Original Article

Effect of passive cigarette smoke on mouse placenta and the role of antioxidants

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Abstract

Objective: To study the effects of passive cigarette smoke on the architecture of mouse placenta and to observe the preventive role of antioxidants if any.

Methods: It was a randomized control trial. Female mice of Balb C strain (51) were mated and grouped as follows: Groups, C: control, S: exposed to smoke and SV: exposed to smoke and given antioxidants (vitamin C, E) and sacrificed at 19 dpc (days post coitus). 14 animals from C, 12 from S and 14 from SV had healthy pregnancies. Their placentae were studied microscopically. The relative thickness of the labyrinthine, spongiotrophoblast-I, spongiotrophoblast-II, and decidual layers were measured. The area of spongiotrophoblast-I was calculated using a computerized software programme.

Results: The mean relative thickness and area of the spongiotrophoblast-I in S (14.46±1.88%, 5.89±0.87%) was significantly more (p=0.03, p=0.035 respectively) as compared to the controls (9.47±1.31%, 3.95±0.24%). In SV these values (13.96±1.2% and 5.74±0.82%) did not show any significant improvement as compared to S (p=0.035).

The mean relative thickness of the labyrinthine, spongiotrophoblast II, and decidual layer in the control group was 44.49±2.51%, 19.06±2.48, 19.44±0.68 respectively. None of these showed any significant difference from each other in the three groups.

Conclusion: Cigarette smoke causes a significant disturbance in the architecture of mouse placenta which could not be prevented by antioxidants. Therefore, these effects may be due to other toxic substances present in the smoke rather than free radicals.

Keywords: Cigarette smoke, Free radicals, Antioxidants, Mouse placenta, Passive smoking (JPMA 61:676; 2011).

Introduction

Epidemiologic studies during the last half century have clearly pointed on the adverse effects of tobacco use on health.[1] Tobacco smoke contains approximately 4000 toxic chemicals[2] and is also potentially capable of generating a high free radical load in the body.[3] Passive smoking, which is breathing other people's smoke, is also considered equally injurious to health as it contains approximately the same toxic substances and free radicals that are present in mainstream tobacco smoke.[4]

Free radicals generated by smoke may lead to oxidative injury in the body,[5] which is counteracted by antioxidants such as vitamin C and E.[6] An excess of free radicals in the absence of sufficient quantities of antioxidants in the body may lead to a state of increased oxidative stress, which has been implicated in the pathology of several diseases.[7] There is therefore a requirement for research to focus on the role of dietary antioxidant supplements which might prove effective in preventing the free radicals associated oxidative damage.

Apart from other injurious effects, studies have demonstrated the effects of cigarette smoke on the morphology of human placenta,[8] which may be the underlying cause of smoke related Perinatal mortality.[9,10] Experimental studies on mouse placenta have also shown a number of histological changes linked with smoke exposure.[11]

In the laboratory mouse three regions can be identified in the main placenta from within outwards: The giant cell layer, spongiotrophoblast layer and labyrinthine layer. The giant cell layer is responsible for accomplishing implantation; Spongiotrophoblast layer is homologous to the cytotrophoblast cell columns in the primates[12] and is responsible for producing hormones.[13] Labyrinthine layer is the major site of maternal foetal exchange and is homologous to the chorionic villi in the primates.[12]

Although studies are available regarding the beneficial effects of antioxidants in preventing some of the harmful effects of cigarette smoke on different parts of the body, however direct histological study of placenta and the extent of the preventive effects produced by antioxidants has not been carried out. Keeping this in mind, this project was designed to study the effects of cigarette smoke on the histological architecture of mouse placenta and to observe the

preventive role of antioxidants if any.

Materials and Methods

In this randomized control trial, a total of 51 nulliparous mice (Balb C strain) were selected by random sampling. The mice were housed in standard cages where temperature was maintained between 20-25°C and humidity ranged between 36-42%. The lights were attuned on 12 hour on/off cycle. They were taken for breeding, and every four of these were placed in a male's cage. They were checked daily for the presence of vaginal plug, which was considered as day 1 of gestation (1 dpc: days post coital). The mice with vaginal plugs were weighed, and six of them were housed in a metal cage of shoe box size and food and water provided to them "ad libitum". They were divided into three groups each containing 17 animals: Group C were control; Group S (Smoke) were further divided into two subgroups and were exposed to passive cigarette smoke in a whole body exposure chamber as described in a previous study.[14] Six of these were exposed to mild smoke i.e 4 cigarettes daily from 7 dpc, with an interval of half hour in between, and 11 were exposed to moderate smoke i.e 12 cigarettes daily from 7 dpc onwards with an interval of 15 minutes in between; Group SV (Smoke plus vitamin) were also subdivided into two subgroups and exposed to mild (n=5) and moderate smoke (n=12). They were injected vitamin C (sodium ascorbate) intramuscularly (35mg/Kg body weight),[15] and were provided with a diet containing vitamin E supplements (400 international units in 20 kg of food).

