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ORIGINAL ARTICLE

Interactions of bexarotene (LGD1069, Targretin) with the coagulation system

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Abstract
Main purpose Bexarotene, (LGD1069, Targretin), is an antitumoral agent used as chemotherapy in the treatment of cutaneous T-cell lymphoma. Therapy with bexarotene is accompanied by adverse events, such as, bleeding, hemorrhage, and coagulopathy
Research question In order to design applications for bexarotene, it was very important to gain an understanding of how bexarotene inhibits blood clotting
Methods We investigated the interaction between bexarotene or vehicle alone, and coagulation factors or blood cells. We used both in vitro and in vivo assays. Anticoagulant activity of bexarotene or vehicle was assessed by clotting time tests (TT, RT, APTT, and PT). Coagulation factors activity was measured by adding diluted test plasma to artificially prepared factor–deficient plasma. Direct interactions between bexarotene and factor Xa were studied by chromogenic substrate assay. A mouse model was used to investigate in vivo effects of the drug on blood system and for evaluation of clinical hematology and organ pathology.
Results Increases in clotting times (prothrombin time and activated thromboplastin time) occurred with bexarotene in in vitro and in vivo experiments. We detected no significant influence of bexarotene on factors II, V, VII, VIII, XI and XII, while factor IX and factors X were affected. Bexarotene exerts anticoagulant effects and acts mainly as a direct factor IX and factor X inhibitor. On the contrary, the vehicle is remarkably inert toward the coagulation system. The number of blood cells was unaffected in mice treated with bexarotene or with the vehicle.
Conclusions Monitoring of the coagulation factors profile should be considered in cancer patients receiving bexarotene, particularly those with a known diagnosis of coagulation factors deficient.

Keywords Bexarotene · Cancer · Coagulation factors · Anticoagulant

Abbreviations

PPP	Human titrated platelet-poor plasma
TT	Thrombin times
RT	Reptilase times
PT	Prothrombin time
APTT	Activated partial thromboplastin times

Introduction

Bexarotene [Targretin, 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphtalenyl) ethenyl] benzoic acid] is an antitumoral agent used as chemotherapy in the treatment of cutaneous T-cell lymphoma [1]. Bexarotene has currently been evaluated for the treatment of other cancers [1–3] and psoriasis [4]. Recently, rexinoids LGD1069 and LG100268

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have been investigated as potential chemopreventive and chemotherapeutic agents in animal breast cancer models [5]. Both drugs have robust chemopreventive efficacy with respect to experimental breast cancer. Bexarotene, a synthetic derivative of 9-cis-RA, is protective in transgenic and carcinogen-induced models, indicating efficacy with respect to both estrogen receptor–positive and estrogen receptor–negative breast cancer [6–9]. Thus, bexarotene is both an element of the current antitumoral therapeutic arsenal and a molecule with emerging and promising effects in various pathologies [10–13]. However, therapy with bexarotene is accompanied by severe adverse effects, such as, bleeding, hemorrhage, and coagulopathy.

Rizvi et al. [14] conducted a phase I study of bexarotene in adults with advanced cancer. These investigators treated a total of 60 patients with oral bexarotene; daily doses ranged from 5 to 1,000 mg/m^2. Pharmacokinetic sampling was performed on days 1 and 15. Four of 60 patients experienced PT prolongation at the 650–1,000 mg/m^2 /day dose levels. No dose-limiting toxicities were observed up to the 500 mg/m^2 dose level. This study concludes that bexarotene possess a more favorable pharmacokinetic and toxicity profile than previously studied retinoids.

In multinational phase II–III trial study [15], the median duration of time in the study was 7 months with a further median 5 months follow up during which there were 17 deaths in the 107 patients (94 in the original database and a further 13 enrolled after the database cut off). One death from bleeding, hemorrhage, coagulopathy, and liver failure was considered possibly related. Moreover, Khuri et al. [16] conducted a phase I/II tumor-specific evaluation of bexarotene in 26 extensively pretreated patients with recurrent head and neck squamous cell carcinoma (HNSCC). Daily doses were increased from 10 to 600 mg/m^2. Coagulation parameters were changed; APTT (intrinsic coagulation pathway) and PT (extrinsic coagulation pathway) were prolonged. Likewise, the results of preclinical studies in several model systems have demonstrated significantly enhanced anticoagulant activity after oral administration of bexarotene. In the repeat-dose rat studies, dose-related mortality due to hemorrhage and serious coagulopathy was associated with bexarotene administered at doses >10 mg/kg/day in sesame oil suspension by gavage or in a diet [17].

