

## LETTER TO THE EDITOR

### Effect of low-molecular-weight fucoidan on experimental arterial thrombosis in the rabbit and rat

S Collic-Jouault<sup>1</sup>, J Millet<sup>2</sup>, D Helley<sup>3</sup>, C Sinquin<sup>1</sup> and A M Fischer<sup>3</sup>

<sup>1</sup>IFREMER, Nantes, France;

<sup>2</sup>Laboratoires Fournier, Daix, France;

<sup>3</sup>INSERM U428/Université Paris V, Paris, France.

Correspondence to: S. Collic Jouault, Laboratoire de Biotechnologie des Molécules Marines, IFREMER, BP 21105, 44311 Nantes Cedex 3, France. E-mail: [Sylvia.Collic.Jouault@ifremer.fr](mailto:Sylvia.Collic.Jouault@ifremer.fr)

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Dear Sir,

The anticoagulant effect of fucoidans (sulphated polysaccharides extracted from brown seaweed)<sup>1,2</sup> depends on a mechanism of action that appears to be somewhat different from that of heparin. On a weight basis, in plasma, fucoidan is about 20 to 30 times less anticoagulant than heparin, as determined by activated partial thromboplastin time or thrombin time<sup>2</sup>. Kinetic studies suggest that thrombin inhibition by heparin cofactor II (HC II) is more responsible than antithrombin (AT) catalysis for the anticoagulant activity of fucoidan<sup>1-3</sup>. *In vitro* studies have shown that fucoidan, like heparin, induces a release of tissue factor pathway inhibitor (TFPI) from endothelial cells in culture that could contribute to the anti-thrombotic effect of fucoidan<sup>4</sup>.

Our previous work showed that a low-molecular-weight (LMW) fucoidan fraction produced venous anti-thrombotic activity in a Wessler model in the rabbit, whereas only a slight anticoagulant effect was observed *ex vivo*<sup>3,5</sup>. The present study tested the arterial anti-thrombotic activity of fucoidan in the rabbit and the rat.

In the rabbit, the technique of Umetsu & Sanai<sup>6</sup> was used to perform an extra-corporeal arteriovenous shunt between the carotid artery and the jugular vein ; mean times for formation of an occlusive thrombus in the carotid artery were determined in control and treated animals. Both fucoidan and unfractionated heparin, injected intravenously 5 min before thrombosis induction, inhibited thrombus formation significantly. LMW fucoidan and heparin increased mean time-to-occlusion about three-fold in the same concentration range (2 mg/kg fucoidan  $43.7 \pm 5.5$  min and 1 mg/kg heparin  $55.4 \pm 2.6$  versus control  $15.6 \pm 1.6$  min ; 5 animals per group ;  $p \leq 0.001$ ). The anti-thrombotic effect of fucoidan was also evaluated in this model after subcutaneous injection 2 h before thrombosis induction. With a dose of 7.5 mg/kg, time-to-occlusion was prolonged significantly ( $22.8 \pm 3.4$  min in the treated group

versus  $12.6 \pm 1.0$  min in the control group ; 5 animals per group ;  $p \leq 0.01$ ). Our previous studies in the rabbit, using approximately the same (1.8 mg/kg i.v.) or even higher (20 mg/kg s.c.) doses, showed that LMW fucoidan inhibited thrombus formation by 80% ( $ED_{80}$ ) in a Wessler model of venous thrombosis<sup>3,5</sup>. The  $ED_{80}$  of unfractionated heparin was 0.1 mg/kg i.v. in this Wessler model. These earlier studies also indicated that neither *ex vivo* coagulation parameters nor bleeding times were strongly affected when LMW fucoidan was injected into the rabbit intravenously or subcutaneously at  $ED_{80}$ , resulting in low bleeding risk compared to unfractionated heparin or dalteparin also administered at  $ED_{80}$ <sup>3,5</sup>. Some studies comparing the anti-thrombotic efficacy of new agents with that of heparin in animal models of arterial thrombosis have reported a poor efficacy/bleeding risk margin for heparin. In arterial thrombosis, anti-thrombotic agents have usually been used at higher doses than in venous thrombosis. Interestingly, LMW fucoidan prevented both venous and arterial thrombosis at the same doses in our study, with low bleeding risk.

LMW fucoidan, unfractionated heparin and a LMW heparin (dalteparin) were compared in another animal species (rat) using the  $FeCl_3$ -induced arterial thrombosis model based on the method of Kurz *et al*<sup>7</sup>. In rats treated by LWM fucoidan, administered intravenously 5 minutes before thrombosis induction, time-to-occlusion was significantly prolonged in a dose-dependent manner from 1.25 to 5 mg/kg (Table 1). The dose (calculated by linear regression) required to increase time-to-occlusion two-fold was close to 2 mg/kg i.v. In animals treated by unfractionated heparin and dalteparin, administered intravenously 5 minutes before thrombosis induction, the doses that increased time-to-occlusion two-fold were 1.25 and 1.88 mg/kg, respectively. Thus, on a weight basis, LMW fucoidan and dalteparin, when tested intravenously, were active in the same concentration range for prevention of artery

occlusion in this animal model. The effects of LMW fucoidan on tail transection bleeding time performed according to Dejana *et al*<sup>8</sup> were compared with those of dalteparin and heparin. When LMW fucoidan or dalteparin was injected intravenously at doses corresponding to 2.5 times the dose doubling the time-to-occlusion of the carotid artery (close to 5 mg/kg), LMW fucoidan induced a low increase of bleeding time (430 s in the treated group versus 285 s in the control group ; 8 animals per group) as compared to dalteparin (>830 s ; 8 animals). Thus, with the same arterial anti-thrombotic activity, LMW fucoidan had a lower haemorrhagic effect than dalteparin, as determined by bleeding time. Moreover, with unfractionated heparin, for a dose as low as that doubling time-to-occlusion (1.25 mg/kg i.v.), bleeding time was markedly increased (>737 s ; 5 animals).

In conclusion, fucoidan, a polysaccharide of non-mammalian origin that prevents arterial thrombosis without major bleeding in animal models, could be a promising new drug for this pathology.

## References

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*Table 1* Effects of intravenously injected LMW fucoidan, heparin or dalteparin on FeCl<sub>3</sub>-induced arterial thrombosis in rats.

Treatment	Dose (mg/kg)	Time-to-occlusion (min)	N
Control	0	11.6 ± 0.6	19
LMW fucoidan	1.25	12.7 ± 1.4	5
	2.5	31.9** ± 9.3	6
	5	41.7*** ± 8.9	7
Heparin	1.25	20.5** ± 0.5	3
	2.5	60.0*** ± 0	2
Dalteparin	1.25	12.2 ± 0.3	3
	1.88	19.6** ± 5.6	8
	3.75	29.5** ± 8.9	4

Arterial anti-thrombotic effect of LMW fucoidan, heparin, dalteparin or saline control administered intravenously 5 min before thrombus induction in a rat model of FeCl<sub>3</sub>-induced arterial thrombosis.

Time-to-occlusion: data are the mean ± SEM. N: number of animals in each group.

\*\*: $p \leq 0.01$  versus control.\*\*\*: $p \leq 0.001$  versus control.