This routine was carried out for 5 days a week. At 19 dpc, the animals were sacrificed and dissected. The abdomen was opened and the uteri were examined. The animals with apparently healthy pregnancies in different groups were included in this study, whereas the remaining animals were used in another study. Therefore, in the present study, the C group contained 14 animals, S group 12 (5 of which had been exposed to mild smoke, and 7 to moderate smoke) and SV group 14 (5 of which had been exposed to mild smoke and 9 to moderate).

The uteri with attached placentae were stored in 10% formalin, and the tissues were processed for paraffin embedding. Five micron thick sections were cut and stained with PAS stain.

The three layers of the placenta; labyrinthine, spongiotrophoblast I, spongiotrophoblast II and decidua were identified, and their average thickness was calculated. The total thickness of the placenta was also measured and the relative thickness of each layer was calculated as a percentage of the total thickness of placenta.

Digital photographs of the slides were taken at low magnification. These photographs were then transferred to the computer software named "Image J 1.33"16. All the photographs had the image of ocular micrometer for reference. The known distance of one division of the ocular was fed into the software for reference. Then, the spongiotrophoblast I layer in each photograph was outlined by freehand tool and the area was calculated with the help of the above referred software. In the end, the relative area of this layer was calculated in reference to the total area of the placenta.

Data analysis for quantitative data which included relative thickness of the various layers, and relative area of the spongiotrophoblast I was performed through SPSS version 10 and presented as mean±SE (Standard Error). The means were compared for significance using paired sample t test at a confidence limit of 95 percent.

Results

The labyrinthine layer made up the major portion of the placental disc. The mean relative thickness of the labyrinthine layer in the C group was 44.49±2.51% of the total thickness of the placenta. It decreased to 40.46±2.78% in the S and was 41.23±2.36% in the SV group. However, none of these showed any significant statistical difference from each other (Statistical significance between C and S: p=0.29; Statistical significance between S and SV: p=0.84).

Relative thickness of spongiotrophoblast I was 9.47±1.31% in the C group which significantly increased in the S to 14.46±1.88% (p=0.035). However, increase in thickness became significant only upon moderate exposure to smoke. In the SV it was 13.96±1.2% which does not show any statistically significant difference from the S group (p=0.822).

Relative thickness of spongiotrophoblast II was 19.06±2.48% in the C group, 16.69±1.55% in the S and 18.29±0.91% in the SV group. These readings do not show

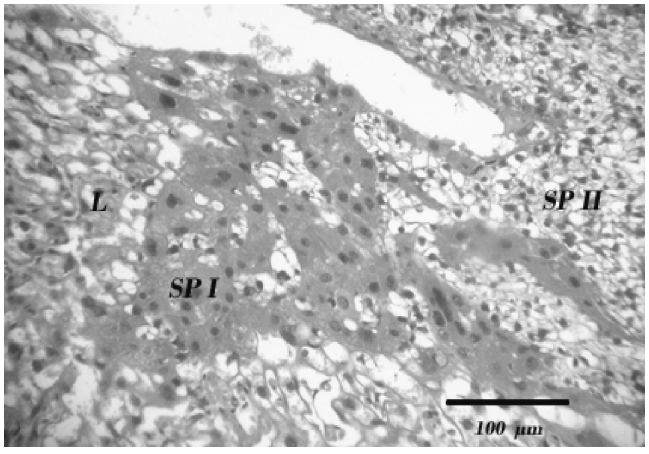

Figure: Section of mouse placenta (case C 19b), showing the labyrinthine (L), spongiotrophoblast-I layer (SP I) and spongiotrophoblast- II layers. PAS stain. Photomicrograph. Bar, 100 µm.

Table: Relative Thickness and area of layers of Placenta.

	n	Relative Thickness of labyrinthine(%) Mean±SE	Relative Thickness of Spongiotrophoblast-I(%) Mean±SE	Relative Thickness of Spongiotrophoblast-II(%) Mean±SE	Relative Thickness of Decidua(%) Mean±SE	Relative Area of Spongiotrophoblast-I (%) Mean±SE
Control Group (as a whole)	14	44.49±2.51	9.47±1.31	19.06±2.48	19.44±0.68	3.95±0.24
Smoke Group (as a whole)	12	40.46±2.78	14.46±1.88	16.69±1.55	18.09±1.21	5.89±0.87
Smoke plus Vitamin (as a whole)	14	41.23±2.36	13.96±1.2	18.29±0.91	21.95±1.63	5.74±0.82
Smoke Group (Mild smoke)	5	45.2±2.2	10.8±1.33	15.51±2.59	18.44±0.42	4.22±0.42
Smoke Group (Moderate smoke)	7	37.08±4.19	17.01±2.27	17.55±2.0	19.27±1.78	7.08±1.31
Smoke plus Vitamin (Mild Smoke)	5	47.46±3.6	10.81±1.85	19.16±1.35	18.91±2.36	4.13±1.00
Smoke plus Vitamin (Moderate Smoke)	9	39.81±2.24	15.86±1.44	18.03±1.33	22.29±1.87	5.48±0.53

any statistically significant difference from each other (p=0.44 and 0.36 respectively)