Bexarotene formulations used in preclinical studies changed during development. In 3- and 6-month studies in rats in which bexarotene was administered by gavage in a PEG/aqueous suspension, hemorrhage due to serious coagulopathy was not observed, despite anticoagulant activity [18]. However, anticoagulant mechanism of bexarotene remained unclear. In order to design applications for bexarotene, it was very important to gain an understanding of how bexarotene inhibits blood clotting. In order to attain this, the present study investigates the interaction between bexarotene or vehicle and coagulation factors.

Materials and methods

Materials

Bexarotene was synthesized in the Laboratoire de Chimie Pharmaceutique (Faculté des Sciences Pharmaceutiques, Universite' de Lille 2, France). Two types of suspensions were tested: bexarotene suspension prepared in the presence of vehicle and vehicle suspension alone (carboxymethylcellulose 1%/polyethylene glycol [PEG] 400/Tween, 90/9.95/0.05, by volume). Human titrated platelet-poor plasma (PPP) was provided by the Laboratoire d'hématologie (Hopital Henri Mendor, Créteil, France), aliquoted, stored at −80°C, and thawed just before use. The buffer used for the clotting tests was PBS Invitrogen (France). Reptilase (FTH50), human thrombin (150 NIH units/mg), human factor Xa, thrombin and factor Xa-specific chromogenic substrate, Neoplastine' (thromboplastin supplemented with calcium ions), CK Prest (cephalin supplemented with kaolin), Factor II, V, VII + X, VIII, IX, XI and XII-deficient plasmas, Fibriprest automate (thrombin 100 NIH/U ml supplemented with calcium ions), and human fibrinogen were provided by Diagnostica Stago (France). Kaolin and calcium chloride were provided by Prolabo (France).

Blood and plasma collection

Human citrated platelet-poor plasma (PPP) from volunteer donors, or mouse PPP was obtained by drawing nine volumes of blood into one volume of 3.8% trisodium citrate and centrifuging at 5,000*g* at 4°C for 10 min. The plasma samples were stored at −80°C until use. Animal facilities, animal care, and study programs were in accordance with the principles in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and its appendix.

Effects of the bexarotene on clotting of human plasma

Thrombin times (TT) and reptilase times (RT): Bexarotene in vehicle 100 µL, in concentrations ranging from 0 to 10 mg/ml or vehicle suspensions or PBS buffer, was incubated with 200 µL of human PPP for 15 min at 37°C. Then, 100 µL human thrombin was added (final concentration, 5.5 NIH units/mL), and the clotting time was recorded. Controls were run using buffer or vehicle instead of bexarotene suspensions, and the level of thrombin was varied. These experiments were repeated with fibrinogen

solution (4 g/L) instead of PPP. Tests in both PPP and fibrinogen solution were repeated using reptilase instead of thrombin.

Prothrombin time (PT): Bexarotene in a concentration of 1–10 mg/mL in vehicle or vehicle suspension, 50 μL, at pH 7.35 was added to 100 μL PPP. The suspensions-plasma mixture and 100 μL thromboplastin were warmed separately for 3 min at 37°C. Then, 100 μL of thromboplastin was added and the clotting time was recorded.

Activated partial thromboplastin times (APTT): Both the suspensions-plasma mixture, as described for the prothrombin test, and 0.1 mL of activated cephaloplastin were prewarmed at 37°C for 3 min. Then, 100 μL of CaCl2 (0.025 M) was added, and the clotting time was recorded.

Effects of the bexarotene on the clotting factors

Coagulation factors activity is measured as reported elsewhere [19] with minor modifications. Coagulation factor II activity is measured by adding diluted test plasma to artificially prepared factor–deficient plasma and comparing the clotting time with the one obtained by the addition of normal diluted plasma. Thus, the clotting time will depend only on the factor II level of the test plasma, all the other factors being in excess. The corrective effect of the test plasma is compared with that of the standard plasma. The same principle applies to the other clotting factors.

Mixture of 10 μl of bexarotene (5 mg/ml, final concentration), vehicle or PBS buffer, and 90 μl of diluted normal plasma (1/10 in PBS buffer) was incubated with 100 μl of coagulation factor–deficient plasma for 10 min at 37°C. The samples were incubated with 100 μl of CK Prest for 3 min at 37°C. Then, 100 μl of 25 mM of calcium chloride was added, and the clotting time was recorded. The clotting time will depend only on the factor level of the test plasma, all other factors being in excess.

The study was validated with curve calibration established in a similar experiment, in which bexarotene solution was replaced by the PBS buffer in human plasma control. Different dilutions of plasma control in factor-deficient plasma were used. Standard curves were constructed by plotting clotting time (APTT or PT) against the coagulation factor concentration (%). The standard curve for the determination of coagulation factor concentrations was linear over the range of the concentrations tested ($r^2 = 0.9965$). The normal plasma (diluted l/10 in buffer)-deficient plasma mixture (50/50) given the value of 100% of factor activity. In coagulation extrinsic pathway, CK Prest replaced by Neoplastine product.

