respectively applied to A549 cells. Cell viability tests showed that cell viability gradually declined with the prolongation of culture time and the increase of drug concentration, exhibiting a time-and-dose manner, which indicated iron-depriving

can inhibit the proliferation of A549 cells. After A549 cells treated with $FeCl_3+$ DFO, the viable cell number and cell viability in each subgroup was similar to those in the control group, and the effect of DFO on A549 basically disappeared, suggesting DFO exerts its effect on the proliferation of A549 cells through interfering in their iron metabolism.

Conclusion

Chloride ferric can promote the growth of A549 cells while deferoxamine can exert the anti-proliferative effect on A549 cells. Iatrogenic iron supplement should be carried out with cautions; thus, if the hemoglobin concentration of a patient is low without declining to such a degree of life-threatening, a trial should be made to avoid blood-transfusion or the volume of blood should be transfused as small as possible.

REFERENCES

Arredondo M, Nunez MT (2005). Iron and copper metabolism. Mol. Aspects Med., 26: 313-327.

Brookes MJ, Hughes S, Turner FE, Reynolds G, Sharma N, Ismail T, Berx G, McKie AT, Hotchin N, Anderson GJ, Iqbal T, Tselepis C (2006). Modulation of iron transport proteins in human colorectal carcinogenesis. Gut, 55: 1449-1460.

Cañete M, Soriano J, Villanueva A, Roca AG, Veintemillas S, Serna CJ, Miranda R, Del Puerto Morales M (2010).The endocytic penetration mechanism of iron oxide magnetic nanoparticles with positively charged cover: A morphological approach. Int. J. Mol. Med., 26: 533-539.

Eisenstein RS (2000). Iron regulatory proteins and the molecular control of mammalian iron metabolism. Ann. Rev. Nutr., 20: 627-62.

Fairweather-Tait SJ (2004). Iron nutrition in the UK: getting the balance right. Proc. Nutr. Soc., 63: 519-528.

Fargion S, Valenti L, Fracanzani AL (2011). Beyond hereditary hemochromatosis: new insights into the relationship between iron overload and chronic liver diseases. Dig. Liver Dis., 43: 89-95.

Huang X (2003). Iron overload and its association with cancer risk in humans: evidence for iron as a carcinogenic metal. Mutat. Res., 533: 153-171.

Jiang XP, Elliott RL, Head JF (2010). Manipulation of iron transporter genes results in the suppression of human and mouse mammary adenocarcinomas. Anticancer Res., 30: 759-765.

Le NT, Richardson DR (2002). The role of iron in cell cycle progression and the proliferation of neoplastic cells. Biochim. Biophys. Acta, 1603: 31-46.

Li D, Sakashita S, Morishita Y, Kano J, Shiba A, Sato T, Noguchi M (2011). Binding of lactoferrin to IGBP1 triggers apoptosis in a lung adenocarcinoma cell line. Anticancer Res., 31: 529-534.

Park S, Yoon SY, Kim KE, Lee SR, Hur DH, Song H, Kim D, Bang SI, Cho DH (2009). Interleukin-18 induces transferrin expression in breast cancer cell line MCF-7. Cancer Lett., 286: 189-195.

Starley BQ, Calcagno CJ, Harrison SA (2010). Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. Hepatol., 51: 1820-1832.

Sun X, Ge R, Cai Z, Sun H, He QY (2009). Iron depletion decreases proliferation and induces apoptosis in a human colonic adenocarcinoma cell line, Caco2. J. Inorg. Biochem., 103: 1074-1081.