The relative thickness of the decidua was 19.44±0.68%, 18.09±1.21%, and 21.95± 0.29% in the C, S and SV respectively which do not show any statistically significant difference from each other (p=0.674 and 0.151 respectively) (Table).

The thickness of the various layers in the subgroups of animals exposed to mild and moderate smoke in the S and SV group is given in Table. None of the results show any significant improvement in the animals exposed to mild or moderate smoke in the SV as compared to the S group.

The mean relative area of the spongiotrophoblast-I layer in the 3.95±0.24 % in the C and 5.89±0.87% in the S (Figure). This shows a statistically significant increase in the S as compared to C (p=0.03). However, this increase became statistically significant only on exposure to moderate smoke. No statistically significant improvement (p=0.91) towards normalization was observed in the SV group where mean relative area of the spongiotrophoblast-I layer was 5.74±0.82%.

Discussion

The present study shows some decrease in the average thickness of the labyrinthine layer on exposure to smoke, but the difference was not significant. As this layer is basically responsible for maternal foetal exchange, a decreased area for this exchange surface may ultimately be responsible for poor foetal outcome. A similar thinning of the labyrinthine layer was also seen in placentae of rat foetuses showing intrauterine growth retardation after exposure to hyperthermia.[17]

The results of the present study show that the relative thickness area of spongiotrophoblast-I layer, was significantly increased in the S group, and the difference became significant only on moderate exposure to smoke. This increase in thickness can be explained on the basis of many mechanisms, any one or more of which can act as the underlying etiological factor: Studies show that placental architecture is regulated by oxygen tension.[18] There is evidence that the development of the human placenta during the first few weeks takes place in a low-oxygen environment.[19] In such conditions, the cytotrophoblast cells tend to proliferate rapidly, but invade poorly. However, once oxygen tension becomes normal, they stop proliferating and differentiate into tumour-like cells that invade the uterus and its vasculature. Thus indicating that oxygen tension strongly influences placental development. Spongiotrophoblasts of the mouse placenta are homologous with cytotrophoblasts in humans,[18] so a similar response to hypoxia can be expected. The results of the present study show an increase in the relative thickness and area of this layer in the smoke exposed group which may be due to an increase in their proliferative capability as a result of decreased oxygen availability to the placenta even beyond the first trimester. This view is further supported by studies which show hyperplasia of the cytotrophoblast cells upon exposure to tobacco smoke in the human placenta.[20] Another explanation for the relative increase in thickness and area of the spongiotrophoblast-I layer can be based on the fact that apoptosis which is observed throughout gestation, increases near the term.[21] Gruslin et al[22] examined the influence of maternal smoking on trophoblast apoptosis throughout development and concluded that exposure to smoke, decreased trophoblast apoptosis near term. However, Ashfaq et al,[8] observed an increase in apoptosis in the placenta of the smoke exposed group. Thus suggesting that, further research is necessary for reaching a conclusive opinion regarding this issue. A still further explanation can be based on studies which show that exposure to tobacco smoke has an anti estrogenic effect[23] and

therefore the increase in number of the hormone producing cells of the spongiotrophoblast-I may be a compensatory mechanism to increase the production of hormones necessary for sustaining a healthy pregnancy.

More knowledge would help us to unveil the underlying mechanisms involved in increasing the area occupied by these cells. Whatever the mechanism involved, there is evidence that maternal smoking leads to increased proliferation of cytotrophoblast cells and their abnormal differentiation.[24]

The present study did not show any significant decrease in the relative thickness or area of the spongiotrophoblast-I towards normalization upon administration of antioxidant supplementation. Thus, some other chemicals present in tobacco smoke might be playing a more important role in producing this effect as compared to free radicals.

Conclusion

Passive exposure to cigarette smoke exposes the body to free radicals and other toxic chemicals. These have effects on the architecture of mouse placenta including an increase in thickness and area of the spongiotrophoblast-I layer. The administration of antioxidants did not ameliorate these effects significantly. Consequently it can be assumed that the toxic affects of cigarette smoke are not only due to oxidative injury produced by free radicals but, may involve other toxic substances present in the smoke.

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