Fibrinogen clotting assay: 100 μl of Fibriprest Automate was added to the mixture of 10 μl of bexarotene (5 mg/ml, final concentration), vehicle or PBS buffer and 90 μl of diluted normal plasma (1/10 in PBS buffer), and the results were compared with controls.

Factor Xa inhibition in a purified system or in plasma in the presence of bexarotene

Bexarotene suspension, 150 μl at increasing concentrations, was incubated with 150 μl of PPP 1/18, AT III 1/18 in PBS (v/v) or vehicle, for 2 min at 37°C. Then, 150 μl of Human factor Xa (final concentration 300 ng/ml) was added. After 1 min, 150 μl of specific chromogenic substrate was added, and absorbance at 405 nm was recorded over 1 min at 37°C. Residual factor Xa was calculated by reference to a calibration curve obtained in the same system with factor Xa in the absence of bexarotene or PPP.

Measurements of the interactions between the particles and calcium ions

In the presence of plasma, l00 μl of CK Prest was added to l00 μl of plasma suspensions. After 3 min, l00 μl of calcium chloride at different concentrations (25, 50 and 100 mM) was added and the clotting time was recorded.

Experimental animals

Female Swiss mice (25–30 g) were obtained from Janvier Laboratories (Le Genst St Isle, France). Mice were allowed to adapt to the conditions of the animal house for 1 week before the experiments. The animals were maintained on a 12 h dark/light cycle at about 22 ± 3°C and allowed free access to a standard laboratory diet (Special Diets Services, Whitam, England) and drinking water during the experiments.

Mice were randomly divided into three groups ($n = 5$/group) and were treated with the bexarotene (suspended in vehicle at the doses of 100 mg/kg body weight), the vehicle, or water alone by oral gavage once daily for 14 days. Clinical signs were observed once daily during the experiments. Four hours after the last bexarotene or vehicle administration, all mice were necropsied after blood samples were collected by cardiac puncture under ether anesthesia for the evaluation of clinical hematology and organ pathology. Hematological parameters, measured with an automated hematology analyzer (Bayer, France), consist of red blood cell count (RBC), hemoglobin (Hb), platelet count (PLT), clotting time (CT), white blood cell count (WBC), and white blood cell differential count.

Plasma activity of coagulation factors was measured by clotting assay using human plasma that was deficient for each specific coagulation factor as described above and according to previously methods [20, 21]. The activities of FVIII, FIX, FXI, and FXII were measured based on the

Table 1 Clotting times: thrombin time, reptilase time, prothrombin time, and activated partial thromboplastin time

Experimental conditions	Thrombin time (s)	Plasma ± reptilase (s)	Fibrinogen ± reptilase (s)	Fibrinogen ± thrombine (s)	Prothrombin time (PT) (s)	Activated partial thromboplastine time (s)
Control	10 ± 2	30 ± 2	30 ± 3	11 ± 2	20 ± 2	110 ± 4
Vehicle	11 ± 1	27 ± 1	32 ± 2	13 ± 3	22 ± 2	105 ± 3
Bexarotene (mg/ml)						
1.25	11 ± 1	28 ± 1	30 ± 3	11 ± 2	20 ± 3	115 ± 6
2.5	12 ± 2	30 ± 2	29 ± 2	15 ± 3	19 ± 2	150 ± 3*
5	16 ± 2*	29 ± 2	32 ± 1	14 ± 1	22 ± 1	200 ± 6*
10	17.5 ± 2*	30 ± 2	30 ± 2	12 ± 2	30 ± 2*	248 ± 5*

Thrombin Time and Reptilase Time: Bexarotene in vehicle 100 μL, in concentrations ranging from 0 to 10 mg/ml or vehicle suspensions, was incubated with 200 μL of human PPP for 15 min at 37°C. Then, 100 μL human thrombin or reptilase was added and the clotting time was recorded. Controls were run using buffer or vehicle instead of bexarotene suspensions. Values are means ± SD of three independent experiments. A significant difference was accepted with $P < 0.05$

Prothrombin time (PT): Bexarotene in a concentration of 1–10 mg/mL in vehicle or vehicle suspension, 50 μL, at pH 7.35 was added to 100 μL PPP. The suspensions-plasma mixture and 100 μL thromboplastin was warmed separately for 3 min at 37°C. Then, 100 μL of thromboplastin was added and the clotting time was recorded. Values are means ± SD of three independent experiments. A significant difference was accepted with $P < 0.05$

Activated partial thromboplastin times (APTT): Both the suspensions-plasma mixture, as described for the prothrombin test, and 0.1 mL of activated cephaloplastin were prewarmed at 37°C for 3 min. Then, 100 μL of $CaCl_2$ (0.025 M) was added, and the clotting time was recorded. Values are means ± SD of three independent experiments. A significant difference was accepted with $P < 0.05$

* $P < 0.05$ compared with control group

activated partial thromboplastin time (APTT) and those of FV, FVII, and FX were measured based on the prothrombin time (PT).

Statistical analysis

Data were presented as means ± SD. The data were analyzed by one-way analysis of variance (ANOVA), and the Student's *t* test was used to determine the level of significance of differences. A significant difference was accepted with $P < 0.05$.

Results

In vitro anticoagulant activity of bexarotene

The anticoagulant activity of bexarotene was investigated in vitro by clotting time tests. In experiments with PPP, the TT was increased in the presence of bexarotene compared with control PPP containing a vehicle or buffer only (Table 1). With bexarotene concentrations, no greater than 10 mg/ml (final concentration), reptilase time (RT) was not significantly modified compared with the control (Table 1). When fibrinogen solution was used instead of plasma, neither TT nor RT was significantly increased by bexarotene compared with the controls vehicle or buffer (Table 1). Thus, under our experimental conditions, bexarotene did not interfere with fibrinogen or thrombin, suggesting the probable interaction between bexarotene and on the clotting factors other than thrombin and fibrinogen. To further investigate the anticoagulant activity of bexarotene, we measured effects on the extrinsic (PT) and intrinsic (APTT) coagulation pathways. The results presented in Table 1 show that when coagulation was initiated through either pathway, the clotting times remained similar to the controls in the presence of vehicle, whereas they were markedly prolonged in the presence of increasing amounts of bexarotene particle suspensions.

Bexarotene inhibits coagulation through clotting factors IX and X reduction

To confirm that bexarotene inhibited coagulation pathways activities, we analyzed coagulation factors activities. The activity in the Table 2 is defined as the percent ratio between the clotting time of the bexarotene and controls vehicle or buffer alone. Thus, the activity is less if bexarotene inhibits the coagulation factor. Compared to the controls, the presence of vehicle during l0-min incubation with PPP did not modify the activity of any factor. Concerning bexarotene, factors XII, XI, VIII, VII, V, II and I levels were similar to the controls, while factors IX and factor X level decreased by 35 ($P < 0.01$) and 30% ($P < 0.01$) respectively. Additional experiments using chromogenic substrate showed time-dependent factor Xa inhibition by bexarotene in purified system. Under these

Table 2 Effects of bexarotene on the activity of different coagulation factors in human (a) or mice (b) plasma

In vitro experiments (a) factor (%)	In vivo experiments (b) ($n = 5$) factor (%)
XII 99.50 ± 2.50	XII 100.50 ± 3.00
XI 124.09 ± 2.20	XI 102.60 ± 2.50
X 70.00 ± 2.00*	X 78.00 ± 1.60*
IX 65.00 ± 3.50*	IX 69.00 ± 1.50*
VIII 125.00 ± 1.50	VIII 104.00 ± 1.80
VII 110.00 ± 2.10	VII 110.00 ± 3.10
V 113.00 ± 3.00	V 105.30 ± 3.00
II 109.00 ± 3.00	II 103.00 ± 2.80
I 100.00 ± 2.00	I 102.20 ± 2.00

Values are means ± SD

* $P < 0.05$ compared with control

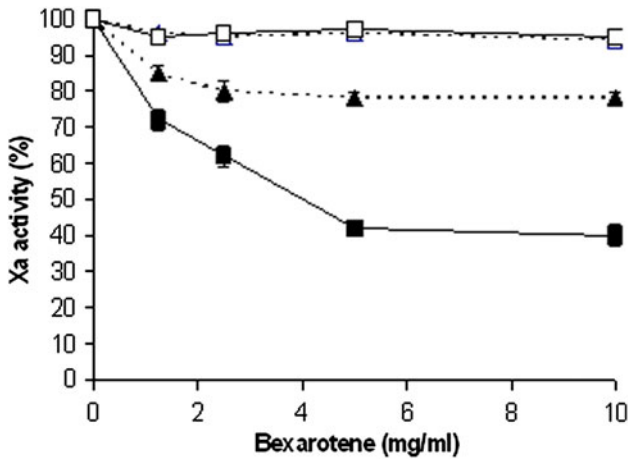

Fig. 1 Investigation into the interaction between bexarotene and factor Xa using chromogenic substrate assays. Various concentration of bexarotene (150 µL) was incubated with 150 µL of plasma (*filled triangle*) or vehicle (*filled square*) for 2 min at 37°C. Factor Xa (150 µL, final concentration 300 ng/mL) was added. After 1 min, 150 µL of chromogenic substrate was added and absorbance at 405 nm was recorded over at 37°C. Controls were run using plasma control (*open triangle*) or vehicle (*open square*) instead of bexarotene suspensions. Values are means ± SD of three independent experiments. A significant difference was accepted with $P < 0.05$

experimental conditions, 5 mg/ml of bexarotene inhibited 60% ($P < 0.01$) of Xa activity (Fig. 1). Similar results were obtained in a nonpurified system. However, in the presence of plasma, 5 mg/ml of bexarotene inhibited 20% ($P < 0.01$) of Xa activity, suggesting binding to other plasma proteins that interfered with binding to Xa.

Interactions between the bexarotene, factor Xa, and the antithrombin III

The hypothesis that bexarotene directly interferes with coagulation factor Xa by combining factor Xa and

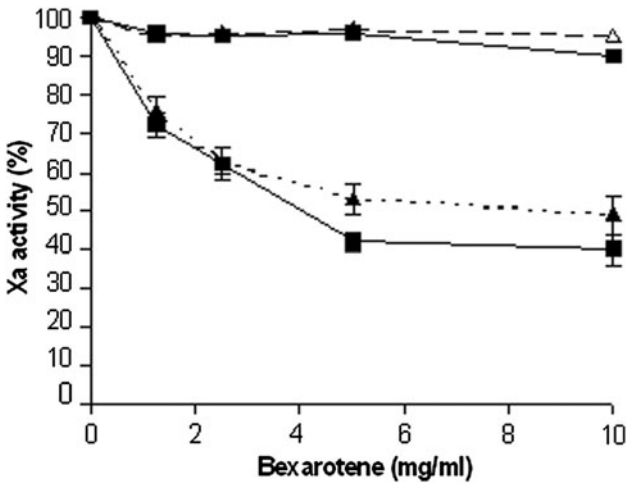

Fig. 2 Investigation into the interaction between bexarotene and factor Xa in the presence of antithrombin using chromogenic substrate assays. Various concentration of bexarotene (150 µL) was incubated with AT IIII (*filled triangle*), or vehicle (*filled square*), for 2 min at 37°C. Factor Xa (150 µL, final concentration 300 ng/mL) was added. After 1 min, 150 µL of chromogenic substrate was added and absorbance at 405 nm was recorded over at 37°C. Controls were run using AT III control (*open triangle*) or vehicle (*open square*) instead of bexarotene suspensions. Values are means ± SD of three independent experiments. A significant difference was accepted with $P < 0.05$

antithrombin III in the presence of bexarotene was investigated. As shown in Fig. 2, no significant difference was found between the anti-Xa activities in the absence or in the presence of antithrombin III, in the presence of bexarotene. This means that these bexarotene suspensions did not catalyze inhibition reaction of factor Xa by antithrombin III.

Interactions between the bexarotene and calcium ions

As bexarotene is charged negatively, we investigated its possible interaction with calcium ions. As shown in Fig. 3, the clotting time in the presence of bexarotene remained higher than the control at different concentrations of calcium ions.

Anticoagulant activity after oral bexarotene

Our finding that bexarotene exerted anticoagulant effects in vitro prompted us to conduct investigations in vivo. We measured the anticoagulant activity of oral bexarotene in a mice model. Oral bexarotene in a dose of 100 mg/kg increased both the PT and the APTT 4 h after the last administration (Table 3). In contrast, no anticoagulant activity was detected when mice were treated with vehicle.

To confirm that bexarotene inhibited coagulation factors, we analyzed coagulation factors activities in plasma

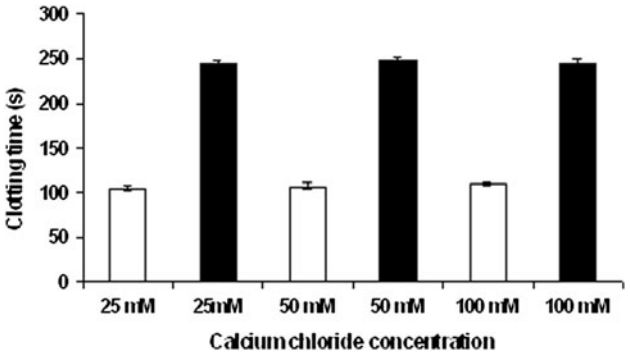

Fig. 3 Variations of the clotting time triggered by the addition of cephalin, kaolin, and calcium ions either to a plasma suspension of bexarotene (*black*) or to control plasma (*white*), as a function of the calcium ions concentration. Clotting system: to 50 µl of a mixture of 25 µl kaolin and 25 µl cephalin was added 100 µl of plasma suspension, and after 3 min, l00 µl of calcium chloride (25, 50, 100 mM). The results are the means ± SD of 4 determinations. A significant difference was accepted with $P < 0.05$

from mice after oral bexarotene administration. Compared with the controls (vehicle or water), coagulation factor IX and X levels decreased in the mice sample that was treated with bexarotene by 31% ($P < 0.05$) and 22% ($P < 0.05$), respectively (Table 2). No significant differences were recorded in any other hematological parameter (Table 3).

In this study, no toxicity was observed in any mice treated, as judged by clinical signs of toxicity during 14 days. In the main study, following oral administration of bexarotene at the dose of 100 mg/kg, mice did not show any signs of toxicity or gross behavioral changes. Gross and microscopic examinations also indicated that there was no evidence of toxicity for any of the mice tested. During the experiment period, no significant abnormality in food intake, feces, hair, and behavior in any group of animals was observed.

At the autopsy, macroscopic observation of the organs did not show any change as a result of the treatment with bexarotene or the vehicle. Gross necropsy and pathological examination of treated mice did not reveal any abnormality in morphology of the brain, lungs, heart, spleen, liver, and kidneys.

Table 3 Hematological data in female Swiss mice ($n = 5$) after oral administration of bexarotene (100 mg/kg) for 14 days

Hematological parameters (i.p)	Control	Vehicle	Bexarotene
Hb (g/l)	11.90 ± 1.9	12.25 ± 1.26	12.13 ± 0.43
RBC (10^6/µl)	7.83 ± 2.5	8.1 ± 1.3	7.87 ± 1.5
Clotting time (s)			
TT	7 ± 2	9 ± 2	8 ± 2
PT	27 ± 2	25 ± 2	34 ± 4*
APTT	107 ± 2	103 ± 3	217 ± 4*
Hématocrit	40 ± 3.25	39.4 ± 1.7	41.4 ± 2
PLT (10^3/µl)	1002 ± 71	906 ± 76	1078 ± 48
WBC (10^3/µl)	7.22 ± 0.2	6.39 ± 1.5	8.1 ± 1
Lymphocytes (%)	77.6 ± 4	79 ± 2.4	80.1 ± 9
Neutrophils (%)	3.8 ± 0.5	4.1 ± 0.4	1.5 ± 0.5
Eosinophils (%)	0.0	0.0	0.0
Basophils (%)	0.9 ± 4	0.8 ± 1.5	0.7 ± 0.5
Monocytes (%)	18.3 ± 2.5	16.4 ± 1.34	17.7 ± 1.85

Values are means ± SD

* $P < 0.05$ compared with untreated group

Discussion

The aim of this work was to show evidence of the interactions of bexarotene and vehicle suspensions with plasma factors of the coagulation system. In the limits of the range of concentrations used, vehicle solutions had no effect on coagulation. The results obtained in the presence of vehicle suspensions were similar to those obtained in the presence of a buffer. This means that the vehicle did not interfere with coagulation initiated by either thrombin, reptilase or through intrinsic or extrinsic pathways. On the contrary, significant interactions were found in the presence of bexarotene. Bexarotene interferes with the coagulation system via intrinsic or extrinsic pathways. The prolongation of the APTT and prothrombin time in the presence of bexarotene seems to be mainly related to interactions with factor IX and factor X. The TT is affected by bexarotene since bexarotene prolong both PT and APTT.

The ability of bexarotene to inhibit coagulation factors of intrinsic and extrinsic pathways is among the most interesting finding from our study. In in vivo experiments, plasma from mice showed that oral bexarotene was found to prolong clotting time (APTT and PT) and to exhibit anti-IX factor and anti-Xa factor activity.

Factor IX is a key component of the plasma system that forms a fibrin clot at a site of vascular injury. Activation of factor IX by factor XIa is required in certain situations to prevent bleeding from premature clot degradation of this system, as demonstrated by the severe bleeding disorder associated with congenital factor IX deficiency (hemophilia B) [22]. It is currently thought that the process leading to fibrin generation is initiated by the formation of a complex between the plasma protease factor VIIa and a membrane protein expressed on cells underlying the blood vessel endothelium known as tissue factor (TF) [23, 24]. The factor VIIa/TF complex activates factor X through proteolytic cleavage to factor Xa, which in turn cleaves prothrombin to generate the enzyme α-thrombin. α-thrombin has many activities in hemostasis, including

conversion of plasma fibrinogen to fibrin to form a clot [25].

Factor VIIa/TF also activates factor IX to factor IXaβ [14]. In fact, factor IX is a major substrate for factor VIIa/TF [15]. The main function of factor IXaβ in coagulation is to activate factor X [26]. Thus, there are two mechanisms for converting factor X to its active form. When the hemorrhagic syndromes associated with deficiency of factors VII or IX are considered, it is clear that both pathways for factor X activation are required for normal hemostasis. The activity of the factor VIIa/TF complex may be temporally limited at a wound site due to the presence of inhibitors such as the TF pathway inhibitor [27] and by accumulation of clot that effectively covers the complex. Factor IXaβ would be required in this scenario for sustained activation of factor X, and bleeding in hemophilia would reflect a failure of this consolidation process, rather than a defect in initiation of coagulation. Phenotypic features of hemophilia, such as the propensity to bleed in tissues low in TF [28] and the requirement for prolonged factor replacement to prevent bleeding after surgery [22], support this interpretation.

The factor IX is structurally similar to factor X [14]; this explains the common inhibition of these factors by the bexarotene. Bleeding complications observed in patients treated by bexarotene were the results of deficit procured by bexarotene. This effect is not surprising because antithrombogenic functional polymers surfaces have been obtained by substituting insolubles polystyrene with sulfonate aminoacids [29] [30]. These polymers are able to inhibit serine proteinases of the coagulation cascade (mainely thrombin and factor Xa) in the presence of human plasma [31]. Comparatively, carboxylic groups present in the bexarotene structure could confer on this drug both anticoagulant activities. In our work, direct interactions between bexarotene and factor Xa were studied by chromogenic substrate assay. In the purified system, active factor X decreased as the bexarotene concentration increased. Similar results were obtained in a nonpurified system. However, in the presence of plasma, bexarotene is not able to bind to factor Xa, suggesting its binding to other plasma proteins that interfered with binding to factor Xa. This effect had been already observed by charef et al. [20] in which anionic compounds interact with many plasma proteins. No catalysis of inhibition of factor Xa was observed in the presence of antithrombin III. No interaction between bexarotene and fibrinogen was detected by the reptilase time with plasma or fibrinogen solution. The fibrinogen clotting time in the presence of thrombin was not prolonged. The addition of increasing amounts of calcium does not shorten the clotting time in the APTT system. This means that bexarotene did not interfere with calcium ions.

In this work, we describe the anticoagulant mechanism of bexarotene action. Bexarotene affects coagulation factors, factor IX and factor X in the blood system, resulting in a defect in initiation of coagulation. Thus, our results have to be considered as new indications in procedure and clinical situations in the case of treatment of patients (especially with congenital factor IX or X deficiency) with bexarotene.

References

1. Farol LT, Hymes KB (2004) Bexarotene: a clinical review. Exp Rev Anticancer Ther 4:180–188
2. Esteva FJ, Glaspy J, Baidas S, Laufman L, Hutchins L, Dickler M, Tripathy D, Cohen R, DeMichele A, Yocum RC, Osborne CK, Hayes DF, Hortobagyi GN, Winer E, Demetri GD (2003) Multicenter phase II study of oral bexarotene for patients with metastatic breast cancer. J Clin Oncol 21:999–1006
3. Rigas JR, Dragnev KH (2005) Emerging role of rexinoids in non-small cell lung cancer: focus on bexarotene. Oncologist 10:22–33
4. Smit JV, Franssen ME, de Jong EM, Lambert J, Roseeuw DI, De Weert J, Yocum RC, Stevens VJ, van de Kerkhof PC (2004) A phase II multicenter clinical trial of systemic bexarotene in psoriasis. J Am Acad Dermatol 51:249–256
5. Liby KT, YoreMM SpornMB (2007) Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. Nat Rev Cancer 7:357–369
6. Gottardis MM, Bischoff ED, Shirley MA, Wagoner MA, Heyman RA, Lamph WW (1996) Chemoprevention of mammary carcinoma by LGD1069 (Targretin): an RXR-selective ligand. Cancer Res 56:5566–5570
7. Lubet RA, Christov K, Nunez NP et al (2005) Efficacy of Targretin on methylnitrosourea-induced mammary cancers: prevention and therapy dose-response curves and effects on proliferation and apoptosis. Carcinogenesis 26:441–448
8. Wu K, Kim HT, Rodriquez JL et al (2002) Suppression of mammary tumorigenesis in transgenic mice by the RXR-selective retinoid, LGD1069. Cancer Epidemiol Biomarkers Prev 11: 467–474
9. Wu K, Zhang Y, Xu XC et al (2002) The retinoid X receptor-selective retinoid, LGD1069, prevents the developmentof estrogen receptor-negative mammary tumors in transgenic mice. Cancer Res 62:6376–6380
10. Bischoff ED, Gottardis MM, Heyman RA, Moon TE, Lamph WW (1998) Beyond tamoxifen: the retinoid X receptor-selective ligand LGD1069 (TARGRETIN) causes complete regression of mammary carcinoma. Cancer Res 58:479–484
11. Bischoff ED, Heyman RA, Lamph WW (1999) Effect of the retinoid X receptor-selective ligand LGD1069 on mammary carcinoma after tamoxifen failure. J Natl Cancer Inst 91:2118–2123
12. Yen WC, Prudente RY, Lamph WW (2004) Synergistic effect of a retinoid X receptor-selective ligand bexarotene (LGD1069, Targretin) and paclitaxel (Taxol) inmammary carcinoma. Breast Cancer ResTreat 88:141–148
13. Agarwal VR, Bischoff ED, Lamph WW, Hermann T (2000) Induction of adipocyte-specific gene expression is correlated-withmammary tumor regression by the retinoid X receptor-ligand LGD1069 (targretin). Cancer Res 60:6033–6038
14. Rizvi NA, Marshall JL, Dahut W, Ness E, Truglia JA, Loewen G, Gill GM, Ulm EH, Geiser R, Jaunakais D, Hawkins MJ (1999) A

Phase I study of LGD1069 in adults with advanced cancer. Clin Cancer Res 5:1658–1664
15. Duvic M, Hymes K, Heald P, Breneman D, Martin AG, Myskowski P, Crowley C, Yocum RC (2001) Bexarotene is effective and safe for treatment of refractory advanced-stage cutaneous Tcell lymphoma: multinational phase II-III trial results. J Clin Oncol 19:2456–2471
16. Khuri FR, Rigas JR, Figlin RA, Gralla RJ, Shin DM, Munden R, Fox N, Huyghe MR, Kean Y, Reich SD, Hong WK (2001) Multi-institutional phase I/II trial of oral bexarotene in combination with cisplatin and vinorelbine in previously untreated patients with advanced non-small-cell lung cancer. J Clin Oncol 19:2626–2637
17. Howell SR, Shirley MA, Grese TA, Neel DA, Wells KE, Ulm EH (2001) Bexarotene metabolism in rat, dog, and human, synthesis of oxidative metabolites, and in vitro activity at retinoid receptors. Drug Metab Dispos 29:990–998
18. Horn TL, Torres KE, Naylor JM, Cwik MJ, Detrisac CJ, Kapetanovic IM, Lubet RA, Crowell JA, McCormick DL (2007) Subchronic toxicity and toxicogenomic evaluation of tamoxifen citrate + bexarotene in female rats. Toxicol Sci 99:612–627
19. Tamada Y, Murata M, Hayashi T, Goto K (2002) Anticoagulant mechanism of sulfonated polyisoprenes. Biomaterials 5:1375–1382
20. Charef S, Petit E, Barritault D, Courty J, Caruelle JP (2007) Effects on coagulation of a synthetic heparan mimetic given intraperitoneally or orally. J Biomed Mater Res A. 83:1024–1031
21. Kung SH, Hagstrom JN, Cass D, Tai SJ, Lin HF, Stafford DW, High KA (1998) Human factor IX corrects the bleeding diathesis of mice with hemophilia B. Blood 3:784–790
22. Lozier JN, Kessler CM (2005) Clinical aspects and therapy of hemophilia. In: Hoffman R, Benz E, Shattil S et al. (eds) Hematology: basic principles and practice, vol 4. Churchill Livingstone: New York, p. 2047
23. Mackman N (2004) Role of tissue factor in hemostasis, thrombosis, and vascular development. Arterioscler Thromb Vasc Biol 24:1015–1022
24. Monroe DM, Key NS (2007) The tissue factor–factor VIIa complex: procoagulant activity, regulation, and multitasking. J Thromb Haemost 5:1097–1105
25. Davie EW, Kulman JD (2006) An overview of the structure and function of thrombin. Semin Thromb Hemost Suppl 1:3–15
26. Furie B, Furie BC (2005) Molecular basis of blood coagulation. In: Hoffman R, Benz E, Shattil S et al (eds) Hematology: basic principles and practice, vol. 4. Churchill Livingstone, New York, p. 1931
27. Lu G, Broze GJ Jr, Krishnaswamy S (2004) Formation of factors IXa and Xa by the extrinsic pathway: differential regulation by tissue factor pathway inhibitor and antithrombin III. J Biol Chem 279:17241–17249
28. Mackman N (2005) Tissue-specific hemostasis in mice. Arterioscler Thromb Vasc Biol 25:2273–2281
29. Charef S, Tapon-Bretaudière J, Fischer AM, Pflüger F, Jozefowicz M, Labarre D (1996) Heparin-like functionalized polymer surfaces: discrimination between catalytic and adsorption processes during the course of thrombin inhibition. Biomaterials 17:903–912
30. Charef S, Jozefowicz M, Labarre D, Tapon-Bretaudiere J, Fischer AM, Bros A (1990) Plasmatic antiproteinase activity enhancement by insoluble functionalized polystyrene surfaces. Biomaterials 11:425–429
31. Douzon C, Kanmangne FM, Serne H, Labarre D, Jozefowicz M (1987) Heparin-like activity of insoluble sulphonated polystyrene resins. Part III: Binding of dicarboxylic amino acids. Biomaterials 8:190–194